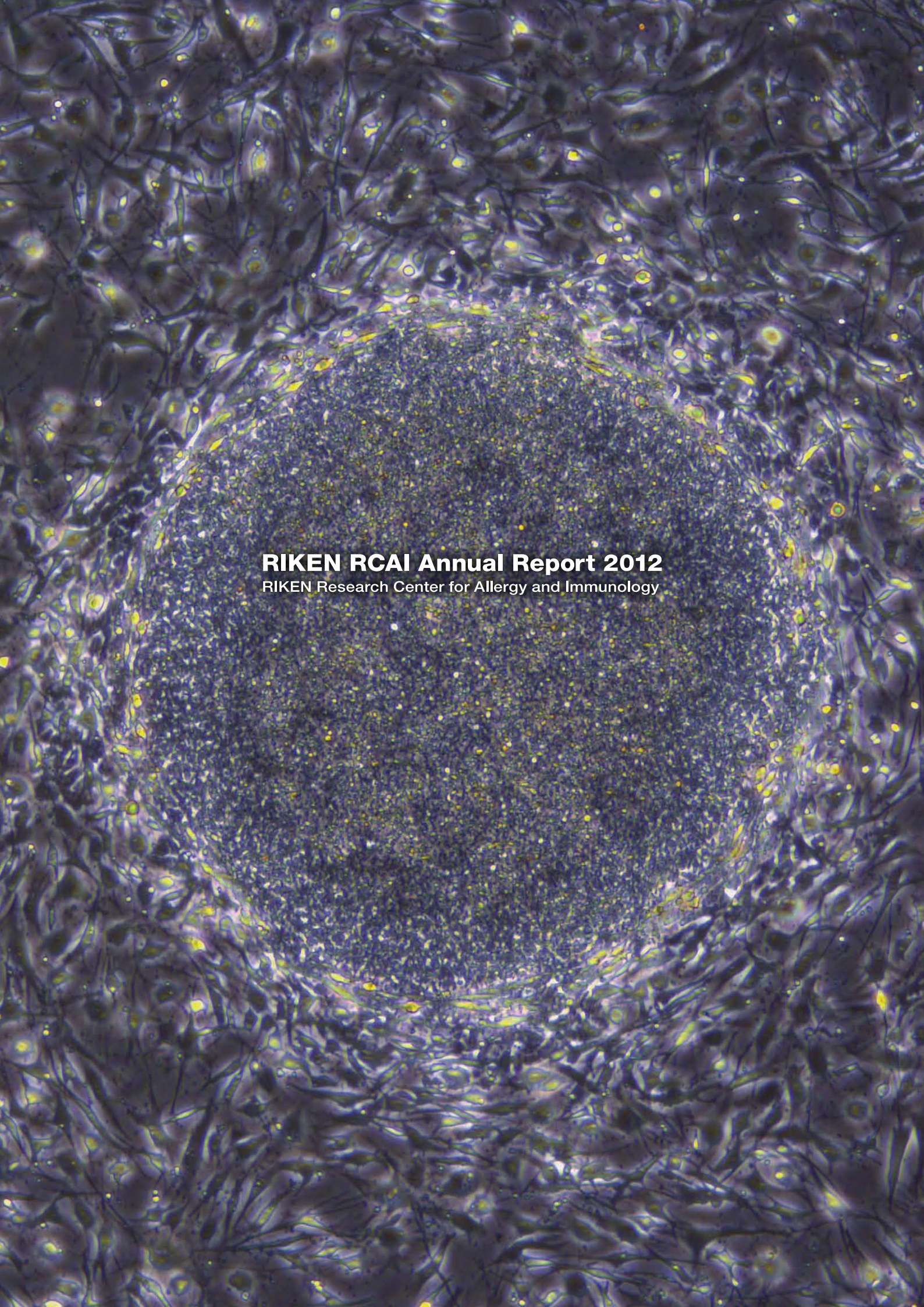

RIKEN RCAI Annual Report 2012

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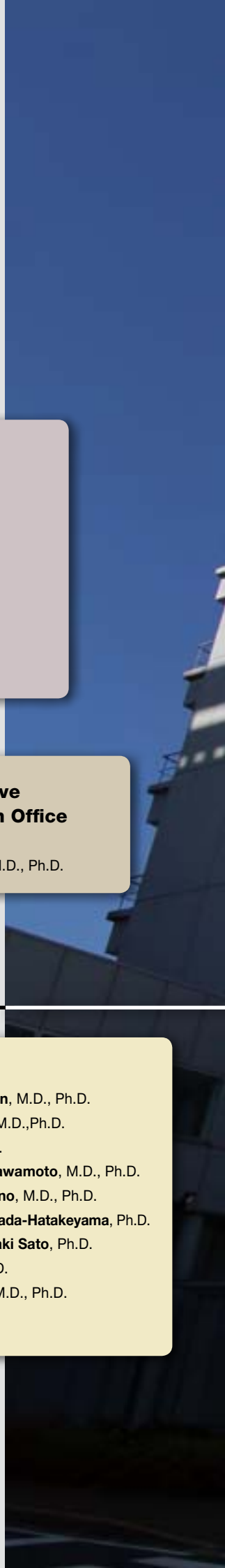

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Director's Report

This is the final annual report as the RIKEN Research Center for Allergy and Immunology (RCAI). In April 2013 RCAI will merge with the RIKEN Center for Genomic Medicine (CGM) to form a new institute, the Center for Integrative Medical Sciences (IMS) focusing on human immunology.

It has been twelve years since RCAI was established on the RIKEN Yokohama Campus. On this particular occasion, I would like to reflect on the promises that I made upon becoming the Founding Director of RCAI; at that time I decided to define seven objectives in establishing the Center.

The first was to establish a system to allow cutting edge research. The outcomes are now quite clear and there have been various epoch-making discoveries in the field of immunology by RCAI investigators.

For lymphocyte signaling, Dr. T. Saito discovered the minimal unit for T cell activation, called the TCR microcluster. This discovery was brought about through the use of single molecule fluorescence microscopy developed by Dr. M. Tokunaga. Dr. Tokunaga received the Commendation for Science and Technology awarded by the Minister of Education, Culture, Sports, Science and Technology (MEXT) in 2011. Dr. T. Hirano discovered that zinc functions as a signaling molecule in two phases, from the early induction of a zinc wave in response to a stimulus to the late induction of specific transporters. He received the Crafoord Prize in 2009 and the Japan Prize in 2011 for his IL-6 research. For lymphocyte development, Dr. H. Kawamoto established a new myeloid-based model of hematopoiesis that is quite different from the classical model with its lymphoid-specific progenitors. Dr. I. Taniuchi defined a transcriptional circuit regulated by Runx and ThPOK for lineage determination in the development of CD4 and CD8 T cell subsets. Drs. Kawamoto and Taniuchi received the Japanese Society for Immunology (JSI) Award in 2010 and in 2013, respectively. For Th2 cell differentiation, Dr. M. Kubo identified a specific region of the IL-4 enhancer

that regulates Th2 responses and a gene, *Mina*, which determines Th2 bias. Dr. T. Kurosaki identified the *in vivo* localization of long-term memory B cells adjacent to the germinal center and received a Commendation for Science and Technology awarded by MEXT in 2012. Dr. T. Okada utilized two-photon microscopy technology to track the process of B cell development in germinal centers. For mucosal immunity, Dr. H. Ohno identified GP2, a pathogen uptake receptor on M cells that provides a target for oral vaccines. Dr. S. Fagarasan, in collaboration with Dr. S. Hori, showed that $Foxp3^+$ T cells in Peyer's patches have unique characteristics that promote IgA generation. Drs. Fagarasan and Hori received the Young Scientist Award from MEXT, Japan in 2005 and 2006, respectively. Regarding innate immunity, Dr. T. Kaisho determined that IκBα is the key player in mediating interferon induction by two Toll-like receptor family members. He received a JSI Award in 2009. Dr. T. Tanaka identified a pathway by which PDLIM2 terminates NF-κB activation through intranuclear sequestration and subsequent degradation. For disease models, Dr. H. Yoshida identified a genetic mutation causing atopic dermatitis through the analysis of ENU-mutant mice and found that atopic dermatitis is not primarily an immune disorder. For systems immunology, Dr. M. Okada developed a systems biology model of a signaling cascade to discriminate activation vs. differentiation of cells through ERK activity.

RCAI's strong publication track record indicates that the research conducted at RCAI is world class. As of 2012, 11.4% of RCAI papers were ranked in the top 1% of papers based on citations. This figure was the highest amongst RIKEN institutes and it clearly demonstrates the high quality of research conducted at RCAI. As of 2013, the average citation per RCAI paper was 46.13, which ranked us third in the world according to Thomson Reuters Essential Science Indicators (See Table 2 in p. 132). Our strong publication record has been maintained through FY2008-2012. About 30% of the papers in these five years have been published in journals with impact factors above 10. I recognize the tireless effort of RCAI researchers and appreciate their scientific contributions during these ten years.

The second purpose was to create a platform for understanding the mechanisms of disease development and discovering the principles for diagnosis and therapy of human diseases. For this purpose, we established the Medical Immunology World Initiative (MIWI), which is an interdisciplinary research platform and an international consortium of multiple research groups.

Third, we have established the Young Chief Investigator Program with its mentor system to nurture talented young investigators who will lead immunology into the next generation and establish interdisciplinary research areas. We have recruited talented young researchers, Drs. S. Nakaoka (mathematical modeling), H. Kaneda (stem cell competency), T. Ikawa (immune regeneration), K. Shiroguchi (integrative genomics), K. Hase (bioenvironmental epigenetics) and T. Kitami (cellular bioenergetics network). Dr. Ikawa received Young Scientist Award from MEXT, Japan in 2012.

Fourth, we have built up a platform from basic research to clinical applications. Dr. F. Ishikawa, in collaboration with Drs. H. Koseki and O. Ohara, has been creating the third generation of humanized mice with a humanized microenvironment. They succeeded in generating mice with a humanized environment in thymus and bone marrow, supporting human T cell development and human HSC maintenance, and showed that human CD8 T cells could develop and differentiate into cells with cytotoxic function in these mice.

Fifth, we succeeded in producing six original seeds toward clinical applications. The first two were anti-allergy drugs. The PEGylated Cryj-1/2 fusion recombinant protein has been licensed to the Torii pharmaceutical company to develop as a biochemical drug for cedar pollinosis. Another version, a chemical compound developed by Dr. Y. Ishii and me, selectively induces apoptosis of IgE but not IgG B cells and preferentially suppresses IgE production. Therefore, it potentially could be applied to any type of IgE-mediated allergic disorder, such as pollinosis and food allergy, as well as allergic asthma. The third seed was NKT cell-targeted therapy. In collaboration with Professors Nakayama, Motohashi and Okamoto at Chiba University, I have conducted NKT cell-targeted adjuvant therapy as a phase I/II study for cancer patients. Advanced lung cancer patients had a very significant prolonged median survival time (MST) of more than 30 months with only a

primary treatment compared to the best supportive care, which had an MST of 4.6 months, or the MST of current advanced therapy, 10 months. This NKT cell-targeted therapy was authorized by the Japanese government as Advanced Medical Treatment B (Senshin Iryo B) in 2011 for advanced non-small lung cancers and in 2013 for head & neck tumors. As a medical scientist, it is a great honor and gives me tremendous satisfaction that I could discover NKT cells and then achieve their clinical application. The fourth seed was the artificial adjuvant vector cell as an anti-tumor vaccine developed by Drs. S. Fujii and K. Shimizu. This vaccine can be dosed with mRNA encoding antigens specific to the malignant tumor, and activates both innate and acquired protective immunity and induces long-term memory for more than one year in mice and dogs with a single administration of the vaccine. The fifth seed was the establishment of iPS cells from human cytotoxic T cells specific for melanoma and of human NKT cells by H. Koseki's group, as well as murine NKT-iPS cells generated by my group. The human iPS project was accepted as a Center for Clinical Application Research (Type B) in the Research Center Network for Realization of Regenerative Medicine, Japan in 2013. The sixth seed was Dr. Ishikawa's work on identification of leukemia stem cells and also development of a drug candidate for effective treatment of leukemia. He received a Young Scientist Award from MEXT, Japan in 2009 and a Japan Society for the Promotion of Science (JSPS) Prize in 2013.

Sixth, we have developed a strong support system for the Center's researchers. I am very proud of the Central Facilities— the Animal Facility managed by Dr. Koseki and Dr. T. Hasegawa, Dr. O. Ohara's Genomics Lab, and the FACS Lab managed by Dr. T. Saito and Ms. H. Fujimoto. Also, the Administrative Coordination Office headed by Ms. H. Tanabe, including Mr. Y. Murahashi's IT-team and Mr. T. Ogata's Facility Support, is operating at a very high level. I also appreciate Drs. P. Burrows, Professor of Alabama Univ., as a science advisor, Drs. T. Takemori and H. Iwano as research coordinators in RCAI.

Seventh, we established a unique Advisory Council system with a two-tiered approach. The Annual Scientific Reviews have a mentoring function and are limited to a review of the research undertaken by a given laboratory whereas the Term Review Meeting, which covers all research activities at the Center as well as the Center's overall research strategy, future plans, organizational policies, and research management, is held every three years. The system has been extremely helpful for RCAI researchers and I would like to express my sincere thanks to all AC members: Drs. M. Cooper (chair), R. Aebersold, A. Coutinho, A. Fischer, R. Germain, P. Kincade, T. Kishimoto, S. Koyasu, B. Malissen, R. Medzhitov, M. Miyasaka, D. Mathis, H. Nakauchi, W. Paul, S. Pierce, K. Rajewsky, T. Sasazuki, R. Steinman, K. Takatsu, D. Umetsu, and A. Weiss.

Finally, I am extremely proud of RCAI researchers, including the seventeen researchers who became university professors during the last 10 years: Dr. K. Hase, YCI, (The Institute of Medical Science, Univ. of Tokyo), Drs. H. Kawamoto, M. Hikida and T. Watanabe (Kyoto Univ.), Dr. K. Hoshino (Kagawa Univ.), Dr. M. Hoshino (Gifu Univ. of Medical Science), Dr. S. Ishido (Showa Pharmaceutical Univ.), Drs. T. Kaisho and Dr. T. Kurosaki (Osaka Univ.), Dr. M. Kubo (Tokyo Univ. of Science), Dr. M. Sakaguchi (Azabu Univ.), Dr. K. Sato (Miyazaki Univ.), Dr. K. Seino (Hokkaido Univ.), Dr. M. Tanaka (Tokyo Univ. of Pharmacy and Life Sciences), Dr. H. Udono (Okayama Univ.), Dr. M. Tokunaga (Tokyo Institute of Technology), and Dr. S. Yamasaki (Kyusyu Univ.).

The new Center aims to create an innovative research field, focusing on the prevention of human disorders using genomic and systems biology approaches in human immunology. The research endeavors by RCAI researchers will continue at a high level in the new center, and the name of our institute will remain as a Research Core for Advanced Immunology, IMS (IMS-RCAI).

Mar. 31, 2013

Masaru Taniguchi
Director,
RIKEN Research Center for Allergy
and Immunology (RCAI)

2012
Part 1

Creation of New Paradigms

Sidonia Fagarasan

Skewed gut microbial communities induce hyperactivation of the systemic immune system

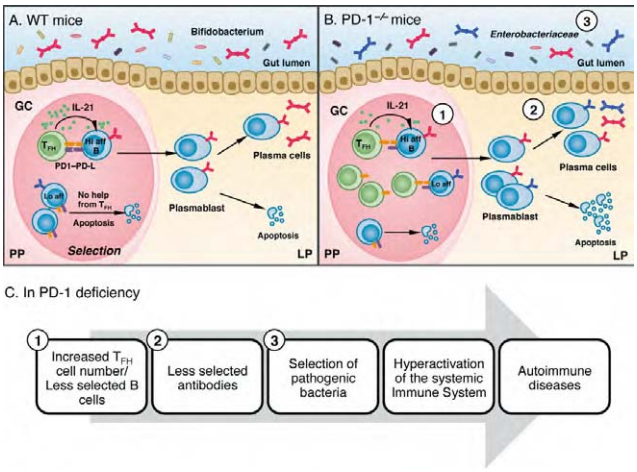

Figure: **Schematic representation of IgA selection within and outside the GCs.** Discrimination of GC IgA B cells with different affinities for gut antigens requires BCR engagement and competition for T_{FH} cell help. A. In WT mice, the limited number of T_{FH} cells implies that only the B cells able to present more antigen (high affinity) will receive T_{FH} help, with B cells capturing less antigen (low affinity) or those loosing the integrity of the BCR undergoing apoptosis. B. In PD-$1^{-/-}$ mice, both the high and low affinity B cells will receive help from the abnormally numerous T_{FH} present within the GCs. In both cases, the IgA B cells generated in the PP GCs migrate out to the MLN where they further proliferate and differentiate into plasmablasts, which via the thoracic duct and blood will reach the lamina propria (LP). In addition to selection in the GCs, IgAs appear to undergo a second, likely commensal-driven selection within the LP. In this way, the proliferating plasmablasts are reselected to fit the geographical distribution of bacteria along the intestine. (This does not exclude the possibility that IgAs are also undergoing selection at their first post-GC station, in MLN). C. The possible sequential events leading to generation of auto-reactive antibodies in PD-$1^{-/-}$ mice.

The gut must keep its trillions of microbial inhabitants contained, and Immunoglobulin A (IgA) plays a central role in the process, maintaining the symbiotic balance between gut bacterial communities and the host immune system. In a process that is not well understood, B cells that produce IgA specific for the gut microflora are selected in Peyer's patches in the gut. Sidonia Fagarasan's group (Laboratory for Mucosal Immunity) used genetically manipulated mice and could show that the inhibitory co-receptor programmed cell death-1 (PD-1) regulates microbial communities and IgA production in the gut.

In PD-1-deficient mice, the total numbers of "healthy" bacteria, such as *Bifidobacterium* and *Bacteroides*, were markedly reduced. By contrast, bacteria of the *Enterobacterobacteiaceae* family, which are minor representatives in wild-type mice, were expanded about 400-fold in PD-1-deficient mice. Flow cytometric analyses of fecal bacteria revealed that the proportion of bacteria coated with IgA was reduced in PD-1-deficient mice, while the concentration of free IgA in intestinal secretions was higher. Thus, the team suspected that the IgAs produced in PD-1-deficient mice have reduced bacteria-binding capacity. By sequencing the immunoglobulin heavy chain variable region genes of IgA-producing cells, they found that the IgA repertoire in PD-1-deficient mice is altered, which could cause the observed alterations in the composition of the microflora.

To investigate the cause of the altered IgA repertoire, they examined the Peyer's patches in mice deficient in the inhibitory receptor PD-1. The absence of PD-1 affected the differentiation of T follicular helper cells (T_{FH}), which provide important differentiation signals to B cells. PD-1 deficiency resulted in an excess number of T_{FH} cells with altered phenotypes, and the ratio of T_{FH} to B cells was increased 2-fold in the germinal centers of Peyer's patches (Fig.). This observation is very telling; it indicates that deregulation of T_{FH} cells leads to inappropriate GC B cell selection when B cells compete for T cell help in the GC.

Along with T and B cell hyperplasia in PD-1-deficient mice, the gut microbiota induced hyperactivation of the systemic immune system. They found that serum from PD-1-deficient mice contained antibodies specific for components of commensal bacteria, a response that does not occur in wild type mice.

"The skewed gut microbial communities that result from the dysregulated selection of IgAs drive the expansion of self-reactive B and T cells," says Fagarasan. "Our studies have implications for how modulation of PD-1 expression promotes tolerance or uncontrolled immune reactions leading to autoimmunity."

(*from left to right*) Thinh Huy Tran, Shimpei Kawamoto and Mikako Maruya

Original paper

Kawamoto, S., Tran, TH., Maruya, M., Suzuki, K., Doi, Y., Tsutsui, Y., Kato, LM., Fagarasan, S. The inhibitory receptor PD-1 regulates IgA selection and bacterial composition in the gut. ***Science*** 336, 485-489 (2012)

Raising an antitumor army

Reprogrammed immune cells might give doctors an edge in rallying the body's defenses against tumor growth

Hiroshi Kawamoto

Haruhiko Koseki

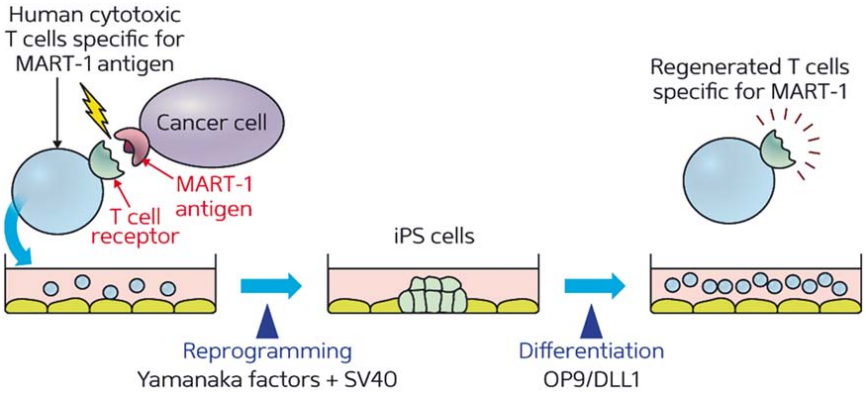

Figure: Scientists reprogrammed purified CTLs into embryonic stem cell-like iPSCs by forcing them to express genes known as the 'Yamanaka factors'. After allowing these reprogrammed cells to proliferate, they then cultivated them alongside specialized cells (OP9/DLL1) that promote their maturation into transplantation-ready, MART-1-specific CTLs.© 2013 Elsevier

Stem cells represent an ideal resource for the production of patient-specific antitumor cytotoxic T lymphocytes (CTLs), but the production process poses special challenges. To deal with the vast array of potential threats that might endanger our health, the immune system uses gene recombination to generate various armies of cells that each expresses receptors capable of recognizing a different target. This means that CTLs produced from either embryonic or adult stem cells would rarely retain the target-recognition capabilities needed to kill a tumor.

Hiroshi Kawamoto's team (Laboratory for Lymphocyte Development), in collaboration with Haruhiko Koseki's group in RCAI, therefore pursued an alternative approach involving the use of a genetic reprogramming technique to transform mature human CTLs into induced pluripotent stem cells (iPSCs). From this undifferentiated state, iPSCs can be cultivated under conditions that favor their development into mature CTLs (Fig.). "All of these lymphocytes will come to express the same antigen receptor as the original T cells," says Kawamoto. "The technique allows us to regenerate antigen-specific T cells with very high efficiency."

(*from left to right*) Raul Vizcardo Sakoda, Hiroshi Kawamoto, Kyoko Masuda and Daisuke Yamada

After proving the soundness of this approach using an isolated pool of mixed human CTLs, the researchers applied their method to a specific line of CTLs that selectively recognize MART-1, a protein commonly overexpressed by the skin cancer melanoma. After reprogramming, they obtained two iPSC lines that contained the same recombined MART-1-specific receptor gene found in the parental CTL cell line. Using one of these as starting material, the researchers were able to produce a 95%-pure pool of new anti-MART-1 CTLs.

Initial experiments using purified MART-1 confirmed that these iPSC-derived CTLs were producing the specialized receptor required to bind this melanoma antigen, and Kawamoto and his colleagues confirmed that their CTLs produced appropriate cytokines when cultured alongside human cells that express MART-1 on their surface. This result is encouraging, as the experiment roughly replicates the context in which CTLs might encounter this antigen in a melanoma patient.

The question of whether this apparent tumor-specific immune response will translate into improved patient outcomes remains to be determined. "We wish to test whether the regenerated T cells can kill tumor cells in *in vivo* situations using mouse models," Kawamoto says. "We are also planning to try the same experiments using different tumor antigens to confirm that this method can be generally used," says Kawamoto. "If this technology can be applied to cancer therapy, an extremely large number of patients would benefit."

Original paper

Vizcardo, R., Masuda, K., Yamada, D., Ikawa, T., Shimizu, K., Fujii, S., Koseki, H. & Kawamoto, H. Regeneration of human tumor antigen-specific T cells from iPSCs derived from mature CD8⁺ T cells. ***Cell Stem Cell*** 12, 31-36 (2013)

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http://www.rikenresearch.riken.jp/eng/hom/7228.html


Shin-ichiro Fujii


Kanako Shimizu

Giving antitumor immunity a helping hand

A two-pronged approach to immune activation could lead to vaccines that effectively shut down tumor expansion

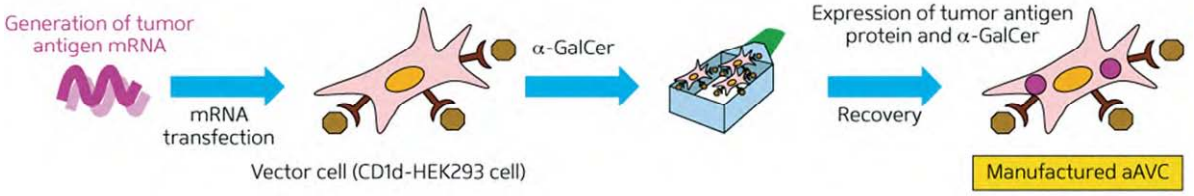


Figure: Artificial adjuvant vector cells (aAVC) were produced by introducing mRNA encoding tumor-specific antigens into human embryonic kidney cells dosed with the synthetic compound α-galactosylceramide, which activates NKT cells.

Tumor cells often express proteins that set them apart from their healthy neighbors. These very same proteins can also help the immune system to recognize and destroy the cancer. Several research groups and companies have already demonstrated proof-of-concept for antitumor therapeutic vaccines based on this principle, typically employing 'retrained' dendritic cells (DCs) harvested from a patient's own immune system. To date, however, such vaccines have demonstrated only limited effectiveness in beating back tumor progression. The research of Shin-ichiro Fujii (Research Unit for Cellular Immunotherapy), Kanako Shimizu (Research Unit for Therapeutic Model) and colleagues led to development of a technique that could supercharge the potency of future cancer vaccines.

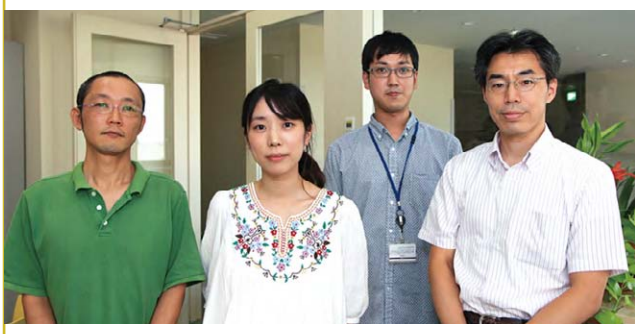
DC-based vaccines directly stimulate 'adaptive' immunity, which is directed against specific molecular targets. "DCs play a pivotal role in determining the character and magnitude of an immune response," explains Fujii, "and the loading of patients' DCs *in vivo* with tumor-specific antigens is one of the most promising current immunotherapeutic strategies."

Using a mouse model, Fujii's team demonstrated that the natural antitumor DC response can be considerably ramped up by stimulating invariant natural killer T (*i*NKT) cells from the 'innate' immune system, which triggers a more generalized response against disease.

Key to their *i*NKT cell-based strategy is the use of artificial adjuvant vector cells, derived from human kidney cells (Fig.). The artificial adjuvant vector cells are induced to take up mRNA encoding a tumor-specific antigen, which is processed by the host DC and expressed on the cell surface, where it can be recognized by T cells as coming from an 'enemy'. The artificial adjuvant vector cells are also treated with α-galactosylceramide, a synthetic glycolipid that is a potent activator of *i*NKT cells. The artificial adjuvant vector cells prepared this way are then injected into patients, where the *i*NKT cells accelerate maturation of host DCs, which in turn promote adaptive immune response against the selected tumor antigen.

Initial experiments demonstrated that artificial adjuvant vector cells producing the model protein ovalbumin activated DCs *in situ* in mice and effectively stalled growth of grafted tumors expressing this protein. Importantly, this treatment proved more effective than a conventional vaccine approach using *ex vivo* DCs transfected with ovalbumin mRNA. Subsequent experiments confirmed that artificial adjuvant vector cells could also elicit immunity against the melanoma antigen MART-1. "Even the injection of one cell could evoke both the innate and adaptive immune response," says Fujii.

Further tests using dog models revealed a clear immune response with no notable adverse effects, even in animals receiving multiple doses of artificial adjuvant vector cells. These promising preclinical results could potentially pave the way for human clinical trials in the near future. "We have some candidate tumor antigens," says Fujii, "and now we need to examine the possibility of using this technique for these antigens in terms of efficacy and safety."


(*from left to right*) Jun Shinga, Miki Asakura, Yusuke Sato and Satoru Yamasaki

Original paper

Shimizu, K., Mizuno, T., Shinga, J., Asakura, M., Kakimi, K., Ishii, Y., Masuda, K., Maeda, T., Sugahara, H., Sato, Y. *et al.* Vaccination with antigen-transfected, NKT cell ligand-loaded, human cells elicits robust *in situ* immune responses by dendritic cells. ***Cancer Res.*** 173, 62-73 (2013)

Enabling rapid recall of familiar foes

A 'rapid response' pathway for immune cell development may improve the body's ability to recognize and fight back against recurring infectious threats

Toshitada Takemori

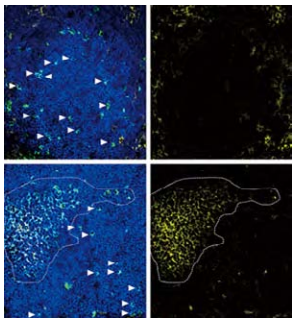


Figure: Fluorescent labeling reveals that both conditionally *Bcl6*-deficient (*top*) and wild-type (*bottom*) mice are able to produce functional memory cells (*left column, arrowheads*). However, these genetically modified animals lack germinal center (GC) B cells (*right column, yellow*), revealing a GC-independent pathway for memory cell development.

Efficient immune protection requires the ability to rapidly recognize intruders that the body has encountered in the past. This is achieved via 'memory' B cells, which develop following immune system activation by a virus, bacterium or other threat.

"Scientists have known about immunological memory for centuries," explains Toshitada Takemori (Laboratory for Immunological Memory), "but certain critical aspects of this process remain incompletely defined." As a case in point, Takemori's team and Klaus Rajewsky at Germany's Max-Delbrück-Center for Molecular Medicine recently uncovered striking proof of a novel memory B cell production pathway with a potentially distinct role in immune defense.

The initial appearance of an immunity-triggering antigen fuels interaction between B and T cells, which in turn yields activated B cells. These can either differentiate into cells that produce antibodies against the target antigen or migrate to structures called 'germinal centers' (GCs) where their antibody-encoding genes undergo extensive mutation. This somatic hypermutation (SHM) process generates antibodies with optimized target affinity and specificity, and the resulting cells mature into antibody-secreting plasma cells or memory B cells.

However, Takemori has observed evidence that some memory B cells never undergo SHM, apparently developing via a GC-independent pathway. This has proven difficult to verify experimentally: mice lacking the *Bcl6* gene fail to develop GCs but also suffer other defects, making them a poor research model. To overcome this, the researchers engineered rodents where *Bcl6* inactivation is limited to a subset of relevant cells.

These animals lacked GCs, but nevertheless generated memory B cells after an immune challenge in numbers roughly equivalent to normal mice (Fig.). Closer examination confirmed that the memory B cells produced by conditionally Bcl6-deficient animals did not undergo SHM. The researchers also isolated non-mutated memory B cells from wild-type animals, although these were eventually outnumbered by mutated memory B cells, indicating that these non-mutated cells represent a distinct subset of memory B cells that develop in advance of the GC maturation process. "Our analysis indicates that immunological memory is established as soon as possible after the onset of an immune response," says Takemori.

As non-GC memory B cells produce relatively low-specificity antibodies, the researchers hypothesize that these cells may complement optimized, post-SHM memory B cells by broadly responding to related but distinct threats: for example, influenza viruses in general rather than one specific strain. "We are now determining whether the GC-independent memory pathway assists the GC-dependent pathway to protect hosts against viral infections," says Takemori.

Conditional Bcl6-deficient mice, generated by Takemori and his colleagues, were selected the "Mouse of the Month" in June, 2013, by RIKEN BioResource Center
http://www.brc.riken.jp/lab/animal/mailnews/nm201306_01.html

Tomohiro Kaji (*left*) and Akiko Ishige (*right*)

Original paper

Kaji, T., Ishige, A., Hikida, M., Taka, J., Hijikata, A., Kubo, M., Nagashima, T., Takahashi, Y., Kurosaki, T., Okada, M. *et al.* Distinct cellular pathways select germline-encoded and somatically mutated antibodies into immunological memory. ***J Exp Med.*** 209, 2079-2097 (2012).

Related publication

Takahashi, Y., Ohta, H. & Takemori, T. Fas is required for clonal selection in germinal centers and the subsequent establishment of the memory B cell repertoire. ***Immunity*** 14, 181-192 (2001)

Ichiro Taniuchi

Hile Cheroutre

Reassigning cells to fight infection

Unexpected flexibility in immune cell development could help the body rapidly marshal its defenses at sites vulnerable to infection

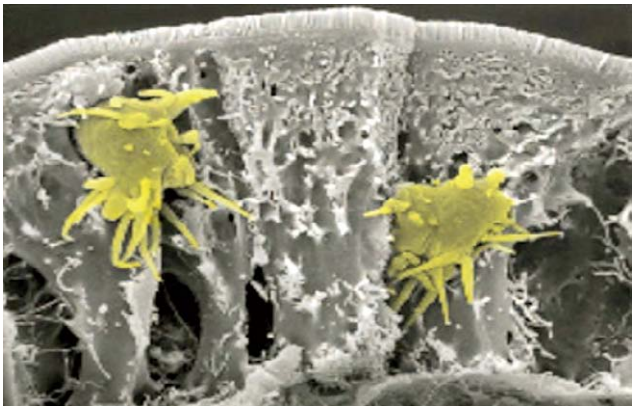


Figure: Electron microscope image of T lymphocytes from the intestinal epithelium. © 2013 I. Taniuchi, RIKEN Research Center for Allergy and Immunology

Just as a uniform helps distinguish a soldier from a police officer, scientists use proteins that immune cells wear on their surfaces to determine their job in the body. T cells, for example, that display the CD8 protein are classified as 'cytotoxic lymphocytes' (Fig.), which kill off cancerous or infected cells, whereas those displaying the CD4 protein are identified as 'helper' T cells that coordinate the immune response.

Immunologists have previously viewed these roles as fixed endpoints in development, but research by Ichiro Taniuchi (Laboratory for Transcriptional Regulation) in collaboration with Hilde Cheroutre (Research Unit for Immune Crosstalk, RCAI, and La Jolla Institute for Allergy and Immunology in California) and colleagues now reveals that helper T cells retain opportunities for a 'career change'.

Cheroutre had noticed that some helper T cells transplanted into immunodeficient recipients unexpectedly began expressing CD8. "This suggested reprogramming of cells from a helper fate into a cytotoxic lineage," says Taniuchi, whose research group studies ThPOK, a 'master' protein that coordinates helper T cell development and suppresses CD8 production.

Teaming up to examine these enigmatic $CD4^+$ cytotoxic lymphocytes (CTLs), the groups of Taniuchi and Cheroutre developed genetically modified mice having fluorescently labeled ThPOK-expressing cells, corresponding primarily to helper T cells. They also isolated unlabeled CD4-expressing cells from the intestine, and closer examination showed that these CD4-positive, ThPOK-negative cells now expressed CD8 and behaved like CTLs.

The researchers followed up with a strategy called 'fate mapping', using genetically modified mice whose cells become fluorescently labeled if they express ThPOK at any point—even transiently—in their lifetime. These experiments showed that intestinal $CD4^+$ CTLs begin as ThPOK-expressing helper cells, but switch to a CD8-expressing CTL fate when external factors cause these cells to inactivate ThPOK production via a genetic 'switch' called the 'ThPOK silencer'. "This demonstrates that $CD4^+$ T cells retain unappreciated plasticity for CTL development," says Taniuchi.

This flexibility appears to arise in response to infectious threats, as mice cultivated to be 'germ-free' do not develop $CD4^+$ CTLs. The researchers subsequently determined that helper T cells must be properly exposed both to foreign antigens as well as interleukin-15 to change roles. Although the intestinal wall is a particularly critical point of entry for pathogens, this mechanism may apply more generally as a means of rallying local defenses by retraining helper T cells in a crisis. "I believe other 'barrier sites' in the body may have these cells as well," says Taniuchi.

Sawako Muroi

Original paper

Mucida, D., Husain, M.M., Muroi, S., van Wijk, F., Shinnakasu, R., Naoe, Y., Reis, B.S., Huang, Y., Lambolez, F., Docherty, M. *et al.* Transcriptional reprogramming of mature $CD4^+$ helper T cells generates distinct MHC class II–restricted cytotoxic T lymphocytes. ***Nat Immunol.*** 14, 281-289 (2013)

A blueprint for the gut's antimicrobial defenses

Hiroshi Ohno

The identification of a developmental 'master switch' helps scientists explore the function of intestinal cells that help prevent infection

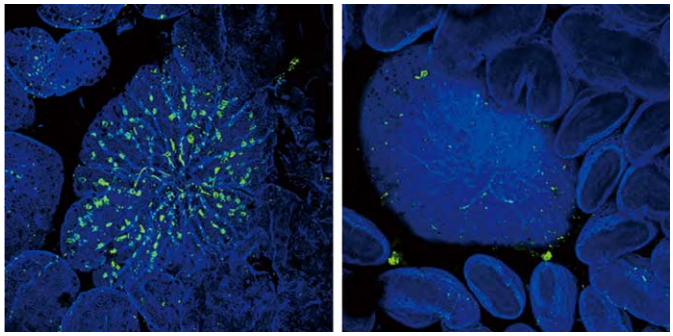


Figure: Fluorescent labeling of GP2, a protein expressed by M cells in wild-type mice *(left)* reveals that this cell population is virtually absent in mice lacking the gene encoding Spi-B *(right)*.

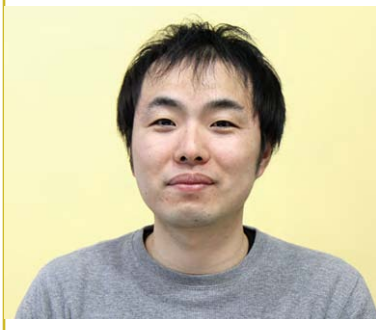
Every bite of food or drink of water is an invitation for potentially harmful bacteria and viruses to set up shop in the body. In order to protect against such invaders, the mucous membrane that lines the intestine contains clusters of specialized microfold cells (M cells), which can absorb foreign proteins and particles from the digestive tract and deliver them to the immune system.

New work from Hiroshi Ohno's group (Laboratory for Epithelial Immunobiology), in collaboration with Ifor Williams and colleagues at Emory University in Atlanta, Georgia, has revealed valuable insights into how these M cells develop. Previous research from Williams' group showed that a signaling protein called RANKL switches on M cell development but virtually nothing was known about the subsequent steps in this process. To find out, Ohno and Williams looked for genes that are switched on when intestinal cells undergo differentiation in response to RANKL exposure.

They discovered that treatment with RANKL causes immature intestinal epithelial cells to sharply increase the production of Spi-B, a protein that regulates the expression of other developmental genes. To test the specific contribution of this protein to M cell maturation, the researchers collaborated with Tsuneyasu Kaisho's group at Osaka University, which had engineered a genetically modified mouse strain lacking the gene encoding Spi-B. The resulting animals were devoid of mature M cells (Fig.). On the other hand, intestinal development as a whole was unaffected by the absence of Spi-B, demonstrating that this protein's impact is limited to this specific class of cells within the gut.

M cells normally localize to immune structures known as Peyer's patches (PPs). Bacteria such as *Salmonella enterica* Typhimurium (*S.* Typhimurium) normally accumulate within these PPs shortly after inoculation. This uptake was considerably reduced in Spi-B-deficient mice, indicating the absence of a functional M cell population. The mice showed a considerably weakened immune response following oral administration of *S.* Typhimurium bacteria relative to wild-type animals, demonstrating the importance of M cell-mediated microbial uptake.

The identification of this critical 'master switch' for M cell development opens exciting new avenues of research into these mysterious cells. Ohno is eager to investigate the details of how the cells perform their critical immunity-training function. "These questions could not be answered previously because of the lack of M cell-deficient mice," he says. "But now, 'knockout' mice that specifically lack Spi-B in their mucosal epithelium will provide the ideal tool for such studies."



Takashi Kanaya

Original paper

Kanaya, T., Hase, K., Takahashi, D., Fukuda, S., Hoshino, K., Sasaki, I., Hemmi, H., Knoop, K.A., Kumar, N., Sato M. *et al.* The Ets transcription factor Spi-B is essential for the differentiation of intestinal microfold cells. ***Nat Immunol.*** 13, 729-736 (2012)

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Knoop, K.A., Kumar, N., Butler, B.R., Sakthivel, S.K., Taylor, R.T., Nochi, T., Akiba, H., Yagita, H., Kiyono, H. & Williams, I.R. RANKL is necessary and sufficient to initiate development of antigen-sampling M cells in the intestinal epithelium. ***J Immunol.*** 183, 5738-5747 (2009)

Medical Innovation World Initiative (MIWI) Kick-off Meeting - Immune System and Primary Immunodeficiency (PID)




MIWI is an international humanized mouse users' consortium and, as well, an integrative network for human immunology research in the next generation. As a part of the MIWI initiative, RCAI launched a collaborative project with INSERM/Hospital Necker in France, Tokyo Medical and Dental University (TMDU) and the Center for Genomic Medicine (CGM) in RIKEN Yokohama Institute for the elucidation of the human immune system and diseases. To celebrate the opening of MIWI, a Mini-symposium was held on May 22nd, 2012 at RCAI.

Dr. Shigeo Koyasu opened the meeting. He described the recently identified innate lymphocytes, including the natural helper (NH) cell. These cells have various effector functions such as limiting the expansion of microorganisms including viruses, bacteria, and parasites. However, unlike T and B cells, innate lymphocytes act without antigen-specific receptors, but cooperation between innate lymphocytes and antigen-specific T and B cells is likely important in protective immunity against specific types of microbes.

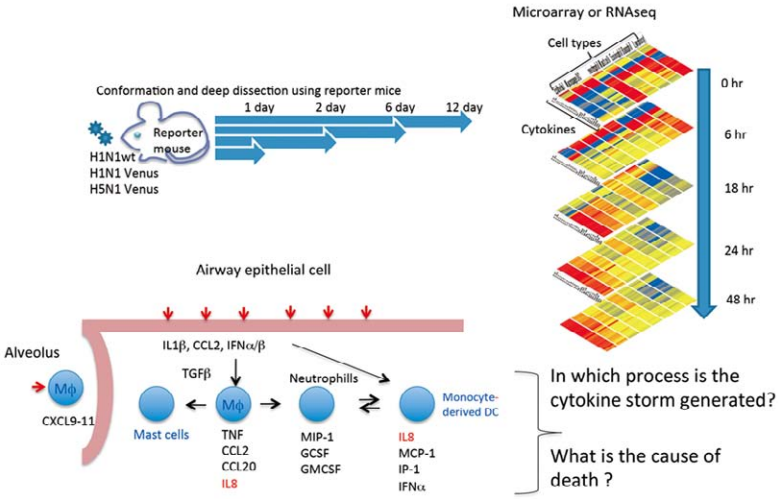
The next speaker, Dr. Tomohiro Morio (TMDU) talked about X-linked agammaglobulinemia (XLA), a human disease caused by Bruton's tyrosine kinase (Btk) deficiency. 11-30% of XLA patients have neutropenia, but it is distinct from that seen in common variable immunodeficiency (CVID) in that the neutropenia seen in XLA is induced by infection and is usually ameliorated after supplementation with immunoglobulin and is not mediated by an autoimmune response. Using neutrophils from patients with XLA, they found that the production of reactive oxygen species (ROS) was augmented. Excessive production of ROS was associated with neutrophil apoptosis, which was reversed by the transduction of wild-type Btk protein, suggesting that Btk is a negative regulator of signal transduction and prevents excessive neutrophil responses.

The third speaker, Dr. Yoshiyuki Minegishi (TMDU) discussed hyper IgE syndrome (HIES). HIES is classified as two diseases, type 1 and type 2. Type 1 HIES is the most common form, in which pneumonia is frequently followed by the formation of pneumatocele and is complicated by fungal infections. A dominant-negative mutation in the signal transducer and activator of transcription 3 (STAT3) has been identified as a major molecular cause of the Type 1 HIES. Type 2 HIES shows no skeletal abnormalities but the patients suffer from recurrent viral infections. A null mutation in tyrosine kinase 2 (TYK2) was identified as a molecular defect in type 2 HIES.

The last speaker, Dr. Anne Durandy (INSERM/Hospital Necker) described immunoglobulin class switch recombination (CSR) deficiencies in humans. CSR deficiencies are rare primary immunodeficiencies whose frequency is estimated as 1 in 100,000 births. They are characterized by normal or increased serum IgM levels and a contrasting, marked decrease or absence of IgG, IgA and IgE. Mutations in the gene encoding the CD40 ligand, a molecule highly expressed by activated follicular helper T cells, result in reduced CSR. Another CSR deficiency, caused by mutations in the *AICDA* gene encoding AID, is characterized by the impairment of both CSR and somatic hypermutation (SHM).

During the meeting, researchers rediscovered the important insights gained from the study of primary immunodeficiencies for the understanding of the normal human immune system. They agreed to collaborate for the analysis of primary immunodeficiencies, especially targeting common variable immunodeficiency (CVID) and CSR deficiency. Using samples collected from TMDU and Necker Hospital, RCAI, in collaboration with CGM, will conduct multiomics analyses, including whole exon sequencing, to identify the causative genes. In addition, once candidate genes are identified, RCAI will generate equivalent humanized mice and gene targeted mice to analyze the mechanisms of the disease onset *in vivo*. To achieve a seamless collaboration, there will be an exchange of scientists, on a short-term basis, between RCAI, TMDU and INSERM/Hospital Necker.

Tackling the enigmas of influenza infection



Influenza virus is one of the most hazardous human pathogens. Despite intensive study in this field, the viral infection pathways and mechanisms of viral escape from the immune system remain obscure. One of the major reasons for this knowledge deficit is the lack of a good animal model. Although some human influenza virus isolates can infect mice, and ferrets are sensitive to most of human viruses, we cannot examine the human immune response to influenza in these animals.

The 3rd generation (3G) humanized mouse is a newly established human hematopoietic cell transfer model based on improvements in the original NOD/SCID IL2Rγ KO. The 3G mice are also transgenic for a human MHCII, and this successful genetic engineering will now allow us to analyze human helper T cell-based immune responses. Taking advantage of this humanized mouse, RCAI launched a collaborative project with Dr. Yoshihiro Kawaoka (The Institute of Medical Science, The University of Tokyo). Under the project, the collaborative team will carry out the following experiments to tackle current enigmas of influenza virus infection.

1) Analysis of influenza virus HA antigenic drift in the 3G humanized mice

Influenza virus pandemics are a major hazard to humankind. On a population scale at present, global surveillance and anti-influenza virus drugs are effective defenses. On the individual scale, innate and acquired immune systems are both important. The viral protein Hemagglutinin (HA) is a dominant antigen in influenza virus infection, and anti-HA neutralizing antibodies play a role in protection against the virus. However, the high frequency of viral genome mutations generates mutated HA, so-called antigenic drift, which allows the virus to escape from the humoral immune response. Therefore, influenza virus expressing a newly mutated HA could be a great public safety risk, since it would not be covered by existing vaccines.

Global surveillance is useful to follow antigenic drift during an infection cycle, but the experimental study of influenza virus antigenic drift is limited, since it is carried out using *in vitro* systems, which do not recapitulate the complexity of virus-antibody competition *in vivo*.

The team will take advantage of the 3G humanized mice that can reconstitute the human acquired immune system to determine which HA mutations result in escape form antibody protection *in vivo*. Specifically, humanized mice will be infected with influenza A virus to induce neutralizing antibodies, then the mice will be infected with viruses that carry random mutations in the HA gene. Only viruses that can escape from the existing neutralizing antibodies will be able to replicate, so we can identify the escape HA mutants by simple sequencing of isolated viruses.

These experiments will provide us information about HA mutations in influenza virus that potentially may cause pandemics.

2) Analysis of pathological mechanisms of highly pathogenic Influenza A (HPIA) infection using the 3G humanized mice

Seasonal influenza virus infection and pathological changes are usually limited to the respiratory system. However, in HPIA H5N1 virus infection, the virus can enter into various types of cells, not just respiratory epithelial cells, where it causes intense and destructive immune responses. These aberrant immune reactions (e.g. a cytokine storm) can cause acute respiratory distress syndrome (ARDS) and also damage non-respiratory organs. However, the mechanism by which HPIA infection causes such severe pathology is largely unknown.

The team will dissect HPIA infection in the 3G humanized mice to elucidate which human cells are infected, and what immune factors determine the damage in each organ, ultimately forcing the system to collapse. Specifically, the team will utilize an infected cell visualization system, which consists of HPIA H5N1 encoding a fluorescent protein in the viral genome, which will allow us to monitor the systemic spread of HPIA all throughout the humanized mice. The team will then isolate mRNA from infected cells sorted based on HPIA fluorescence at various time points after infection. The mRNA will be analyzed by RNA-seq and the team will establish a map of the cytokine and chemokine network during HPIA spreading, including chronological information.

These experiments will provide new insights into HPIA infection and will provide a framework for further studies to establish new biomarkers for more effective therapeutic strategies and more efficient vaccine development.

From Genetic Analysis to Mechanistic Interpretation of Immune Diseases

Important features of the immune system have been elucidated during the long history of immunology. However, there still remains a large black-box in understanding of immune homeostasis and its dysregulation. This lack of knowledge, as a matter of course, is a large hurdle in the development of better therapies for many immune diseases. To address this, we must bridge numerous gaps between 1) the information about how the immune system is regulated in mouse and human, 2) human genetic information obtained by genome-wide association studies (GWAS) and mechanistic interpretation of disease etiology, and 3) global cell populations and particular subsets of immune cells.

Because human genetic data based on advanced genome technologies have already proven to be very powerful in detecting the association between genotype and phenotype, close collaborations with human genetics groups are very likely to bear fruit in tackling unsolved mechanisms of immune diseases. Our efforts to understand how the immune system is regulated have been confined largely to studies in mice, but, after serious deliberation, we think that it is now time to boldly take a step towards conquering human immune diseases. In this regard, it is fortunate that RIKEN has a strong and prominent research institute in this field, the Center for Genomic Medicine (CGM), in the same Yokohama campus. Because the mission of CGM is to understand individual genetic variations and to apply this knowledge to healthcare, we certainly have several overlaps in our research interests.

Besides intramural collaboration with CGM in RIKEN, we have begun to discuss the possibility of formal collaboration with extramural genetics research groups carrying out the SardiNIA project (http://sardinia.nia.nih.gov/Project_Team/project_team.html) because they are particularly interested in the relationship between genotype and immune-phenotype. The SardiNIA project is a joint effort of investigators at the National Institute of Aging in the National Institutes of Health (USA), at the Italian National Research Council Institute of Neurogenetics and Neuropharmacology in Cagliari, and statistical geneticists at the University of Michigan. Most countries have a heterogeneous population, and the unique advantage of the SardiNIA project is that the lineage of most Sardinians can be traced directly back approximately 8,000 years to the island's original settlers. Their relative genetic homogeneity greatly simplifies population genetic analyses, so that study of this highly interrelated population cohort is expected to have a high probability of identifying genetic factors affecting specified phenotypic traits.

It is quite likely that collaboration with human genetics groups will give us many insights into the etiology of immune diseases. However, there still remains the issue of how to approach mechanistic interpretations. In particular, because the immune system is a highly sophisticated and complex biological system, approaches using human samples have an obvious limitation since only non-invasive analyses are possible in most cases due to ethical reasons. The use of model animals is thus an indispensable approach towards this end, and the question becomes, how to fill the gap between knowledge obtained from human and model animals. One possible approach is to use and analyze knock-in animal models with a disease-related genetic variation identified in human genetic analysis by integrative genomic approaches from molecular to whole body levels. This course of endeavor has just been launched in a systematic manner at the center.

RCAI Advisory Council 2012

The RCAI Advisory Council met on October 1-2, 2012 and what follows is a summary of comments and recommendations from that meeting. This was a particularly important meeting since RCAI will undergo significant changes in the next fiscal year beginning April 2013: Dr. Taniguchi will step down as director after a remarkably successful ten year tenure. There is a significant new push at RIKEN to prioritize translational medicine coupled with systems biology. RCAI will merge with the RIKEN Center for Genomic Medicine (CGM) to form a new institute called "Center for Integrative Medical Sciences (IMS)". Taken together, all of these factors pose an exciting but potentially hazardous challenge to the hard won successes of RCAI.

Within less than a decade of its inception, RCAI has become a leading world center in immunology research. Its success is indicated by the high quality, quantity and impact of (1) publications in the best scientific journals, (2) development of a steady stream of successful independent investigators and (3) regional and international educational contributions. RCAI scientists have achieved leading positions in understanding the mucosal immune system and immune cell signaling mechanisms through innovative biochemical, imaging and gene targeting studies in mouse models. Although successful translation of research findings into health benefits typically requires 12-15 years, RCAI investigators have already developed promising leads for a cedar pollen allergy vaccine and NKT cell-based therapy for refractory lung cancer. RCAI has truly become a crown jewel of RIKEN, for which RCAI, Director Taniguchi and all of the RCAI researchers and staff are to be heartily congratulated. Future plans for changes in research direction or administrative organization should have the preservation of this excellence as a major priority.

Merger of RCAI and CGM

The merger of RCAI and CGM will have major, unpredictable and likely unforeseen consequences on the productivity, direction and future of both institutes as they combine into the new hybrid institute. It was unclear to the AC whether or not this fusion is a *fait accompli*, and thus whether any of our comments and recommendations will have any impact. Despite some general descriptions about the new institute, it remains unclear at this point how the new institute will be structured, precisely what each side will bring to the merger, and how the two will interact in a synergistic way. These topics are apparently under current intensive discussion; however, since the merger is scheduled to take place by April 1, 2013, there is concern that there is not yet a clearer vision as of October 1, 2012.

The proposed merger seems only weakly justified from a scientific perspective. RCAI is a world-class immunology pow-

erhouse that could likely draw from the scientific expertise of CGM as needed on an *ad hoc* basis by natural collaborations arising between RCAI and CGM investigators. The merger seems to be based more on administrative considerations than on a firm scientific rationale.

As a leading immunology institute, RCAI has brought RIKEN into the international limelight of this important discipline. The RCAI "brand" has become very well known in a remarkable eight short years. To have it disappear and be subsumed into an institute titled "Center for Integrative Medical Sciences (IMS)" without the word "Immunology" in the name is, in the opinion of the AC, an incomprehensible move. In the absence of this label, the new institute will, at least initially, no longer be so attractive and visible to outside scientists and potential recruits.

The administrative and budgetary framework of the new institute has not been worked out; in particular the incipient administrative leadership vacuum in the new hybrid at this late date is a concern. Dr. Taniguchi is stepping down as RCAI director and, based on their English website, Dr. Kubo is presently acting and not permanent director of CGM. During the AC meeting it was revealed that Dr. Koyasu has recently been appointed to a position where he will oversee the transition and establishment of the new institute and help identify potential director candidates. Such essential movement should proceed as quickly as is judiciously possible.

RCAI Outstanding Contribution of the Year 2012

RIKEN RCAI Outstanding Contribution of the Year 2012 was awarded to Dr. Shin-ichiro Fujii, and Ms. Hiroko Tanabe.

Dr. Shin-ichiro Fujii (Photo 1), Leader of Research Unit for Cellular Immunotherapy, received the award for the establishment of human artificial adjuvant vector cells, a new cell-based therapy to coordinate the activity of innate and adaptive immunity against tumors.

Photo 1 : Dr. Shin-ichiro Fujii

Dendritic cells (DC) are extremely efficient at activating T cells, and the use of DCs in anti-tumor therapy has been a goal of many investigators since the 1990's, when it was found that DCs could be generated *ex vivo* by culture with GM-CSF and that inclusion of tumor-derived peptides or irradiated tumor cells could help eradiated tumor cells. Development of effective DC anti-tumor therapy has been a lifelong dream of Dr. Fujii, who spent several years in the laboratory of Professor Ralph Steinman, the Novel Laureate who discovered these cells. Dr. Fujii published several important studies on the roles of DCs against cancer, but was looking for a new breakthrough to make their application more efficient and powerful.

Around that time, Dr. Masaru Taniguchi discovered a unique anti-cancer immune system. He showed that α-GalCer activates NKT cells and induces very strong direct anticancer effects. Dr. Fujii was very surprised to hear the story, and wanted to know how NKT cells work on DCs. He was able to show that activated NKT cells induce DC maturation *in vivo* (Fujii et al., *J Exp Med.*, 2003), but this result contradicted those from *in vitro* experiments, where α-GalCer had no such effect on DC maturation.

Dr. Fujii devoted his efforts to understand the difference between *in vivo* and *in vitro*, and found a specific adjuvant for DC, CD40 ligand, that could link innate and adaptive immunity (Fig. 1).

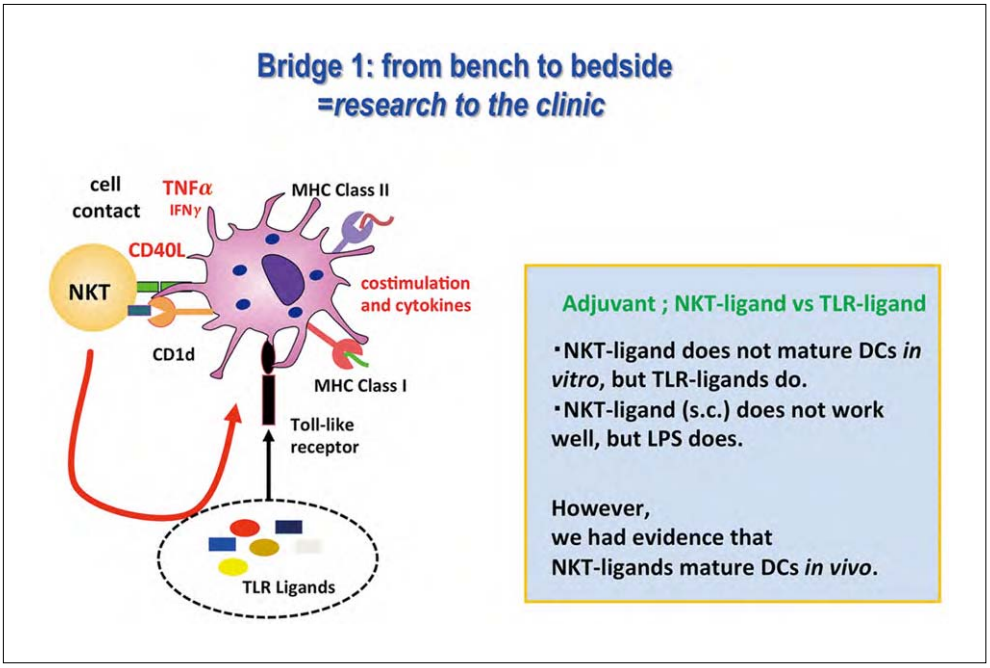


Figure 1: Bridge 1. From bench to bedside=research to the clinic

This result gave Dr. Fujii a new idea; co-administration of NKT ligand (α-GalCer) and irradiated dead tumor cells might trigger both adaptive and innate immunity. However, in testing this idea in mice he discovered a serious problem; very large numbers of tumor cells were required for an effective treatment, and many animals died because of pulmonary embolisms due to entrapment of the cells in the lung. Pressing forward, they were able to overcome this problem by injecting $CD1d^+$ cells expressing tumor antigen and α-GalCer, which required many fewer cells (Shimizu *et al.*, *J Immunol*, 2007, Shimizu *et al.*, *J Exp Med*, 2007).

This year, Dr. Fujii published another important breakthrough in the application of this anti-tumor therapy. They can now use allogenic $CD1d^+$ cells carrying α-GalCer and mRNA encoding tumor antigen, artificial adjuvant vector cell, to efficiently generate both innate and adaptive anti-tumor immunity (Fig. 2) (Shimizu *et al.*, *Cancer Res.* 2013). Because artificial adjuvant vector cell utilize RNA to generate protein, this therapy is different from gene therapy. Dr. Fujii rather thinks that this therapy should be used as a prodrug. Prodrugs undergo chemical conversion by metabolic processes before becoming an active pharmacological agent. Indeed, artificial adjuvant vector cells activate NKT cells and release IFN-γ which activates NK cells and innate immunity. Also, neighboring DCs capture the artificial adjuvant vector cell, undergo maturation, and induce adaptive immunity, along with immunological memo-

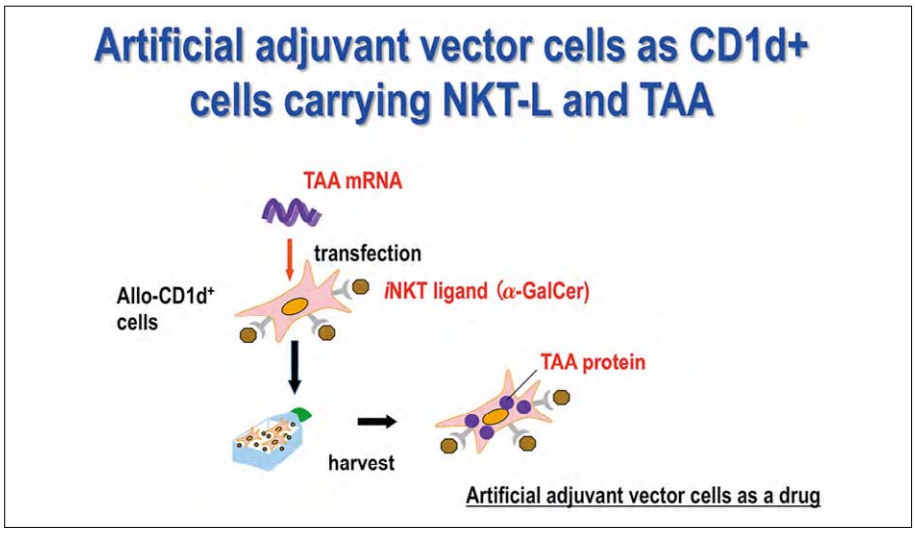


Figure 2: Artificial adjuvant vector cells (aAVC) as $CD1d^+$ cells carrying NKT-L and TAA

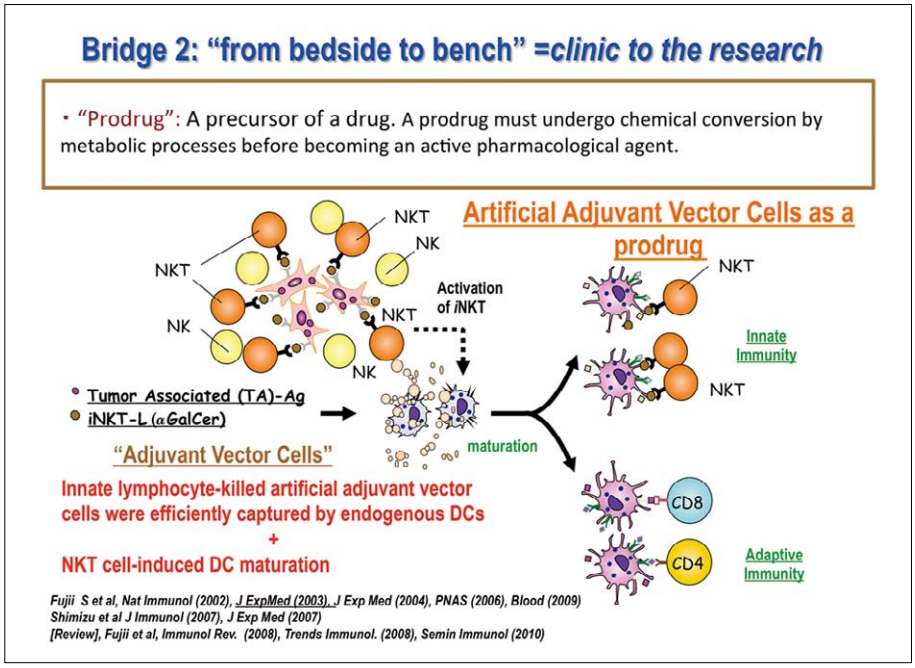


Figure 3: Bridge 2: From bedside to bench=clinic to the research

ry that persists for longer than a year (Fig. 3).

When artificial adjuvant vector cells were administered to dogs, there were no adverse events. NKT cells initially increased but one month later had almost disappeared; however $CD8^{+}$T cell responses were still present (Fig. 4). Currently, artificial adjuvant vector cells therapy is being tested using human samples.

Dr. Fujii thinks that the artificial adjuvant vector cell makes three bridges. First, it bridges innate and adaptive immunity. The American Cancer Research Association gave this comment to Dr. Fujii, "preclinical proof-of-principle studies establish a new cell-based therapy to coordinate the activation of innate and adaptive immunity against tumors, as an effective strategy for cancer immunotherapy." The second bridge is between the clinical and basic sciences. Artificial adjuvant vector cells are expected to be a new type of cell therapy that can be used as a prodrug. Thirdly, artificial adjuvant vector cell has come out of two immunology fields; the NKT field, pioneered by Masaru Taniguchi, and dendritic cells, pioneered by Ralph Steinman. Dr. Fujii thinks of artificial adjuvant vector cell as an artificial cell that bridges NKT and DC (Fig. 5). "We are honored to concentrate on using these systems in humans. I would like to thank all the laboratory members, Drs.

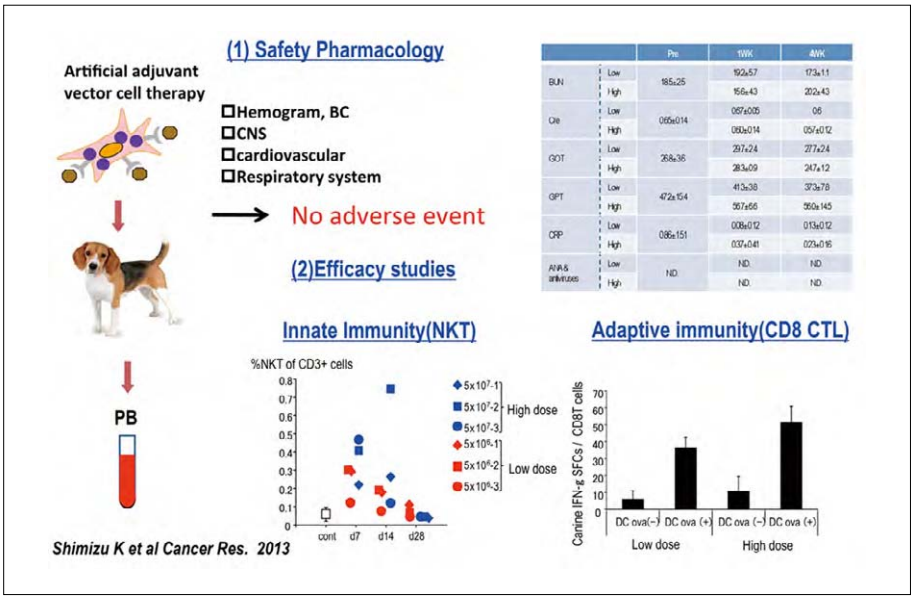


Figure 4: Preclinical study of artificial adjuvant vector cell using large animals

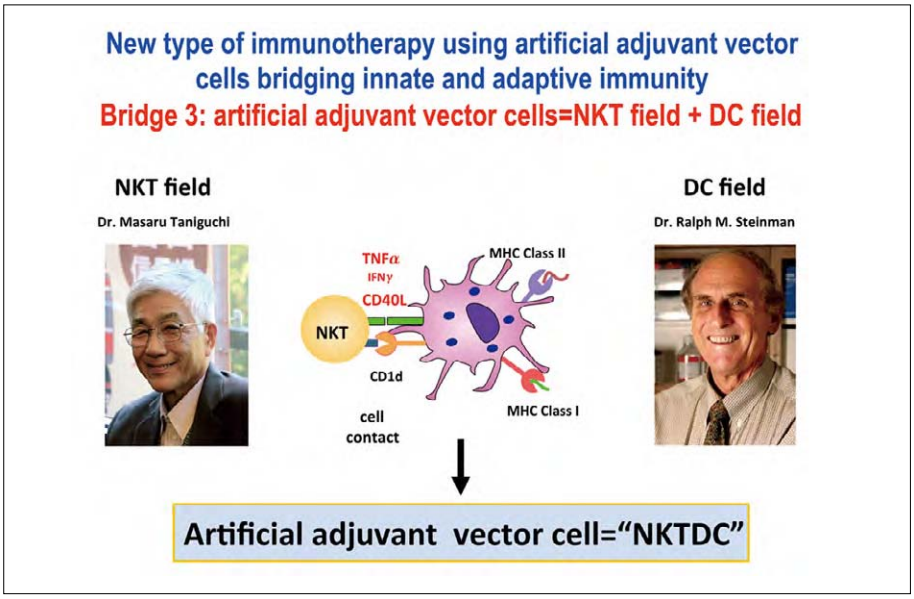


Figure 5: Bridge 3: Artificial adjuvant vector cells=NKT field + DC field

Shimizu, Shinga, Sato, Yamasaki, and Ms. Asakura. We'd like to thank Dr. Ishii, Dr. Taniguchi, and external collaborators, especially Dr. Schuler for generation of GMP grade mRNA," Dr. Fujii said in his speech at the award ceremony.

Photo 2 : Ms. Hiroko Tanabe

Ms. Hiroko Tanabe (Photo 2), Chief Assistant of the Administrative Coordination Office, received the award for the establishment and management of the Administrative Coordination Office (AdmCO). The AdmCO was established within the RCAI in April, 2010 to manage a wide range of administrative tasks related to RCAI laboratories and the Center.

Until then, each leader had an assistant and the assistant's tasks differed greatly depending on the laboratory. In addition, tasks related to the Center were managed by the Director's assistant or by the organizers' assistants, and they had to ask for volunteers to support the Center's events.

To make these dispersed administrative duties more efficient, Ms. Tanabe centralized all of their work, as well as IT related work and facility support. Thanks to the tremendous efforts by Ms. Tanabe and other chief assistants, Mses Hiroko Yamaguchi and Mari Kurosaki, the reorganization made great progress in a short time and enhanced the Center's activities. A new data sharing system has been introduced, which enables assistants to share information, creating an environment where they easily interact and teach each other. Ms. Tanabe has also launched an RCAI ordering system, which completely separates three parties' activities; order placement by laboratories, delivery verification by the Yokohama Research Promotion Division (YRPD), and payment processing by the AdmCO. This has proven a useful system to manage various budgets simultaneously and to prevent any potential fraudulent activity. Ms. Tanabe centralized the MTA related tasks, and AdmCO currently processes 200 MTAs per year. Using this centralized approach, the AdmCO is now able to provide the same high standards to all RCAI laboratories.

It is not an exaggeration to say that the RCAI could not have been reorganized smoothly to become the IMS without the support of the AdmCO and Ms. Tanabe. The AdmCO arranged construction of new laboratories and provided people arriving and leaving with constant administrative support. Although many people longed for her continued efforts in the new Center, she decided to leave, handing over her work to Ms. Yamaguchi. "I have no words to express our appreciation. Tanabe-san always showed us how to work with pride, and she always stood by us when we felt so small," said Ms. Yamaguchi. Ms. Tanabe in her speech thanked all the members of AdmCO; all assistants, IT members and Mr. Ogata. "Without them, I could not have done anything," she said.

Dr. Taniguchi retires from the Director.

The ceremony was the last chance for all RCAI members to gather together. In the end of the ceremony, Dr. Cooper (Photo 3), the chairman of the RCAI Advisory Council, sent a video message to Dr. Taniguchi (Photo 4).

Photo 3 : Dr. Max Cooper

In the message, he said, "I want to tell you that anyone who was ever associated with you was very impressed with what you and your colleagues accomplished with RIKEN. In a decade, you've become an international leader in the field of immunology. I realize you owe a lot to your colleagues and to RIKEN as well, but I would like to pay a special tribute to your leadership. You've done the job in exceptional and incredible ways to draw cooperation from everyone around you. You've had a lot of difficult situations, both personal and professional and through all of that, you maintained a calm leadership, listened to people around you, and responded to advice from Scientific

Advisory Board members. You've treated all this with an even-handedness that was absolutely remarkable and admirable. I think you had learned a lot from being a dean of a medical school, and you brought that experience and knowledge with you when you came to RCAI. I think though, that probably you've been able to maintain your balance and even serenity by the fact that you continued to run your own laboratory and do research, and thus had a way to test whether your opinions or ideas were right or wrong. It is easy to get off track, but having laboratory-based research activity can keep you on track to a much better extent. I don't know what you will be doing next, but I hope that you maintain the research activities. That will continue to give you pleasure. You will have many good memories of your times as the Director of RCAI, and all of us who had any chance to experience your leadership or to be with you will treasure those memories, and we hope you will, too."

In the end of the message, he performed a song by Louis Armstrong, "Summer Day."

♪
I hear laughter, from the swimming hole
Kids out fishin', with the willow pole
Boats come float, round the bend
Why must summer, ever end...

Love, to me, is like a summer day
If it ends, the memories will stay
Warm and sweet, and tender
I can hear my summer song
♪

Dr. Taniguchi closed the ceremony by citing a phrase from Samuel Ullman's poem *Youth* and one of Gen. Douglas MacArthur's speeches.

Nobody grows only by merely living a number of years;
people grow old only by deserting their ideals.
You are young as your faith, as old as doubt;
as young as your self-confidence, as old as your fear;
as young as your hope, as old as your despair.

Old soldiers never die, they just fade away.

Photo 4 : Dr. Masaru Taniguchi

References:

Fujii, S., Shimizu, K., Smith, C., Bonifaz, L., and Steinman, R.M. Activation of natural killer T cells by alpha-galactosylceramide rapidly induces the full maturation of dendritic cells in vivo and thereby acts as an adjuvant for combined CD4 and CD8 T cell immunity to a coadministered protein. ***J Exp Med.*** 198, 267-279 (2003)

Shimizu, K., Goto, A., Fukui, M., Taniguchi, M., and Fujii, S. Tumor cells loaded with alpha-galactosylceramide induce innate NKT and NK cell-dependent resistance to tumor implantation in mice. ***J Immunol.*** 178, 2853-2861 (2007)

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

Shimizu, K., Mizuno, T., Shinga, J., Asakura, M., Kakimi, K., Ishii, Y., Masuda, K., Maeda, T., Sugahara, H., Sato, Y., et al. Vaccination with antigen-transfected, NKT cell ligand-loaded, human cells elicits robust in situ immune responses by dendritic cells. ***Cancer Res.*** 73, 62-73 (2013)

Prize Winners 2012

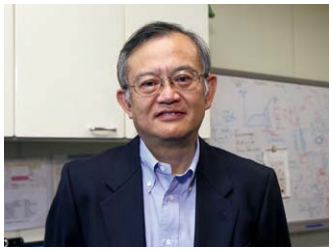


Photo 1 : Tomohiro Kurosaki

Tomohiro Kurosaki (Photo 1), Group Director of the Laboratory for Lymphocyte Differentiation, received The Commendation for Science and Technology from the Minister of Education, Culture, Sports, Science and Technology (MEXT), 2012. He won the Research Category Prize for his studies on identification of regulatory factors in allergic reactions. The prize is awarded to investigators whose research is highly original and contributes to the science and technology of Japan.

Photo 2 : Sidonia Fagarasana

Sidonia Fagarasan (Photo 2), Team Leader of the Laboratory for Mucosal Immunity, received the 15th Japanese Society for Immunology Award for her research on IgA synthesis: a form of functional immune adaptation extending beyond gut. Through this research, she discovered that IgA production is essential for the symbiosis of microorganisms and immunity in the gut, and it even has strong effects on systemic immunity. She is currently expanding her studies on the relationship between gut bacteria and autoimmune diseases, and further important research achievements in this new area are very likely.

Photo 3 : Tomokatsu Ikawa

Tomokatsu Ikawa (Photo 3), Young Chief Investigator of the Laboratory for Immune Regeneration, received the MEXT Prize for Young Investigators. This prize is awarded to young scientists (under 40 years of age) in recognition of creative and original research and an outstanding ability to develop scientific research projects. He received the award for his research on molecular mechanisms of T cell lineage developmental decisions.

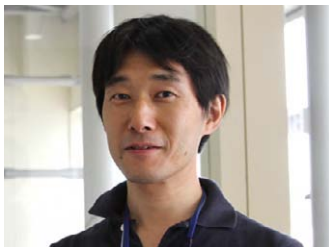


Photo 4 : Tadashi Yokosuka

Tadashi Yokosuka (Photo 4), Senior Researcher of the Laboratory for Cell Signaling, received The Chiba Medical Society Award 2012. He won the basic medical science category prize for his research on immune signal units that achieve T cell antigen recognition and immune responses. This award is given to scientists whose research is greatly expected to contribute to advanced medical science, and the winners receive ¥500,000.

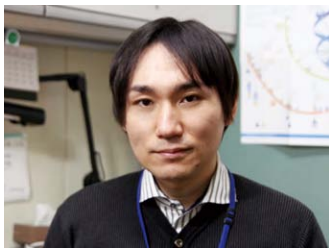


Photo 5 : Hayato Kaneda

Hayato Kaneda (Photo 5), Young Chief Investigator of the Laboratory for Stem Cell Competency, received The Keio University School of Medicine Sanshikai Encouragement Award 2012. He won the basic medical science category prize for his research on requirement for COUP-TFI and II in the temporal specification of neural stem cells in centeral nervous system development. This award is given to young scientists under 40 years of age who has greatly contributed to the fields of basic or clinical medical sciences.

Masanaka Sugiyama (Photo 6), Junior Research Associate of the Research Unit for Inflammatory Regulation, received the Young Investigator Award at the 20th International Symposium on Molecular Cell Biology of Macrophages (MMCB) 2012. He received the award for his poster presentation: "*In vivo* ablation of XC chemokine receptor 1-expressing dendritic subset."

Photo 6 : Masanaka Sugiyama

Osamu Masui (Photo 7), Researcher of the Laboratory for Developmental Genetics, received a Genetic Society of America Poster Award at the 2012 Mouse Molecular Genetics Conference held at Pacific Grove, CA, USA. He received the award for his presentation: "Live-cell imaging of X-chromosome inactivation in differentiating ES cells: visualization of X-inactivation centre locus and Xist RNA".

Photo 7 : Osamu Masui

Shimpei Kawamoto (Photo 8), Special Postdoctoral Researcher of the Laboratory for Mucosal Immunity, and **Takashi Kanaya** (Photo 9), Researcher of the Laboratory for Epithelial Immunobiology, received Poster Awards at The 34th Naito Conference "Infection, Immunity and their Control for Health: Mucosal Barrier" held on October 16-19, 2012, in Sapporo. Kanaya's presentation "Ets transcription factor Spi-B is essential for the differentiation of intestinal microfold cells" and Kawamoto's presentation "The inhibitory receptor PD-1 regulates IgA selection and bacterial composition in the gut" were selected as excellent poster presentations, and The Naito Foundation Subsidiy for Promotion of Specific Research Projects (¥500,000 each) were granted to them.

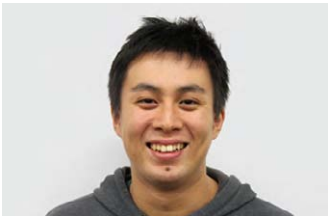


Photo 8 : Shimpei Kawamoto

Photo 9 : Takashi kanaya

The RIKEN Research and Technology Incentive Awards 2012 were presented to **Raul Eduardo Vizcardo Sakoda** (Photo 10) and **Shimpei Kawamoto** (Photo 8). Sakoda, Researcher of the Laboratory for Developmental Genetics, received the award for his study on regeneration of human tumor antigen-specific T cells from iPSCs derived from mature $CD8^+$ T cells. Kawamoto, Special Postdoctoral Researcher of the Laboratory for Mucosal Immunity, received the award for his study of how the inhibitory receptor PD-1 regulates IgA selection and bacterial composition in the gut. The RIKEN Research Incentive Award is given to researchers under age 40 who have contributed to furthering RIKEN's ideals by achieving exemplary results in their research.

Photo 10: President Ryoji Noyori (*left*) and Raul Eduardo Vizcardo Sakoda (*right*)

Excellent Paper of the Year 2012

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

In 2012, 6 excellent papers were selected from 11 candidates for this award.

Takashi Kanaya, Tsuneyasu Kaisho and Hiroshi Ohno

Laboratory for Epithelial Immunobiology
"The Ets transcription factor Spi-B is essential for the differentiation of intestinal M cells"
Nature Immunology, Vol. 13, pp. 729-736, 2012

Tomohiro Kaji and Toshitada Takemori

Laboratory for Immunological Memory
"Distinct cellular pathways select germline-encoded and somatically mutated antibodies into immunological memory"
The Journal of Experimental Medicine, Vol. 209, pp. 2079-2097, 2012

Sawako Muroi, Chizuko Miyamoto, Ichiro Taniuchi and Hilde Cheroutre

Laboratory for Transcriptional Regulation and Research Unit for Immune Crosstalk
"Transcriptional reprogramming of mature CD4 T helper cells generates distinct MHC class II restricted cytotoxic T lymphocytes"
Nature Immunology, Vol. 14, pp. 281-289, 2013

Kanako Shimizu and Shin-ichiro Fujii

Research Unit for Therapeutic Model and Research Unit for Cellular Immunotherapy
"Vaccination with antigen-transfected, NKT cell ligand–loaded, human cells elicits robust *in situ* immune responses by dendritic cells"
Cancer Research, Vol. 73, pp. 62-73, 2013

Shimpei Kawamoto, Mikako Maruya and Sidonia Fagarasan

Laboratory for Mucosal Immunity
"The inhibitory receptor PD-1 regulates IgA selection and bacterial composition in the gut"
Science, Vol. 336, pp. 485-489, 2012

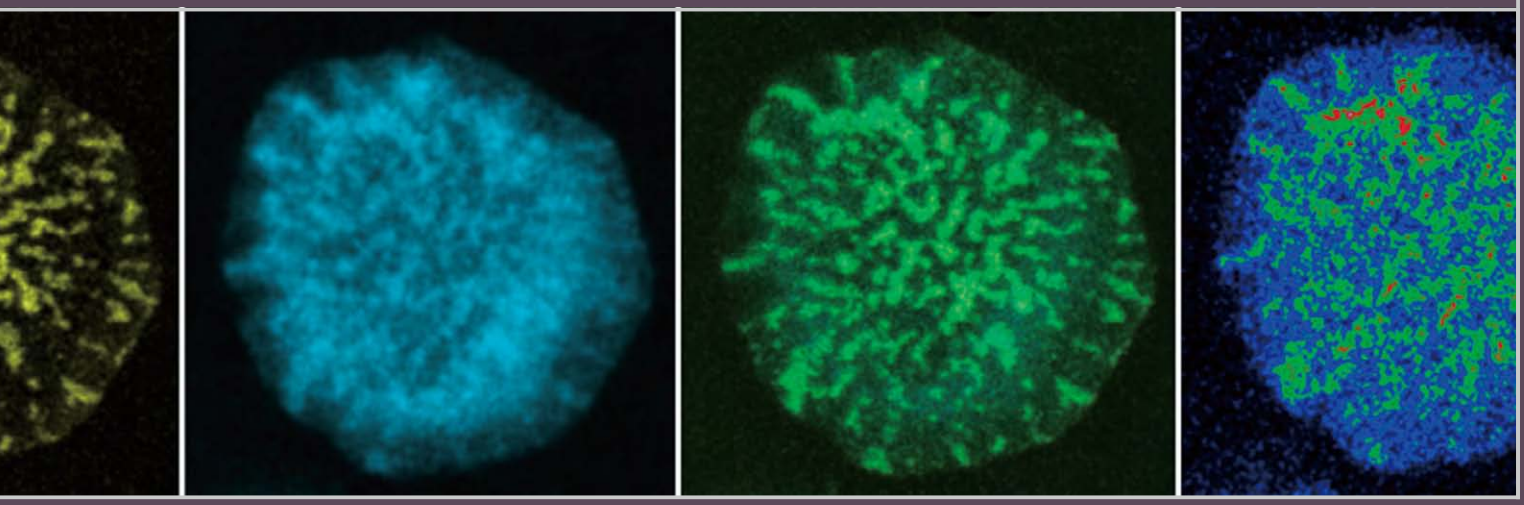
Raul Vizcardo, Kyoko Masuda, Daisuke Yamada, Haruhiko Koseki and Hiroshi Kawamoto

Laboratory for Developmental Genetics and Laboratory for Lymphocyte Development
"Regeneration of human tumor antigen-specific T cells from iPS cells derived from mature $CD8^+$ T cells"
Cell Stem Cell, Vol. 12, pp. 31-36, 2013

2012

Part 2

Technology and Innovation



RCAI and Torii collaborate for development of an allergy vaccine

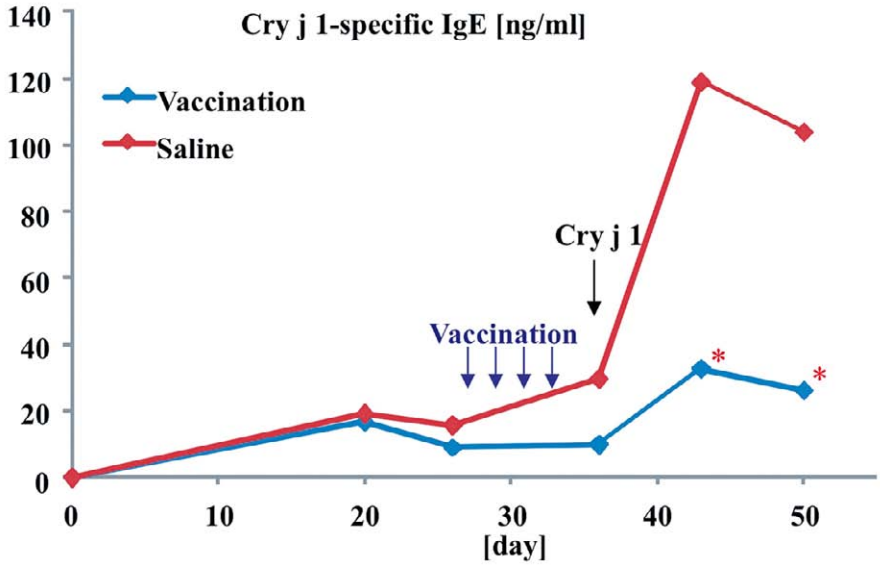


Figure: **Subcutaneous vaccination significantly prevents the increase of specific IgE after Cry j 1 sensitization.** The recombinant vaccine was administered subcutaneously into Cry j 1-sensitized mice four times; the mice were sensitized again with Cry j 1 again after the vaccination. The increase of Cry j 1-specific IgE after the last sensitization with Cry j 1 was significantly attenuated in the vaccinated mice compared the control saline injected mice. The figure shows the average Cry j 1-specific IgE titer in each group. *p<0.01 by Mann-Whitney U-test.

Japanese cedar (*Cryptomeria japonica*) pollinosis is a common allergy in Japan, with a prevalence estimated to be 26.5% in a nationwide survey conducted in 2008. Antigen-specific immunotherapy (SIT) is considered to be the only curative treatment for allergy. Only a crude extract from Japanese cedar pollen has been approved for clinical use by the Ministry of Health, Labor and Welfare in Japan. However, it will be very difficult to gather enough cedar pollen from the male flower of Japanese cedar to prepare enough vaccine for pollinosis patients, because of the limited source of the pollen and the limited pollen scattering season, usually early February to middle March. Therefore, development of a recombinant vaccine independent of the natural source is expected to be very valuable for clinical use as a SIT vaccine. Vaccines using allergoids and modified Cry j 1, a major allergen of Japanese cedar pollen, have been developed and used in pre-clinical trials; however, none of them has become commercially available for medical use due to poor clinical outcomes at later stage clinical trials or to the failure to find a cooperative pharmaceutical company to introduce them into market. To fill the critical gap between basic research and later stage of drug development, RCAI offered a program named the 'exchange zone', where RCAI, universities, hospitals, and pharmaceutical companies work together for drug development, including allergy vaccine development, especially for Japanese cedar pollinosis. Based on this program, RIKEN and TORII pharmaceutical Co., Ltd. set up joint research laboratory in RCAI in May 2010 and started research and development of the recombinant SIT vaccine. The aims of the joint research laboratory are to develop a recombinant SIT vaccine with high safety and tolerability, and to introduce the vaccine into the Japanese market.

Development of recombinant SIT vaccine

For preparation of the vaccine, recombinant technology is used to conjugate two major allergens from Japanese cedar pollen, namely Cry j 1 and Cry j 2, and then it is further modified with polyethylene glycol (PEG) to prevent binding with immunoglobulin E (IgE) and for improvement of its solubility. The research

team conjugates PEG with recombinant Cry j 1 and Cry j 2 fusion protein via a cysteine residue on the fusion protein. To accomplish this, all cysteine residues except one in the cDNA encoding the fusion protein had to be substituted with serine residues to control the number and location for conjugation with PEG. The vaccine is expected to be unable to bind human IgE, which could induce adverse events after vaccine administration *in vivo*.

The research team compared the yield of the recombinant fusion protein using several host/vector combinations in eukaryotic cells. Based on this analysis, they selected the best combination and, using a jar fermenter and, based on knowledge of safety and usage to produce vaccine or medicine already approved by the Ministry of Health, Labor and Welfare in Japan, are optimizing the culture conditions to produce the maximum amount of fusion protein. They are also developing simple methods for purification of the recombinant SIT vaccine from the host cells, taking into consideration cost-performance and purity. They isolated hybridomas that produce vaccine-specific IgG and set up a procedure to specifically determine the quantity of the vaccine by sandwich ELISA. They also set up a system to evaluate uniformity and purity of the vaccine using a high performance liquid chromatography system.

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

Systemic injections of Cry j 1-sensitized mice with the vaccine prevented the increase of serum Cry j 1-specific IgE following sensitization with native Cry j 1. (see Fig.) The research team evaluated therapeutic potential of the vaccine by comparing the reduction of local inflammation or IgE production among various doses and frequencies of vaccination using sensitized mice in the mouse model of pollinosis. They confirmed the ability of the vaccine to prevent the increase of specific IgE following systematic or local sensitization in a dose-dependent manner. To improve the therapeutic efficacy of the vaccine, elucidation of the therapeutic mechanisms and identification of biomarkers are important issues. It is also important to find biomarkers to distinguish responders and non-responders to the vaccine. Therefore, they are looking for biomarkers to monitor therapeutic responses during and after vaccination. The research team plans to evaluate therapeutic effects of the vaccine in humans and the availability of biomarkers in translational research, after which they will produce the vaccine at GMP grade.

Members of RIKEN-TORII Joint Research Team

Team Leader :	**Masaru Taniguchi**
Senior Research Scientist :	**Takashi Fujimura**
Visiting Scientist :	**Hiroyuki Miyazaki**, **Yasushi Okumura**, **Koji Fujinami**, **Ryosuke Ishikawa**
Technical Staff :	**Hisako Motokawa**
Administrative Staff :	**Akiko Imai**

Recent Publications:

1. Fujimura T, Okamoto Y, Taniguchi M. Therapeutic effects and biomarkers in sublingual immunotherapy: a review. ***J Allergy (cairo).*** 2012, 381737 (2012)

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

3. Fujimura T, Yonekura S, Taniguchi Y, Horiguti S, Saito A, Yasueda H, Nakayama T, Takemori T, Taniguchi M, Sakaguchi M, Okamoto Y. The induced regulatory T cell level, defined as the proportion of $IL10^{+}Foxp3^{+}$ cells among $CD25^{+}CD4^{+}$ leukocytes, is a potential therapeutic biomarker for sublingual immunotherapy: a preliminary report. ***Int Arch Allergy Immunol.*** 153, 378-387 (2010)

4. Fujimura T and Okamoto Y. Antigen-specific immunotherapy against allergic rhinitis: the state of the art. ***Allergol Int.*** 59, 21-31 (2010)

iPS technology development for immunological research and therapeutics

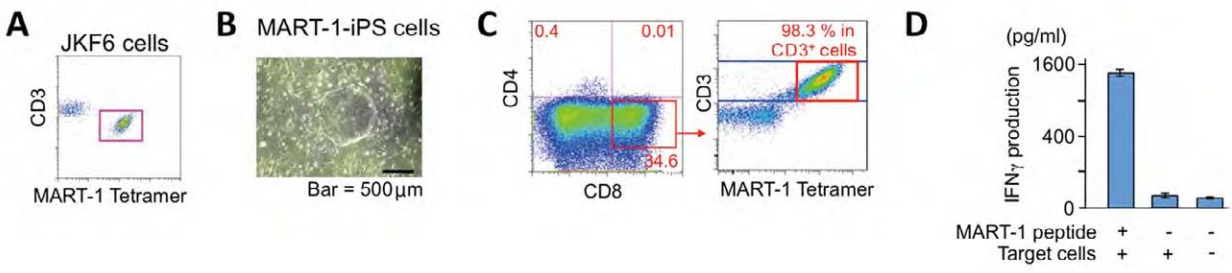


Figure: Efficient generation of antigen-specific mature T cells *in vitro* from T-iPS cells originally derived from antigen-specific T cells
(A) Flow cytometric profile of JKF6 cells.
(B) Photomicrograph of iPS cells derived from JKF6 cells.
(C) Efficient generation of antigen-specific mature T cells *in vitro* from T-iPS cells. Left panel shows the CD4 vs. CD8 profile of cells generated in the coculture system using OP-9/DLL1 cells. Right panel shows the CD3 vs. MART-1 tetramer staining of $CD8^+$ T cells gated in the rectangle in the left panel.
(D) Production of IFNγ by $CD8^+$ T cells upon antigen -specific stimulation. IFNγ secretion was measured after co-culturing CD8 SP cells for 24 hours with EBV-lymphoblastoid cells (CIRA0201) pulsed or not with MART-1 peptide.

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

Aberrant reprogramming of polycomb protein Ring1B in B-iPSCs

The iPS Group successfully established B cell (B)-iPSCs in mouse and human by using overexpression of defined factors. Furthermore, by transcriptome analysis, the Group identified polycomb (Ring1B) target genes as candidates for an aberrant reprogramming region. To address this issue, they generated B-iPSCs from Ring1A/B conditional knockout mice. They found that Ring1 deleted B-iPSCs showed more apoptotic cells than ESCs. Moreover, a Ring1B target gene, Ink4a/Arf, was upregulated in B-iPSCs after Ring1 protein deletion. These results suggest that B-iPSCs are reprogrammed when cells are in a pluripotent state, but have aberrant phenotypes when cells are in a differentiated state. Next, they addressed the impact of polycomb during somatic cell reprogramming to pluripotency using Ring1B and Cdkn2a double knockout (dKO) or Cdkn2a single knockout (sKO) mice. They found that there were two fold more ESC-like colonies in dKO than in the sKO by an alkaline phosphatase (ALP) assay. Although they were statistically low, more clones were established as B-iPSCs in dKO than in the sKO by an iPSCs establishment assay. These results indicate that polycomb impact during reprogramming has at least two phases (Early and Late). Polycomb represses the reprogramming in the early phase while promoting it in the late phase. Thus, aberrant reprogramming by polycomb may be occurring in the late phase. Polycomb impact during reprogramming in the early phase may be addressed using a reprogrammable mouse strain (kindly provided from CiRA, Kyoto University).

Efficient induction of iPSCs from human circulating T cells

The Group used an improved version of a temperature sensitive (TS) SeV vector to generate iPSCs in which the SeV could be efficiently depleted by a simple temperature shift. Pluripotency of the resulting iPSCs was validated by generating germ line chimeric mice. They then showed that human T cells were reprogrammed to iPSCs (T-iPSCs), which are also virus-free in the host genome, using the TS SeV vector. Furthermore, they showed more efficient induction of human T-iPSC using SV40 combined with defined Yamanaka factors (SV40+OSKM), compared to using defined Yamanaka factors alone (OSKM). Finally, they induced T-iPSCs from several types of human T cells such as $CD4^+$ or $CD8^+$. These results suggested that the TS SeV is qualified as a future standard vehicle to reprogram human cells for clinical purposes.

Generation and analysis of NKT-iPSC-derived cloned mice

The Group has previously succeeded in generating iPSCs harboring NKT cell-specific rearranged TCR loci by a conventional protocol with retroviral vectors starting with highly purified B6 splenic NKT cells. The

iPSC clones can efficiently develop into NKT cells producing large amounts of IFN-γ *in vitro* by co-culturing with the OP9/Dll-1 stromal cell line in the presence of IL-7 and Flt3L. These NKT cell-derived iPSCs also generated coat color chimeras when injected into BALB/c blastocysts. The male chimeras tested were found to be germline chimeras that transmitted the rearranged Vα14-Jα18 and Vβ7-Dβ2-Jβ2.3. The NKT-iPSC-derived cloned mice have reduced numbers (1/5 to 1/20) of total MNCs in the thymus but not in the periphery. They also had a 5- to 100-fold increase in absolute number of NKT cells and a severe reduction of αβT cells (1/20-1/100) and loss of γδT cells. The function and phenotype of NKT cells in cloned mice is comparable to those in B6 mice, indicating that the cloned mice are a feasible model for the analysis of NKT cells and are adapted for NKT cell-targeted therapy.

Induction of hematopoietic lineages from human iPSCs

The Group aims to induce hematopoietic stem/progenitor cells from human iPS cells to understand human hematopoietic development and to elucidate whether human iPS cells can recapitulate hematopoiesis through progenitor cells with myeloid, lymphoid, and erythroid potential.

To this end, they set up *in vitro* culture of human iPSCs using stromal cells. Compared with primary human osteoblasts or mesenchymal stem cells, OP9 induces human $CD34^+CD38^-$ cells from iPSCs more efficiently, regardless of Notch signaling. The Group then examined the extent to which human iPS-derived $CD34^+CD38^-$ cells recapitulate the transcriptional signature of normal HSCs. Expression levels of iPS-associated genes including NANOG, SOX2 and OCT3/4 in human iPS-derived $CD34^+CD38^-$ cells were downregulated to the levels seen in CB/BM HSCs. Of the gene sets associated with the HSC-specific transcriptional signature, HOXB4, HOPX, STAT5 and CBFA2TE were expressed by iPS-$CD34^+$ cells at comparable levels to those in HSCs. Furthermore, they analyzed the expression of transcription factors associated with lineage specification. iPS-derived $CD34^+CD38^-$ cells at day 7 and at day 14 expressed GATA3, GFI1, GATA2 at levels similar to those in normal BM/CB HSCs. Consistent with the transcription profile of human iPS-derived $CD34^+CD38^-$ cells, a colony forming capacity (CFC) assay demonstrated that these iPS-derived $CD34^+CD38^-$ cells generated granulocyte-, monocyte-, and erythroid colonies in methylcellulose agar. Furthermore, 750-3000 $CD34^+CD38^-CD43^+$ cells exhibited potent capacity to differentiate into T cells and NK cells in the OP9-DLL1 culture system. Together, human iPSCs generate human $CD34^+CD38^-CD43^+$ cells with multi-lineage differentiation capacity, and the putative progenitor could be a key cellular component in developing hematopoiesis therapies in humans.

Regeneration of antigen-specific T cells from iPS cells derived from human $CD8^+$ mature T cells

In this study, the Group established iPSCs from mature cytotoxic T cells specific for the melanoma epitope MART-1 (Fig. A). JKF6 cells are long-term cultured tumor infiltrating lymphocytes that were originally derived from a melanoma patient, and have been maintained at the Surgery Branch of the National Cancer Institute (Yang *et al., PLOS One, e22560*, 2011). JKF6 cells are specific for the complex of MART-1-peptide and HLA-A*02:01, which can be visualized as MART-1-$tetramer^+$ cells by flow cytometry (Fig. A). MART-1-specific T cells were transduced with Yamanaka factors, and they established a clone that forms colonies with human ESC-like morphology (MART-1-iPSCs, Fig. B). When co-cultured with OP9/DLL1 cells, these MART-1-iPSCs efficiently generated $CD8^+$ T cells, and more than 90% of these cells were specific for the original MART-1 epitope (Fig. C). Stimulation of these $CD8^+$ T cells with HLA-A*02:01-expressing cells pulsed with MART-1-peptide resulted in the secretion of IFNγ (Fig. D). The present study thus provides a novel method for cloning and expanding functional $CD8^+$ T cells specific for a given antigen, which can potentially be applied for cell therapy against cancer.

Members of the iPS Group

Leader : **Hiroshi Kawamoto**, **Fumihiko Ishikawa**, **Osamu Ohara**
Senior Research Scientist : **Hiroshi Watarai**
Research Scientist : **Daisuke Yamada**, **Kyoko Masuda**, **Yoriko Saito**, **Raul Eduardo Vizcardo Sakoda**
Technical Staff : **Momoko Okoshi-Sato**, **Genta Kitahara**, **Masako Fujita**, **Chieko Tezuka**, **Sakura Sakata**, **Yuko Nagata**, **Mariko Tomizawa**

Recent Publications

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

2. Pereira CF, Piccolo FM, Tsubouchi T, Sauer S, Ryan NK, Bruno L, Landeira D, Santos J, Banito A, Gil J, Koseki H, Merkenschlager M, Fisher AG. ESCs require PRC2 to direct the successful reprogramming of differentiated cells toward pluripotency. ***Cell Stem Cell*** 6, 547-556 (2010)

3. Watarai H, Yamada D, Fujii S, Taniguchi M, Koseki H. Induced pluripotency as a potential path towards iNKT cell-mediated cancer immunotherapy. ***Int J Hematol.*** 95, 624-631 (2012).

RCAI innovation projects in drug discovery and medicine

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

Several RCAI researchers collaborate with DMP. Toshitada Takemori is the Unit Leader of Drug Discovery Antibody Platform Unit of DMP, in order to develop monoclonal antibodies that can be used as therapeutic drugs for treatment of cancer and other diseases.

Besides the Antibody Platform Unit, six other R&D projects were conducted under the collaboration between RCAI and DMP (Table 1).

Table 1

Project	Project Leader
NKT cell therapy for cancer	**Masaru Taniguchi**
Vaccine for cedar pollinosis	**Masaru Taniguchi**
Artificial adjuvant vector	**Shin-ichiro Fujii**
Stem cell-targeting therapy for leukemia	**Fumihiko Ishikawa**
Mucosal vaccine	**Hiroshi Ohno**
Antibody therapy for $CD8^+$ T cell-related diseases	**Tsuneyasu Kaisho**

NKT cell-targeted adjuvant cell therapy for cancer patients

RIKEN and the National Hospital Organization (NHO) reached an integrative collaboration agreement for NKT cell-targeted therapy and medical innovations in 2012. NHO manages a Japanese network of 144 hospitals including 53,000 beds and a staff of 54,000. Under this agreement, Nagoya Center and Kyusyu Cancer Center of the NHO will begin NKT cell-targeted therapy for lung cancer.

A phase I/IIa clinical study of the application of the NKT cell-targeted therapy for advanced lung cancer had been conducted in collaboration with Chiba University (Drs. Toshinori Nakayama and Shin-ichiro Motohashi) and RIKEN RCAI. In this study, the median survival time (MST) of the responder group was significantly longer than the poor-responder group (29.3 months versus 9.7 months). Following the outcome of this clinical trial, NKT cell-targeted therapy for lung cancer was approved by the Advanced Medical Care Assessment System by the Japanese Ministry of Health, Labor and Welfare (MHLW) in 2011. This system allows partial health insurance coverage to ease the financial burden on patients who need state-of-the-art medical treatment.

After the agreement between RIKEN and NHO, a collaboration between RIKEN RCAI, Chiba University and NHO was started. NHO created Cell Processing Centers in Nagoya Center and Kyusyu Cancer Center. RCAI coordinated the agreement, provides α-GalCer to stimulate the NKT cells, and conducts SNP analysis

of responder and poor-responder groups in collaboration with the RIKEN Center for Genomic Medicine and NHO. Chiba University provides advice on the clinical aspects of the therapy and technical training to the NHO.

In 2012, NKT cell-targeted therapy for head and neck squamous cell carcinoma, in collaboration with Professor Yoshitaka Okamoto, Chiba University Hospital, was newly approved by the Advanced Medical Care Assessment System. Although translational research on NKT cell-targeted therapy has been progressing, there are still problems in its clinical applications. There is no clear regulation of cell therapy in Japan; some cell therapies are conducted for beauty treatment or cancer without enough safety studies. Moreover, different regulations are applied depending on where the cell therapy is conducted, in hospitals or other places. In addition, to apply for the Pharmaceuticals and Medical Devices Agency's review, it is not clear how cell therapy should be categorized. The Japanese Pharmaceutical Affairs Law (PAL) has only two categories, "pharmaceuticals" and "medical devices". Regenerative medicine, such as transplantation of skin or cartilage cells is currently categorized as "medical devices", but NKT cell-targeted therapy may be rather closer to "pharmaceuticals". A draft amendment of PAL was submitted to the parliament and it will be discussed in the Diet in 2013.

Development of artificial adjuvant vector cells as a novel therapy

In collaboration with Drs. Kakimi (Univ. of Tokyo), Maeda (Iwate Medical Univ.) and Mizuno (Yamaguchi Univ.), RCAI's Shin-ichiro Fujii, Kanako Shimizu and Yasuyuki Ishii have been developing unique adjuvant vector cells that stimulate NKT cell activation. They generated glycolipid-loaded, mRNA-transfected allogenic fibroblasts that act as artificial adjuvant vector cells to promote NKT cell activation, leading to dendritic cell maturation and antigen-specific T cell immunity. In preclinical studies, they observed the safety profile and immune response. Administration of these artificial adjuvant vector cells to dogs activated NKT cells and also elicited antigen-specific T cell responses with no adverse events. This unique tool could prove clinically beneficial in the development of immunotherapies for malignant and infectious diseases. (See *Research Highlights* p. 4 and p. 13)

A drug lead for leukemia

Acute myeloid leukemia (AML) is a difficult cancer to cure. Although most people with the disease achieve a period of remission following a standard dose of chemotherapy, the leukemia will typically return. Now, in a breakthrough that could lead to the development of a drug that promises to prevent such relapses, Dr. Fumihiko Ishikawa and his colleagues discovered a small-molecule drug that wipes out AML cells in a mouse model (Saito *et al. Sci Trans Res*. 2013).

Ishikawa and his team previously found that the expression of hematopoietic cell kinase (HCK)—an enzyme involved in blood cell differentiation and proliferation—was greater in leukemia stem cells taken from individuals with AML than in blood stem cells obtained from people without cancer.

In their latest research, Ishikawa's team assessed the potential of targeting this enzyme as a possible therapeutic agent for human use. They found that by knocking down HCK expression in cell culture with counteracting RNA molecules, they could achieve a significant reduction in both the growth and survival of the AML cells.

The team then enlisted the help of Dr. Toshio Goto and colleagues at the RIKEN Program for Drug Discovery and Medical Technology Platforms to search for a

chemical agent that possessed the same beneficial activity against AML. A high-throughput screen of 50,000 compounds for HCK inhibitors, in which the most likely compounds were compared with the results of a parallel computer model-based screen, identified one molecule as the most promising drug candidate. This chemical, a pyrrolo-pyrimidine derivative, was structurally analyzed by x-ray crystallography and subsequently optimized to yield the small molecule RK-20449.

Experiments with human AML cells, both *in vitro* cultures and implanted into mice, showed that the drug reduced the number of leukemia cells and shrank the tumor burden of the disease (Fig.) (Saito, Y. et al. *Sci Trans Med*. Vol. 5, 181ra52, 2013). If these results are translatable to the clinic, the drug could help prevent and overcome cancer relapse in AML patients.

"If RK-20449 or its derivatives can eliminate AML cells, including leukemia stem cells, in patients, that will advance AML treatment significantly," Ishikawa says. "After thorough and careful examination of the toxicity of RK-20449, we hope that our findings can be translated into a safe and effective treatment for AML."

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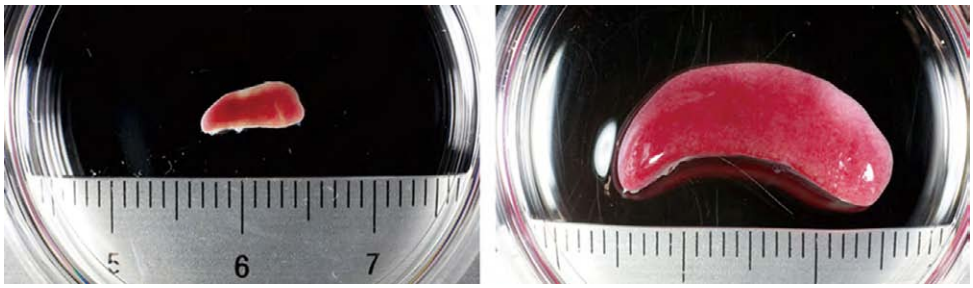


Figure: Spleens from mice engrafted with human AML cells after 52 days of treatment with the small-molecule drug RK-20449 (*left*) and prior to treatment (*right*).

Longitudinal birth cohorts and the humanized mouse model

The incidence of atopy, allergic disease and asthma is increasing in industrialized nations. Allergies are considered to result from the combined effect of genes and environmental factors and their interactions. To clarify the incidence of allergic disease, RCAI, in a close collaboration with Chiba University and the National Center for Child Health and Development (NCCHD), has been working on longitudinal birth cohorts. They recruited babies born of mothers who, along with their family and relatives, have histories of allergy. In this process, Chiba University and NCCHD are studying the environmental factors associated with the development of allergic disease from birth to 3 years of age (the original cohort is now at 2 year of age). For its part, RCAI has already stored white blood cells (now 300 samples) that were

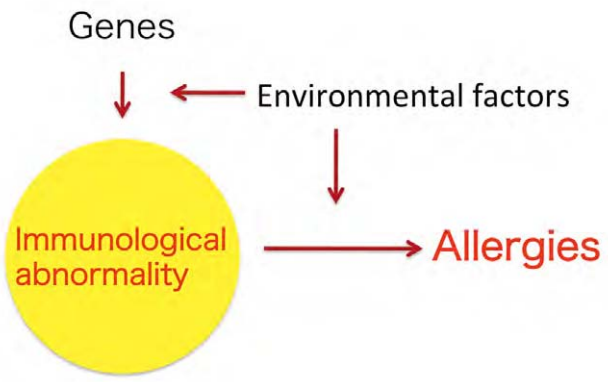


Figure: The incidence of allergic diseases

purified from umbilical cord blood of all participants. After completing the cohort study, RCAI will purify hematopoietic stem cells from stored samples and use them to establish humanized mice that will be reconstituted with the immunological state of the allergic infants before the onset of disease. This approach will enable us to evaluate the environmental and genetic risk factors in allergy development.

As of December 2012, 191 babies at 1 year of age and 55 babies at 2 years of age were examined. Until now, the presence or absence of atopic dermatitis and sensitization to major food and airborne allergens has been evaluated in 180 babies at 1 year of age. The rate of atopic dermatitis at 1 year of age was 12.7%. Sensitization to any of the allergens tested was 39.3%, with egg white showing the highest (32%). Colonization of the cheeks by *Staphylococcus aureus* at 1 month and 6 months of age was 46.1% and 46.7%, respectively. The clinicians found several early life exposures that could be risk factors for allergy development in infants at one year of the study and will continue the analysis up to two years of age. When candidate factors are identified for each of the participants, RCAI will be able to evaluate their roles by using humanized mice reconstituted with the participants' immune system at birth.

Establishing an efficient delivery system for mucosal vaccines

Mucosal (oral) vaccination can be a better vaccination protocol in that it can induce both mucosal secretory IgA as well as systemic serum IgG, whereas systemic (e.g., subcutaneous) vaccination can only induce serum IgG. However, an oral vaccine has to be resistant to gastrointestinal digestion to reach gut-associated lymphoid tissue (GALT), the inductive site for mucosal immune responses, in a sufficient quantity to induce an efficient immune response. Because of this requirement, successful oral vaccines to date are often attenuated live viruses, such as poliovirus and rotavirus, which can propagate in the body to generate enough immunogenicity, even when the initial amount delivered to GALT is very small.

RCAI's Hiroshi Ohno and his team identified a bacterial uptake receptor GP2 on M cells, a subset of intestinal epithelial cells that overlay the GALT, and have shown that GP2 is important for efficient delivery of the internalized bacteria to GALT for induction of mucosal immune responses. Therefore, GP2-targeting should be an ideal vaccination strategy, since this could enable efficient delivery of vaccine antigens to GALT for mucosal immune responses, even if the vaccines are killed or attenuated viruses unable to propagate in the body. With support from the RIKEN Program for Drug Discovery and Medical Technology Platforms, they are now trying to develop a novel GP2-targeted mucosal vaccine delivery system.

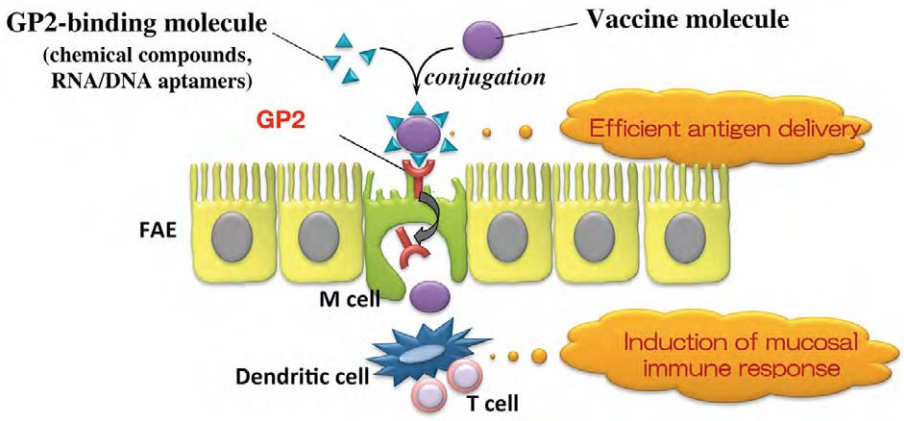


Figure : **Schematic overview of the GP2-targeted vaccine delivery system**

Host gastrointestinal immune response upon exposure to probiotic bacteria

Intestinal Microbe Symbiosis Laboratory, Integrated Collaborative Research Program with Industry, RIKEN Innovation Center

Many commercial probiotic bacterial strains have been developed to help manage a number of health issues such as gastrointestinal (GI) disorders, inflammatory bowel diseases, allergies, and pathogenic infections. Among these probiotic strains, *Lactobacillus acidophilus* L-92, with anti-allergic effects for pollen allergy, perennial allergy, atopic dermatitis, and the ability to control GI disorders has been developed by CALPIS Co. Ltd. To understand the mechanisms of the beneficial effects of L-92, the Intestinal Microbe Symbiosis Laboratory has been established under the Integrated Collaborative Research Program with Industry of RIKEN in collaboration with CALPIS and the Laboratory for Epithelial Immunobiology.

The health benefits from L-92, especially immunomodulatory effects, are thought to result from interactions between L-92 and the host mucosal immune system in the GI tract (Fig.). They hypothesized that these health benefits are most likely initiated with the association between the L-92 and host intestinal epithelium, and are focusing on the immune response of the epithelium.

To understand host GI epithelial immune responses upon exposure to L-92, microarray analysis was performed by using the well-established human adenocarcinoma epithelial Caco-2 cell line. Genes classified as cell adhesion, MAP kinase pathway, transmembrane proteins, and immune related pathway were up-regulated after 20 h of treatment, whereas no significantly down-regulated genes were observed. Information obtained in the present study could be an important step for consideration of the impact of L-92 on the mucosal immune system in the GI tract and on host health (Yanagihara, S. *et al.*, *J. Med. Food* 15(6): 511-519, 2012).

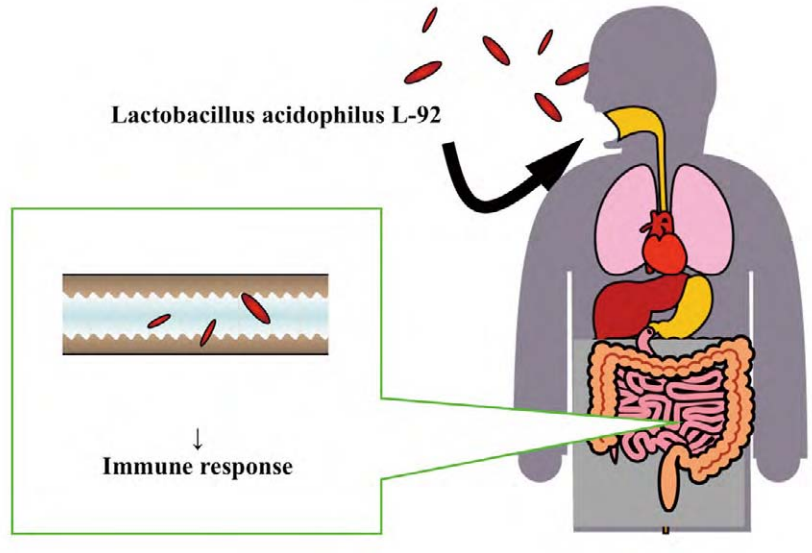
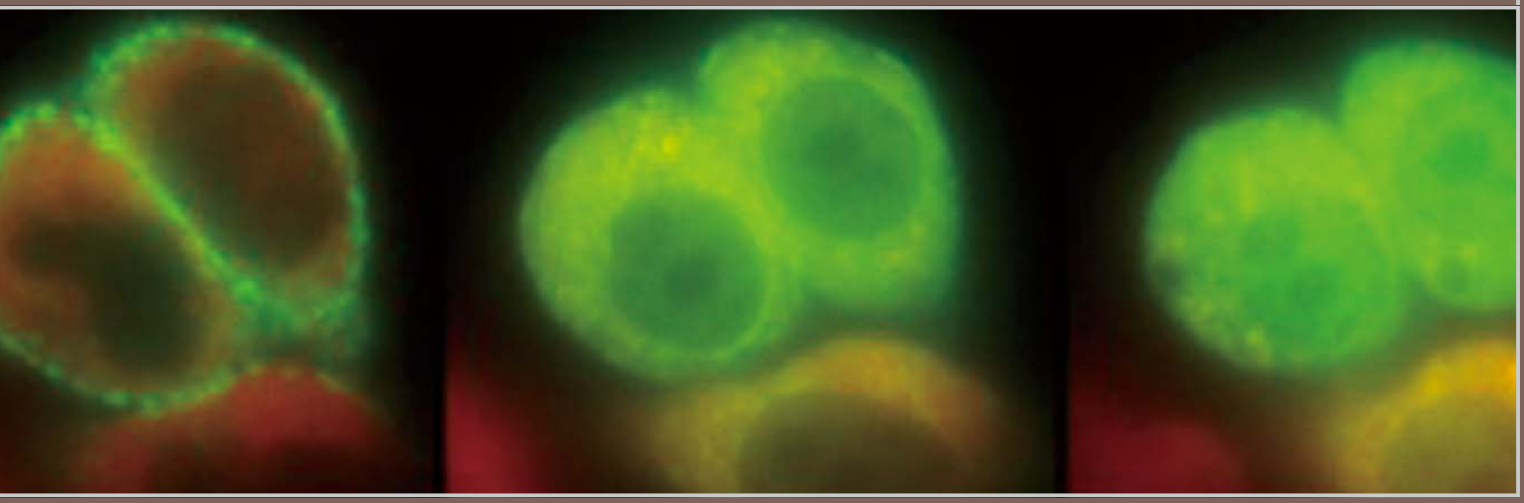


Figure: The mucosal immune system in the GI tract forms the largest part of the entire immune system, where the health benefits from L-92, especially immunomodulatory effects, are induced.

2012
Part 3

Nurturing Young Scientists



YCI

Young Chief Investigator Program

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

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

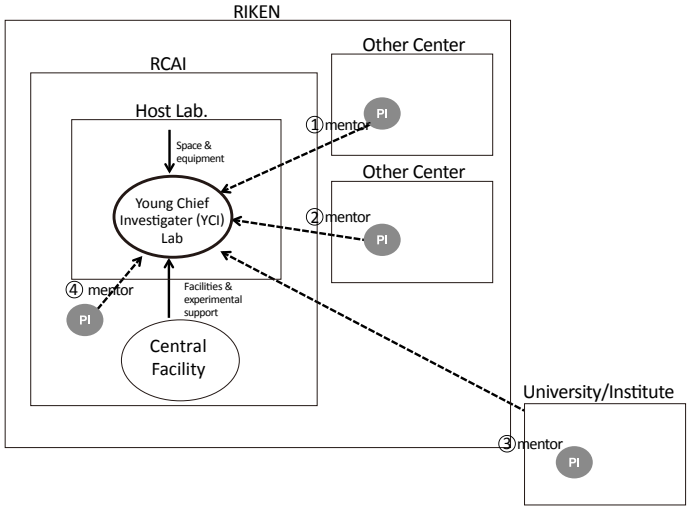


Figure: Scheme of the Young Chief Investigator Program

In 2012, four researchers conducted their studies as Young Chief Investigators, Dr. Hayato Naka-Kaneda (Stem Cell Competency), Dr. Shinji Nakaoka (Mathematical Modeling of Immune System), Dr. Tomokatsu Ikawa (Immune Regeneration) and Dr. Katsuyuki Shiroguchi (Integrative Genomics). Their mentors and host laboratories are listed in Table.

Table: Young Chief Investigators and their mentors 2012

YCI lab	Mentors	Affiliation
Shinji Nakaoka Laboratory for Mathematical Modeling of Immune System Host Lab: **Osamu Ohara**, RIKEN RCAI	**Masahiro Ueda**	Group Director, Laboratory for Cell Signaling Dynamics, RIKEN QBiC
	Kenji Kabashima	Associate Professor, Department of Dermatology, Graduate School of Medicine, Kyoto University
	Osamu Ohara	Group Director, Laboratory for Immunogenomics, RIKEN RCAI
	Masato Kubo	Professor, Research Institute for Biological Science, Tokyo University of Science / Senior Visiting Scientist, Open Lab. for Signal Network, RIKEN RCAI
	Ronald Germain	National Institutes of Health, USA
Hayato Kaneda Laboratory for Stem Cell Competency Host Lab: **Ichiro Taniuchi**, RIKEN RCAI	**Hitoshi Niwa**	Team Leader, Laboratory for Pluripotent Stem Cell Studies, RIKEN CDB
	Piero Carninci	Team Leader, Functional Genomics Technology Team, RIKEN OSC
	Hiroshi Kawamoto	Team Leader, Laboratory for Lymphocyte Development, RIKEN RCAI / Professor, Institute for Frontier Medical Sciences, Kyoto University
	Haruhiko Koseki	Group Director, Laboratory for Developmental Genetics, RIKEN RCAI
Tomokatsu Ikawa Laboratory for Immune Regeneration Host Lab: **Haruhiko Koseki**, RIKEN RCAI	**Takashi Nagasawa**	Professor, Institute for Frontier Medical Sciences, Kyoto University
	Piero Carninci	Team Leader, Functional Genomics Technology Team, RIKEN OSC
	Toshio Kitamura	Professor, The Institute for Medical Science, The University of Tokyo
	Haruhiko Koseki	Group Director, Laboratory for Developmental Genetics, RIKEN RCAI
Katsuyuki Shiroguchi Laboratory for Integrative Genomics Host Lab: **Osamu Ohara**, RIKEN RCAI	**Yoshie Harada**	Professor, Institute for Integrated Cell Material Sciences, Kyoto University
	Yutaka Suzuki	Associate Professor, Graduate School of Frontier Sciences, The University of Tokyo
	Yoshio Kodera	Director, Center for Disease Proteomics, Kitasato University
	Osamu Ohara	Group Director, Laboratory for Immunogenomics, RIKEN RCAI

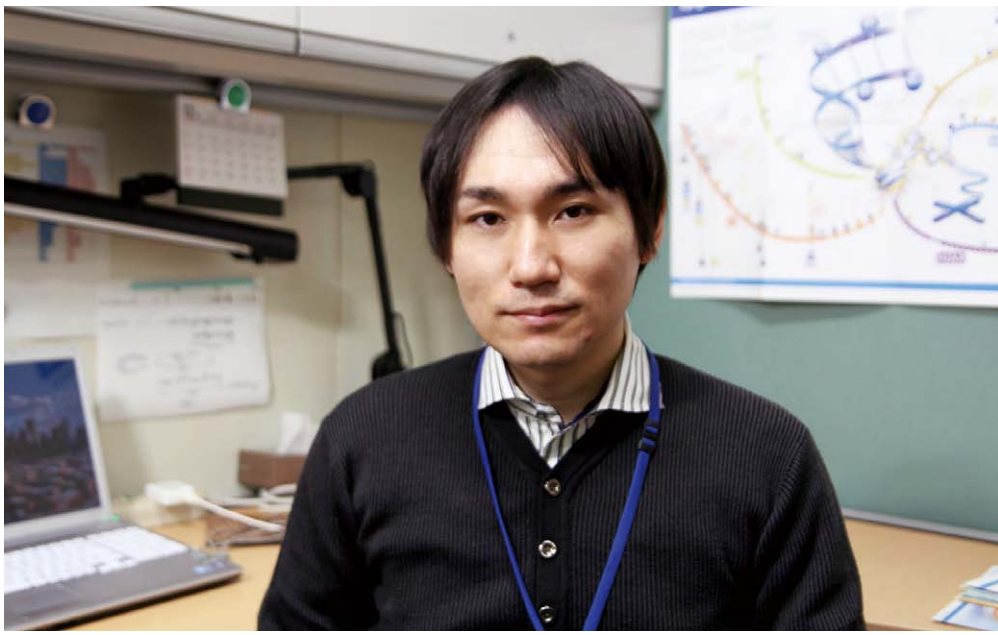
YCI Laboratory for

Stem Cell Competency

Young Chief Investigator :
Hayato Kaneda

Research associate :
Michiko Ohno-Oishi

Technical staff :
Shiho Nakamura



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

Competence regulation of aged stem cells

Although neural stem cells (NSCs) have the capacity to differentiate into neurons and glial cells, neurogenesis largely precedes gliogenesis during central nervous system (CNS) development in vertebrates. The neurogenesis-to-gliogenesis switch requires the temporal identity transition of NSCs. In a previous study, we found that the “competence change” is a key molecular mechanism for the temporal identity transition of NSCs. Inhibition of the competence change causes loss of gliogenesis and sustained neurogenesis. Remarkably, the competence change does not promote or repress the differentiation processes directly. Instead, it controls changes in responsiveness to extrinsic signals such that NSCs cannot respond to the gliogenic cytokines and express their gliogenic potential before they acquire the gliogenic competence.

Recently, we found that the competence regula-

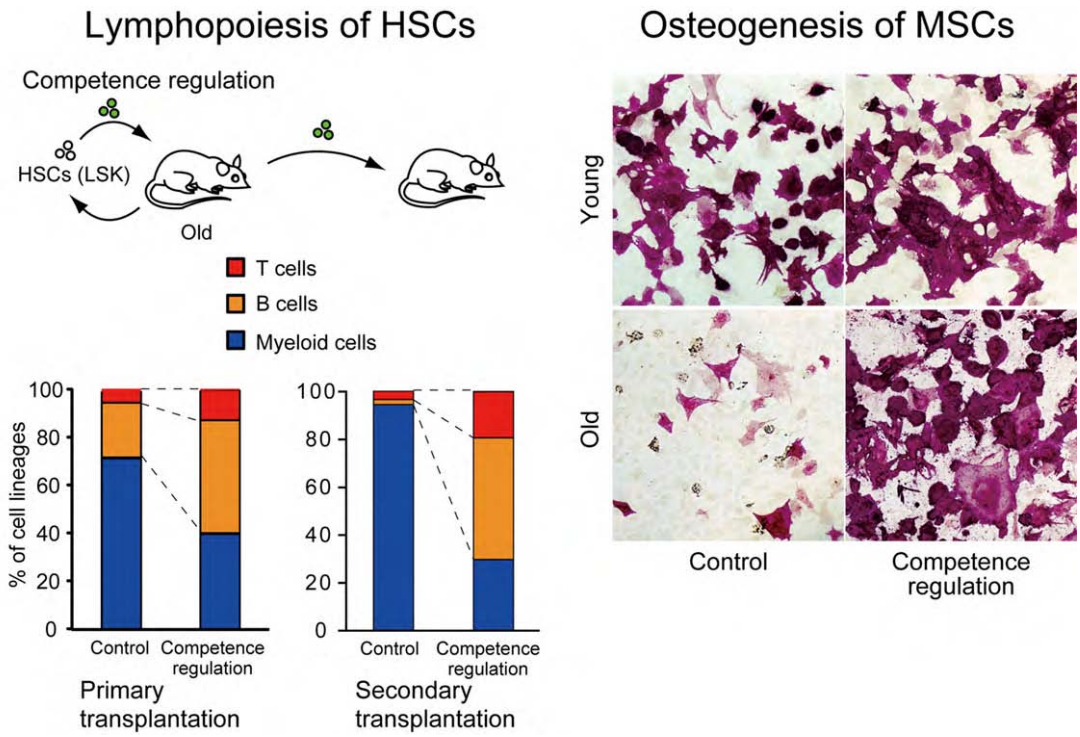


Figure: **Functional restoration of aged stem cells**
Forced competence regulation of aged HSCs and MSCs restored diminished lymphopoiesis and osteogenesis ability, respectively.

tion also has important roles in stem cell ageing. Forced competence regulation of aged hematopoietic stem cells (HSCs) or mesenchymal stem cells (MSCs) restored diminished lymphopoiesis and osteogenesis ability, respectively (Fig.). We will try to reveal the molecular mechanisms underlying stem cell ageing in terms of competence regulation.

Screening of systemic anti-ageing factors derived from competence-regulated MSCs

A recent study about restoration of the regenerative capacity of muscle stem cells by heterochronic parabiosis of young and old mice suggests that there is a systemic ageing-control factor. Several studies have described pro-ageing factors such as CCL11 and C1q, however, no critical systemic anti-ageing factors have been identified.

Recently, we found that transplantation of competence-regulated MSCs has anti-ageing effects on the reduction of bone mass and the decline of lymphopoiesis. These effects seem to be due to systemic factors because transplanted MSCs engrafted in the lungs.

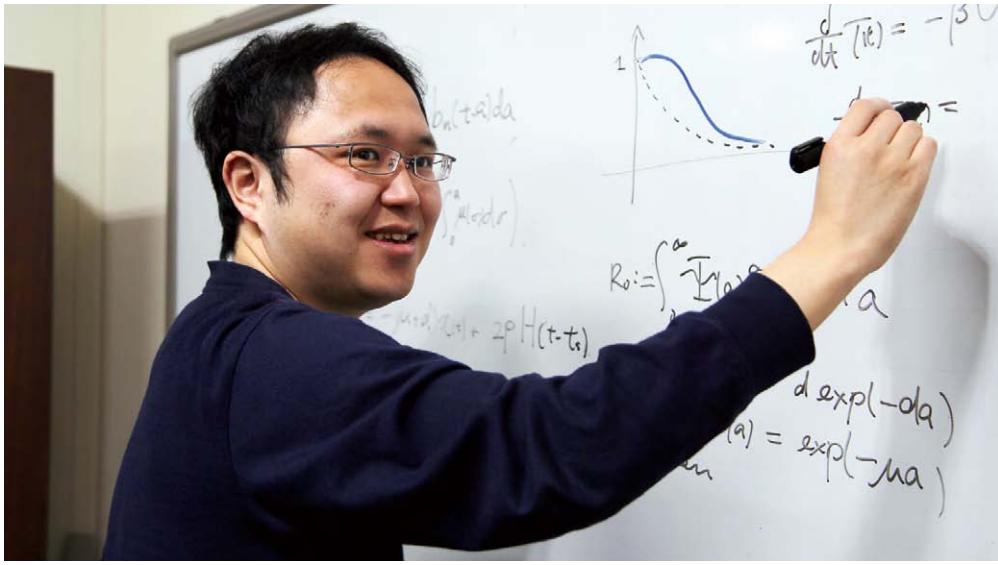
Recent Publications

1. Sato T., Shimazaki T., Naka H., Fukami S., Satoh Y., Okano H., Lax I., Schlessinger J., Gotoh N. FRS2alpha regulates Erk levels to control a self-renewal target Hes1 and proliferation of FGF-responsive neural stem/progenitor cells. ***Stem Cells*** 28, 1661-1673 (2010)
2. Naka-Kaneda H., Shimazaki T., Okano H. Neurogenesis to gliogenesis switching. ***Experimental Medicine*** 28, 815-822 (2010)
3. Tao O., Shimazaki T., Okada Y., Naka H., Kohda K., Yuzaki M., Mizusawa H., Okano H. Efficient generation of mature cerebellar Purkinje cells from mouse embryonic stem cells. ***J Neurosci Res***. 88, 234-247 (2010)

YCI Laboratory for

Mathematical Modeling of Immune System

Young Chief Investigator :
Shinji Nakaoka



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

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

(i) Integrative knowledgebase for atopic dermatitis

The onset of atopic dermatitis (AD) is diverse because there are several routes to induce skin barrier dysfunction, for example, impaired protease activity and excessive oxidative stress. To treat static diversity in the progression of AD, bioinformatics offers useful tools. Recently, I constructed an integrative knowledgebase for atopic dermatitis that provides gene-centered information on AD found in the AD literature and on public databases. The knowledgebase also provides automatic keyword-based classification for the entire AD literature with which we can generate hypotheses via associative inferences (Fig. 1).

(ii) Stochastic dynamics of skin inflammation

In order to treat dynamic diversity in the progression of AD, it is necessary to decompose spatio-temporal multi-scale dynamics of atopic dermatitis into characteristic

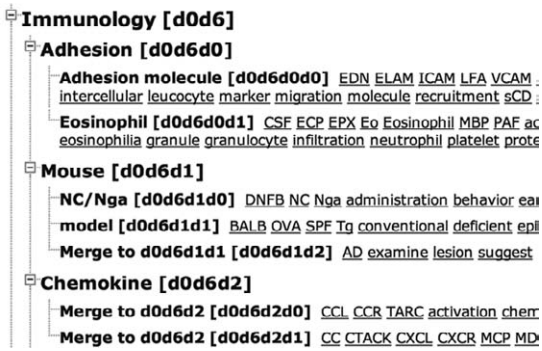


Figure 1: Automatic keyword-based classification for the entire literature on atopic dermatitis. Each sub-research field in atopic dermatitis study is represented and summarized in a hierarchical tree.

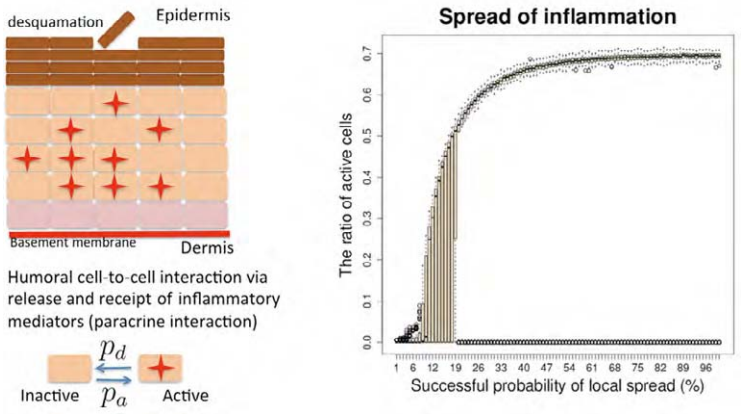


Figure 2: *Left*: A scheme of inflammation spread in the epidermis. *Right*: stochastic simulation result for the propagation of inflammation among keratinocytes via paracrine interactions: the horizontal axis represents successful probability of a cell to become an active secretor of inflammatory mediators (0-100%), while the vertical axis represents the fraction of active cells in a local epidermal tissue (0-100%). For each fixed successful probability, we carried out stochastic simulations100 times. The results are shown as a box-plot. A wide-ranged box-plot for relatively small successful probabilities suggests that stochastic variability might largely affect outcomes (success or failure of inflammation spread).

spatio-temporal time scales. At the microscopic scale, signal transduction pathways associated with the inflammatory response play an important role in determining the behavior of cells in the onset of atopic dermatitis. We will investigate the role of the JAK/STAT signaling pathway to reveal how differences in signal transduction can result in diverse outcomes. At the mesoscopic scale, we need to describe the process of recruitment of immune cells into the epidermis by sensing chemokines released from cells of the epidermis. At the macroscopic scale, it is necessary to describe the destruction process of the epidermis and the invasion of various substances that would initiate inflammatory responses. We will construct a tissue-level mathematical model to describe macroscopic barrier function of the epidermis against air-liquid substance or allergen invasion. Epidermal permeability will be also investigated with the tissue-level model to link clinically available measurements, such as trans-epidermal water loss, with the degree of barrier integrity. I have recently constructed a mesoscopic-level mathematical model and a performed simulation study to link intracellular molecular dynamics with intercellular communication among keratinocytes via release and receipt of pro-inflammatory cytokines. Preliminary stochastic simulation results suggest that local propagation of the inflammatory response can be determined by stochastic variability, which might endow an on/off switch-type information processing machinery within the epidermis to flexibly control the initiation of inflammation (Fig. 2).

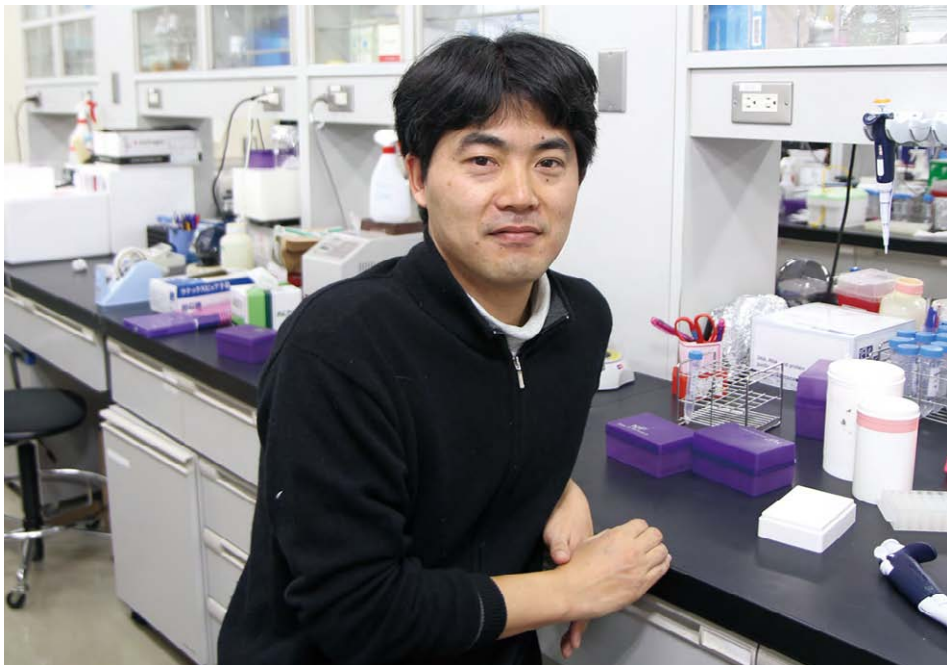
Recent Publications

1. Katsuyama C., Nakaoka S., Takeuchi Y., Tago K., Hayatsu M., Kato K., Complementary cooperation between two syntrophic bacteria in pesticide degradation, ***J Theor Biol.*** 256 644-654 (2009)
2. Iwami S., Nakaoka S., Takeuchi Y., Miura Y., Miura T., Immune impairment thresholds in HIV infection, ***Immunol Lett.*** 123, 149-154 (2009)
3. Diekmann O., Gyllenberg M., Metz J.A.J., Nakaoka S. and de Roos A., Daphnia revisited: local stability and bifurcation theory for physiologically structured population models explained by way of an example, ***J Math Biol.*** 61, 277-318 (2010)
4. Nakaoka S., Aihara K., Mathematical study on kinetics of hematopoietic stem cells -- theoretical conditions for successful transplantation, ***J Biol Dyn.*** 6, 836-854 (2011)
5. Nakaoka S., Aihara K., Stochastic simulation of structured skin cell population dynamics, ***J Math Biol.*** 66, 807-835 (2013)

YCI Laboratory for

Integrative Genomics

Young Chief Investigator :
Katsuyuki Shirogouchi



I came to Yokohama from Boston last summer and joined this center, RCAI, as a young chief investigator (YCI). I think my mission here is to expand the research area of RCAI by creating my own research direction. Since I settled in, I have been thinking of what and how I can generate a new research field, and also have been discussing with many new colleagues in order to understand what RCAI is, so that I can start unique projects related to this center. One possible way is to study immunology and related issues, e.g. diseases, based on my own experience and my original techniques. Below, I will introduce my research background and current ideas toward my new research direction here.

I had been working on a motor protein, myosin V. Myosin V is a linear molecular motor which moves unidirectionally along actin filaments using energy obtained in the ATP hydrolysis reaction. Myosin V apparently has two "feet" (each has an actin binding site and an ATP binding site), and is supposed to "walk" like a human. One of the outstanding questions was how the lifted (rear) foot can bind a forward site on the actin filament. To answer this question, first, I developed a rod-shape probe and a microscopic observation system. Then, I attached the probe to a leg of myosin V and observed under an optical microscope the change in the angle of the probe when a single myosin V molecule was walking along the actin filament. Using this biophysical approach, I elucidated walking mechanism of myosin V (Shiroguchi and Kinosita, *Science* 2007).

When I joined a research group at Harvard University in Boston, I dramatically changed my research filed, from biophysics to genomics. I have developed "Digital RNA Sequencing" using a next generation sequencer for gene expression profiling. This technique allows one to count nucleic acid molecules accurately with single molecule resolution genome-wide, which opens a window of oppor-

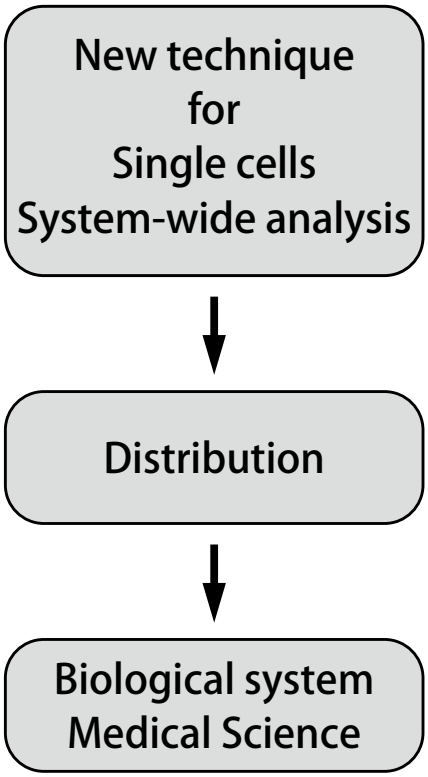


Figure: **My approach**
A new technique for single cell and/or system wide analysis may provide a new angle to visualize a new distribution of cell states that is related to biological systems and diseases.

tunity to define a cell state with single cell transcriptome analysis.

Thus, I have been developing techniques to "see" something unknown. Here at RCAI, based on my experience and skills, I am going to keep developing new techniques in order to understand biological systems and to contribute to medical science and our understanding of diseases. Especially, I am interested in visualizing the distribution of cell states by accurate system-wide measurements with single molecule and/or single cell resolution (Fig.) I believe that this approach may provide significant insights in these research fields since heterogeneity of cell populations may be one of the key factors in medical science and diseases. For example, at the beginning of a disease, homeostasis starts to be destroyed at the single cell level. I have just started some projects based on this approach. I hope I can show some interesting results next time.

Recent Publications

1. Shiroguchi K., Jia T. Z., Sims P. A., Xie X. S. Digital RNA Sequencing Minimizes Sequence-Dependent Bias and Amplification Noise with Optimized Single Molecule Barcodes ***Proc Natl Acad Sci USA.*** 109, 1347-1352 (2012)
2. Shiroguchi K.*, Chin H. F., Hannemann D. E., Muneyuki E., De La Cruz E. M.*, Kinosita K. Jr. Direct Observation of the Myosin Va Recovery Stroke That Contributes to Unidirectional Stepping along Actin" ***PLoS Biol.*** 9: e1001031 (2011) (* corresponding author)
3. Kohori A., Chiwata R., Hossain M.D., Furuike S., Shiroguchi K., Adachi K., Yoshida M., Kinosita K. Jr. Torque Generation in F_1-ATPase Devoid of the Entire Amino-Terminal Helix of the Rotor That Fills Half of the Stator Orifice ***Biophysical J.*** 101, 188-195 (2011)

YCI Laboratory for

Immune Regeneration

Young Chief Investigator :
Tomokatsu Ikawa

Technical Staff :
Asako Shibano-Satoh

Hematopoietic stem cells (HSCs) give rise to various cell types in hematopoietic and immune systems, including erythrocytes, megakaryocytes, myeloid cells (macrophages and granulocytes) and lymphocytes. HSCs and their progenitors exhibit multiple patterns of gene expression during differentiation, and the development of hematopoietic cells is regulated by a network of transcription factors. Although many essential transcription factors, such as PU.1, Ikaros, C/EBPs, GATA1-3, E2A, EBF1 and Pax5 have been implicated in regulating the cell fate choice of each hematopoietic cell lineage, molecular mechanisms underlying the generation of these patterns during cell fate determination remain unexplored because of an absence of suitable experimental systems.

We have recently established an ideal system that can examine gene regulatory networks during lymphoid lineage specification from HSCs. This novel system enabled the analysis of a large set of regulatory molecules that control the generation of T and B lymphocytes. It can also be applied for *ex vivo* expansion of human hematopoietic stem/progenitors, which will be required for immune cell therapy or transplantation of HSCs. Thus, the aims of our study are 1) from a basic science perspective, to elucidate the mechanisms that orchestrate cell fate specification, commitment and differentiation during lymphocyte development and 2) from a clinical medicine perspective, to establish a novel method to expand human hematopoietic stem/progenitors for the development of HSC transplantation as a clinical strategy.

Generation of induced Leukocyte Stem (iLS) cells

E2A is a basic helix-loop-helix transcription factor essential for B cell development. We have previously shown that B cell development in the bone marrow of E2A-deficient mice is blocked at the myelo-lymphoid progenitor stage, and that such progenitor cells have a multi-differentiation potential both *in vitro* and *in vivo* (Ikawa et al. *Immunity*, 2004). These results indicate that (1) the progenitors whose differentiation potential is blocked at the myelo-lymphoid progenitor stage display self-renewal activity, (2) these progenitors are able to proliferate indefinitely in pro B cell culture conditions. Therefore, we hypothesized that myelo-lymphoid progenitors can be expanded by suppressing E2A activity in multipotent progenitors that are in the process of differentiating into B lineage cells.

We overexpressed Id3, one of the Id proteins that are natural inhibitors of E2A, in normal murine HSCs, and cultured the transduced cells on TSt-4 stromal cells in the presence of added cytokines (SCF, IL-7 and Flt3-L). As a result, these cells expanded extensively without losing their multilineage differentiation potential. They were able to generate T, B, and myeloid lineage cells for several months, upon transfer into irradiated immunodeficient ($Rag1^{-/-}$) mice. We named such induced

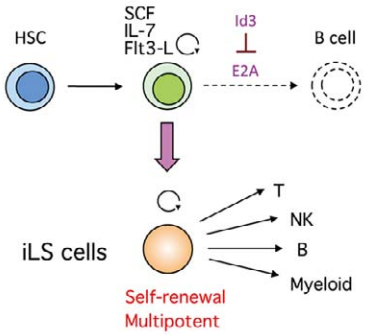


Figure 1: Induced Leukocyte Stem (iLS) cells
We have established multipotent progenitors that have self-renewal activity and multipotency. This was done by overexpressing Id3 in hematopoietic stem/progenitor cells and culturing the cells under B cell differentiating conditions. Id3 suppresses the E2A activity and the multipotent progenitors acquire self-renewal activity. The cells extensively proliferate for at least several months, still maintaining their multipotency.

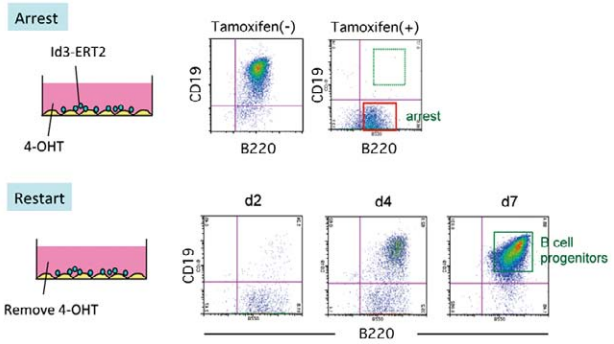


Figure 2: Inducible culture system to examine transcriptional networks in B cell lineage specification
Hematopoietic stem/progenitor cells were transduced with the Id3-Estrogen receptor (ERT2) retrovirus. The cells were cultured in the presence or absence of 4-Hydroxytamoxifen (4-OHT). 4-OHT blocks B cell differentiation, whereas following its removal, B cell generation ensues. Large numbers of $CD19^+$ B cells were generated within 7 days.

multipotent cells as iLS (induced leukocyte stem) cells (patent applied for, manuscript in preparation; Fig. 1). These unique cells provide an ideal system that can be induced to proliferate or differentiate at will.

The advantages of the iLS cells are (i) iLS cells are homogenous, (ii) iLS cells are normal and not neoplastic, (iii) time course analysis of T, B and myeloid lineage cell differentiation can be performed under appropriate culture conditions, (iv) a large number of cells (>1x10^6) are available, allowing us to do genome-wide assays such as transciptome or epigenetic (DNA methylation and histone modification) analyses. Therefore, this system has tremendous potential to answer basic developmental biology questions, especially about the mechanisms of cell fate determination. We are currently translating this novel method for the expansion of human leukocyte progenitors that later can also be applied in clinical application such as immune cell therapy and reconstitution of hematopoietic cells in immunocompromised patients.

Establishment of an inducible system that can clarify the transcriptional network in B-lymphoid lineage specification

We have recenlty succeeded in modifying the iLS cells by using an Id3-ERT2 (Estrogen receptor) retroviral construct whose expression can be induced by 4-Hydroxytamoxifen (4-OHT). Mouse HSCs were transduced with the Id3-ERT2 retrovirus and cultured on TSt-4 stromal cells in the presence of 4-OHT. One month later, a homogenous population of iLS cells was obtained. The iLS cells kept proliferating and could be stably maintained by continuous addition of tamoxifen. However, once tamoxifen was removed, they began differentiating into B cells under B cell generating culture conditions (Fig. 2). They easily give rise to $CD19^+$ cells, and the B lineage commitment can be induced within 7 days. Using this inducible system, we will attempt to elucidate the transcriptional network regulating B cell fate specification.

Recent Publications

1. Ikawa T., Hirose K., Masuda K., Kakugawa K., Satoh R., Shibano-Satoh A., Kominami R., Katsura Y., Kawamoto H. An essential developmental checkpoint for production of the T cell lineage. ***Science*** 329, 93-96 (2010)
2. Kawamoto H., Ikawa T., Masuda K., Wada H., Katsura Y. A map for lineage restriction of progenitors during hematopoiesis. ***Immunol Rev.*** 238, 23-36 (2010)
3. Seo W., Ikawa T., Kawamoto H., Taniuchi I. Runx1-CBFβ facilitates early B lymphocyte development by regulating expression of Ebf1 gene. ***J Exp Med.*** 209, 1255-1262 (2012)
4. Isoda T., Takagi M., Piao J., Nakagama S., Sato M., Masuda K., Ikawa T., Azuma M., Morio T., Kawamoto H., Mizutani S. Process for Immune defect and chromosomal translocation during early thymocyte development lacking ATM. ***Blood*** 120, 789-799 (2012)
5. Sakamoto S., Wakae K., Anzai Y., Murai K., Tamaki N., Miyazaki M., Miyazaki K., Romanow WJ., Ikawa T., Kitamura D., Yanagihara I., Minato N., Murre C., Agata Y. E2A and CBP/p300 act in synergy to promote chromatin accessibility of the immunoglobulin κ locus. ***J Immunol.*** 188, 5574-5560 (2012)

RCAI International Summer Program 2012

The 2012 RCAI International Summer Program (RISP) was held on June 22-29. This was the seventh annual RISP and was co-sponsored by the Global Center of Excellence at Chiba University. It was a special event, since RISP 2011 had to be canceled because of the Great East Japan Earthquake and tsunami on March 11, 2011 and subsequent nuclear accident at Fukushima Nuclear Power Plant. Although repercussions of this disaster are still being felt, the situation in Yokohama was stable and the RCAI leadership was pleased to resume the program, which is one of the major annual events at the Center. Forty one young scientists from nineteen countries participated in RISP 2012; approximately 80% were graduate students and the remainder postdoctoral fellows. The first part of the program (June 22-27) was held in Yokohama at RCAI. There were morning oral presentations by the participants and these were supplemented by two early evening poster sessions, where there was time for more detailed discussions of the data. The afternoon sessions featured lectures by prominent scientists invited from RCAI, Japanese universities, and abroad. On June 28, the RISP participants joined the annual RCAI-JSI International Symposium on Immunology at the Pacifico Yokohama Conference Center. This year's theme was "New Horizon in Immune Regulation – Bridging Innate and Acquired Immunity." Some of the RISP lecturers also spoke at this venue, but on new topics and there were also many new speakers, making for an exciting two day meeting. Most of the participants returned home after the close of the meeting, but six participants remained at RCAI for a month-long research internship.

The RISP lectures at RCAI were quite diverse. Topics included lymphocyte development and differentiation, the function of microRNAs in these processes, transcriptional regulatory networks, immunoreceptor signaling, mucosal immunity, and the inflamasome. Because of their breadth, these lectures provided an expansive overview of the immune system, with experimental approaches ranging from sophisticated imaging in cancer and in the germinal center reaction, to whole animal and "humanized" mouse analyses.

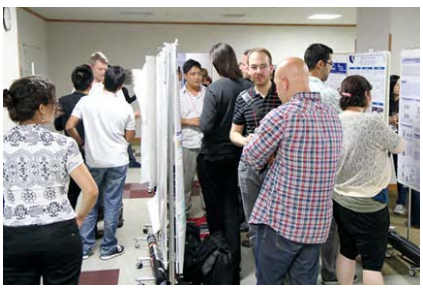
The invited speakers incorporated introductory material as well as recent highlights from their own research into their talks. The research interests of the participants were similarly varied, making for a unique opportunity for cross-fertilization among immunology subdisciplines during the oral and poster presentations and in more informal settings. The question periods following talks by the invited lecturers as well as the participants were spirited and stimulating. Awards for best RISP posters were presented at a farewell party held at the close of the RCAI-JSI meeting. All of the participants expressed their appreciation to the organizers for inviting them to RISP, and some of them established collaborations as a result of the RISP that are likely to be long-lasting.

The RISP 2012 participants had a full schedule, but had some opportunities to explore the Yokohama/Tokyo area and many of them also visited nearby Kamakura. RISP provided an exceptional experience from both scientific and cultural perspectives. In a survey completed after the school, the participants unanimously agreed that they would recommend the program to colleagues. Half indicated that they would consider a postdoctoral position at RIKEN and nearly all would consider returning to RCAI for a short-term period of collaborative research. The success of this unique program was due to the efforts of the Organizing Committee, chaired by Dr. Kurosaki, and the RISP Secretariat, Mr. Fukushima, Mss. Haraguchi, Iyama, Nomura and Yoshioka, who kept the entire operation running smoothly, as well as to the efforts of the outstanding participants. Planning is already underway for RISP 2013.

Table: :Lectures

Toshinori Nakayama, Chiba University, Japan	Protective and pathogenic function of memory Th2 cells
Ichiro Taniuchi, RIKEN RCAI, Japan	Transcriptional regulation of thymocyte development
Hilde Cheroutre, La Jolla Institute for Allergy & Immunology, USA and RIKEN RCAI, Japan	Mucosal T cells: Shaped by the challenge
Rudolf Grosschedl, Max Planck Institute of Immunobiology and Epigenetics, Germany	Transcription networks regulating B lymphopoiesis
Thomas Boehm, Max Planck Institute of Immunobiology and Epigenetics, Germany	Design principles of adaptive immune systems
Gary A. Koretzky, University of Pennsylvania, School of Medicine, USA	Integration of immunoreceptor signaling pathways by adaptor proteins
Hisataka Kobayashi, National Cancer Institute, NIH, USA	Molecular cancer imaging; New optical diagnostic technologies and beyond
Takaharu Okada, RIKEN RCAI, Japan	Imaging of lymphocyte dynamics during the germinal center formation
Axel Kallies, Walter and Eliza Hall Institute of Medical Research, Australia	Transcriptional control of conventional and regulatory effector T cell differentiation
K. Mark Ansel, University of California San Francisco, USA	MicroRNA regulation in helper T cell differentiation
Matthias von Herrath, La Jolla Institute for Allergy and Immunology, USA	Peripheral insulin recognition determines pathogenic versus Foxp3^{+} regulatory CD4 T cell formation and susceptibility to type 1 diabetes
Shigeo Koyasu, Keio University School of Medicine and RIKEN RCAI, Japan	Role of natural helper cells in helminth infection
Kiyoshi Takeda, Osaka University, Graduate School of Medicine, Japan	Regulation of gut homeostasis by innate immunity
Jenny Ting, University of North Carolina at Chapel Hill, USA	NLRs: Basic science and disease relevance
Fumihiko Ishikawa, RIKEN RCAI, Japan	Humanized mouse: a research tool to investigate normal & diseased human immunity





7th RCAI-JSI International Symposium on Immunology

New Horizon in Immune Regulation –Bridging innate and acquired immunity–

The seventh RCAI-JSI International Symposium on Immunology was held on Jun 28-29, 2012, at Pacifico Yokohama Conference Center. Three hundred and ninety people, including 57 from abroad, gathered to learn about the most advanced topics in immunology and to discuss their results.

This year's symposium focused on immune regulation bridging innate and acquired immunity. Twenty one invited researchers introduced their cutting edge research. The first session 'Novel innate cell subsets' started with a hot discussion on the recently discovered 'natural helper (NH) cells' and 'nuocytes', which play critical roles in Th2 immune responses in innate immunity. Shigeo Koyasu (Photo 1) introduced GATA-3 as a master regulator for differentiation of NH cells, and then Andrew McKenzie (Photo 2) explained that transcription factor RORα plays an important role in nuocyte development. In the 'Innate cell function' session, David Mosser (Photo 3) explained the characteristics of so-called 'regulatory macrophages' that produce the anti-inflammatory cytokine IL-10. In the 'Recognition by innate sensor' session, Tadatsugu Taniguchi (Photo 4) beautifully summarized 'classical' secreted cytokines and 'new' cytokines that are present in distinct cell compartments. Alexander Rudensky (Photo 5) discussed the biological functions of thymus Tregs (tTregs) and peripheral Tregs (pTregs) in the final session 'Lymphocyte subsets and effecter function'.

The RCAI-JSI International Symposium on Immunology is a series of annual conferences started in 2005 and hosted by RIKEN RCAI in conjunction with the Japanese Society for Immunology (JSI). The symposium provides a forum for active discussions on the cutting edge of immunological research. The steering committee is composed of representatives of RCAI and JSI, and organizes the program with advice from an international advisory committee. The RCAI-JSI international symposium is complementary to JSI International Symposia that are held during its annual meeting. These meetings promote the progress of immunology and encourage young researchers not only in Japan but also in Asia and all over the world.

Direct interaction among people is one of the most effective ways to spread knowledge and inspire researchers to explore new directions. In this rapidly advancing era of science, the symposium connected researchers and provided an opportunity to consider both newly solved and yet unsolved problems in immunological science.

Table: Program

Session I: Novel innate cell subsets

Shigeo Koyasu, Keio University / RIKEN RCAI, Japan	Critical role of GATA3 in natural helper cell differentiation and function
Andrew N.J. McKenzie, MRC Laboratory of Molecular Biology, UK	RORα and Notch play important roles in type-2 ILC development
Dmitry I. Gabrilovich, H Lee Moffitt Cancer Center & Research Institute, USA	Myeloid-derived suppressor cells in regulation of immune responses

Session II: Innate cell function

Foo Y. Liew, University of Glasgow, UK	The role of Interleukin-33 in infection and inflammation
David M. Mosser, University of Maryland, USA	Macrophage heterogeneity and inflammatory diseases
Joseph C. Sun, Memorial Sloan-Kettering Cancer Center, USA	The NK cell response against viral infection: Crossing the boundaries of innate immunity
James P. Di Santo, Institut Pasteur, France	IL-22-producing innate lymphoid cells (ILC22): Regulators of immune defense and tissue homeostasis
Mitchell Kronenberg, La Jolla Institute for Allergy & Immunology, USA	Recognition and responses to microbial antigens by NKT cells

Session III: Recognition by innate sensor

Jenny P.Y. Ting, The University of North Carolina, USA	NLRs in infection, inflammation and cancer
Eicke Latz, University of Bonn, Germany / University of Massachusetts, USA	Inflammasome activation in chronic inflammatory diseases
Sho Yamasaki, Kyushu University, Japan	Immune responses through C-type lectin receptors
Tadatsugu Taniguchi, The University of Tokyo, Japan	Regulation of innate and adaptive immune responses by old and new cytokines

Session IV: Interface between innate and acquired immunity

William R. Heath, The University of Melbourne, Australia	Immunity to viral infection of the skin
Yong-Jun Liu, Baylor Research Institute, USA	Biochemical characterization of nucleic acid sensors in dendritic cells
Hiroshi Ohno, RIKEN RCAI, Japan	Function and differentiation of M cells, a unique subset of intestinal epithelial cells specialized for mucosal antigen-uptake
Shin-ichiro Fujii, RIKEN RCAI, Japan	NKT cell-mediated licensing of dendritic cells (DCs) *in vivo*
Ken J. Ishii, National Institute of Biomedical Innovation, Japan	Making immune sense of nucleic acids in inflammation and vaccination

Session V: Lymphocyte subsets and effecter function

Alexander Y. Rudensky, Memorial Sloan-Kettering Cancer Center, USA	Biological roles of extrathymic differentiation of regulatory T cells
Claudia Mauri, University College London, UK	Regulatory B cells maintain regulatory T cells whilst inhibiting Th17 and Th1 differentiation.
Masato Kubo, Tokyo University of Science / RIKEN RCAI, Japan	Regulation of cytokine expression in follicular helper T cell and humoral immunity
David Artis, University of Pennsylvania, USA	Mechanisms of immune regulation at barrier surfaces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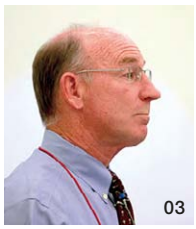
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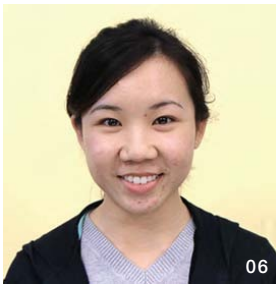
RIKEN Joint Graduate School Program International Program Associate

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

The IPA students who studied at RCAI in 2012 were :



Sebastian Nieke
(Tübingen Univ., Germany) studied in the Laboratory for Immune Transcription (Photo 1)

Li Shuyin
(China Agriculture University, China) studied in the Laboratory for Immune Diversity (Photo 2)

Yue Ren
(Jilin University, China) studied in the Laboratory for Immune Regulation (Photo 3)

Mohamed El Sherif Gadelhaq Gadelhaq Badr
(Tokyo Medical and Dental University) from Edypt studied in the Laboratory for Cell Signaling (Photo 4)

Zhang Yanfei
(Peking University, China) studied in the Laboratory for Immune Diversity (Photo 5)

Joo Ann Ewe
(Universiti Sains Malaysia, Malaysia) studied in the Laboratory for Epithelial Immunobiology (Photo 6)

Huey Shi Lye
(Universiti Sains Malaysia, Malaysia) studied in the Laboratory for Epithelial Immunobiology (Photo 7)

RIKEN Special Postdoctoral Researcher (SPDR) Program

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

This year, 5 postdocs conducted their research at RCAI through the SPDR program.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Hirokazu Tanaka (Laboratory for Transcriptional Regulation) Photo 1
Yuki Horisawa-Takada (Laboratory for Developmental Genetics) Photo 2
Shimpei Kawamoto (Laboratory for Mucosal Immunity) Photo 3
Saya Moriyama (Research Unit for Immunodynamics) Photo 4
Shinsuke Ito (Laboratory for Developmental Genetics) Photo 5

RIKEN Foreign Postdoctoral Researcher

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

In 2012, Jafar Sharif (Photo) studied in the Laboratory for Developmental Genetics as a RIKEN FPR.

RIKEN Junior Research Associate (JRA) Program

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

This year, 16 JRA students studied in RCAI.

Norihiko Inoue (Lab. for Cellular Systems Modeling) Photo 1
Chie Kano (Lab. for Immune Diversity) Photo 2
Nanako Shimura (Lab. for Immunogenomics) Photo 3
Misao Hanazato (Lab. for Epithelial Immunobiology) Photo 4
Yuki Obata (Lab. for Epithelial Immunobiology) Photo 5
Akemi Fujiwara (Lab. for Epithelial Immunobiology) Photo 6
Yuuhou Najima (Lab. for Human Disease Model) Photo 7
Yuki Aoki (Lab. for Human Disease Model) Photo 8
Shinsuke Takagi (Lab. for Human Disease Model) Photo 9
Masanaka Sugiyama (Research Unit for Inflammatory Regulation) Photo 10
Rikiya Ishikawa (Lab. for Infectious Immunity) Photo 11
Yusuke Sato (Research Unit for Cellular Immunotherapy) Photo 12
Hisashi Wada (Lab. for Transcriptional Regulation) Photo 13
Takashi Ikeno (Research Unit for Immunodynamics) Photo 14
Satoshi Koga (Lab. for Immune Cell System) Photo 15
Ryuichi Murakami (Research Unit for Immune Homeostasis) Photo 16

Adjunct Professorship Programs

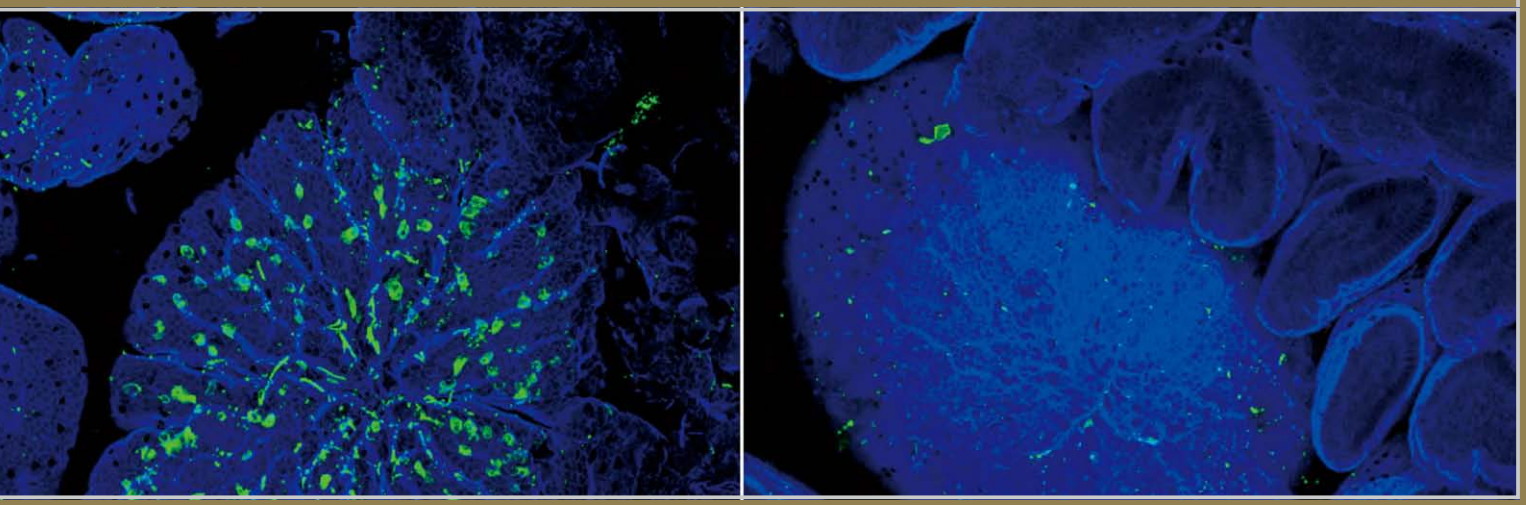
RCAI accepts graduate students through the mechanism of adjunct professorships at various Japanese universities. RCAI collaborates with and accepts graduate students from 10 domestic university graduate schools. There are now a total of 23 adjunct professors/associate professors in RCAI. Fifty-three students studied at RCAI in 2012.

Table: Adjunct professorship programs

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

2012
Part 4

Collaborative Networks



Open Laboratory at RCAI

RCAI's Open Laboratory was established in 2009 to provide a framework for researchers from external institutes, universities or hospitals that would allow them access to the Center's resources and the opportunity for collaborative research projects.
In 2011, two researchers, Drs. Masato Kubo (Open Laboratory for Signal Network) and Koji Hase (Open Laboratory for Bioenvironmental Epigenetics), had their own space and staff in RCAI and have joined various internal research meetings and discussions to promote extensive research communication with RCAI investigators.

Open Laboratory for
Signal Network

Leader:
Masato Kubo
(Research Institute for Biomedical Science, Tokyo University of Science)

Research Scientist: **Kosuke Miyauchi**
Visiting Scientist: **Yasutaka Motomura**
Technical staff: **Yoshie Suzuki**

T cells play a central role in the effector and regulatory functions of immunological surveillance and aberrations in these functions can lead to various immunological disorders. The different subsets of helper T cell secrete distinct cytokines that define their role in controlling the outcome of immunological surveillance. Type 1 helper 1 (T_H1) cells secrete IFN-γ and TNF-α during the cellular immune response to intracellular pathogens. T_H2 cells produce interleukin-4 (IL-4), IL-5, IL-6, IL-10 and IL-13, which account for protective against extracellular pathogens and allergic immune responses. T_H17 cells produce IL-17, which is associated with autoimmune responses. All the helper T cell subsets differentiate from a common precursor cell, the naive T cell, and these differentiation programs occur in milieus of certain types of cytokines. IL-4 was originally identified as a T_H2 cytokine responsible for class switch recombination to IgG1 and IgE, and recent studies suggest that follicular helper T cells (T_{FH}) cells may be an alternative IL-4 source to regulate humoral immune responses. However, the regulation of cytokine gene expression in T_{FH} cells remains a mystery because of the limited availability of *in vivo* tracking systems to follow cell specific cytokine expression. The overriding goal of our laboratory is to understand the molecular mechanisms underlying cytokine gene expression in helper T cell subsets and innate type immune cells.

The 3' enhancer CNS2 is a crucial regulator in follicular helper T cells of interleukin-4 mediated humoral immunity

B cells are the antibody factories that selectively target foreign threats as a component of the humoral immune response. This process requires T cells to secrete IL-4, which promotes a mechanism called 'class switching' that enables production of functionally specialized antibody subtypes. However, it remains unclear exactly which T cells generate the signal required for the class switching. Nevertheless, most textbooks say that T_H2 cells control the antibody response. We provided strong evidence that a recently-discovered class of T_{FH} cells generates the IL-4 signal that facilitates B cell help in the antibody responses. T_{FH} are a specialized T cell subset localizing in germinal centers (GCs) and there secreting cytokines, IL-4 and IL-21. We demonstrated that conserved noncoding sequence 2 (CNS2) is an essential enhancer element for IL-4 expression in T_{FH} cells but not in T_H2 cells. Mice with a CNS2 deletion had a reduction in IgG1 and IgE production and in IL-4 expression by T_{FH} cells. Tracking of CNS2 activity using a GFP reporter mouse demonstrated that CNS2-active cells expressed several markers of T_{FH} cells: CXCR5, PD-1, and ICOS; the transcriptional master regulator Bcl6; the cytokines IL-21 and IL-4, and were mainly localized in B cell follicles and germinal centers. The GFP^+ T_{FH} cells were derived from GFP^- naïve T cells after *in vivo* system-

ic immunization. These results indicate that CNS2 is an essential enhancer element required for IL-4 expression in T_{FH} cells that control humoral immunity. Moreover, they have proven that IL-4 is a critical cytokine for controlling IgG1 and IgE antibody responses, although its expression in T_H2 and T_{FH} cells is independently regulated by distinct regulatory elements. Furthermore, T_{FH} and not T_H2 cells are the T cell subset responsible for T_H2-type humoral immune responses.

Dysregulation of suppressor of cytokine signaling 3 (SOCS3) in keratinocytes caused skin inflammation induced by pathogenic IL-20 receptor-related cytokines

Homeostatic regulation of epidermal keratinocytes is controlled by the local cytokine milieu. However, a role of a negative feedback regulator to control the cytokine networks, suppressor of cytokine signaling (SOCS), remains unclear in skin homeostasis. Keratinocyte specific deletion of *Socs3* (*Socs3* cKO) resulted in severe skin inflammation with IgE hyper-production, epidermal hyperplasia, and S100A8/9 expression, although *Socs1* deletion caused no such inflammation. The inflamed skin showed constitutive STAT3 activation and up-regulation of IL-6 and IL-20 receptor I (IL-20RI) related cytokines, IL-19 and IL-24. Disease development was rescued by deletion of the *Il6* gene, but not by deletion of the *Il23*, *Il4r*, or *Rag1* genes. The expression of IL-6 in *Socs3* cKO keratinocytes increased IL-19 and IL-24 expression, which further facilitated STAT3 hyperactivation, epidermal hyperplasia and neutrophilia. These results demonstrate that skin homeostasis is strictly regulated by the IL-6-STAT3-SOCS3 axis. Moreover, the SOCS3 mediated negative feedback loop in keratinocyte has a critical mechanistic role in the prevention of skin inflammation caused by hyperactivation of STAT3.

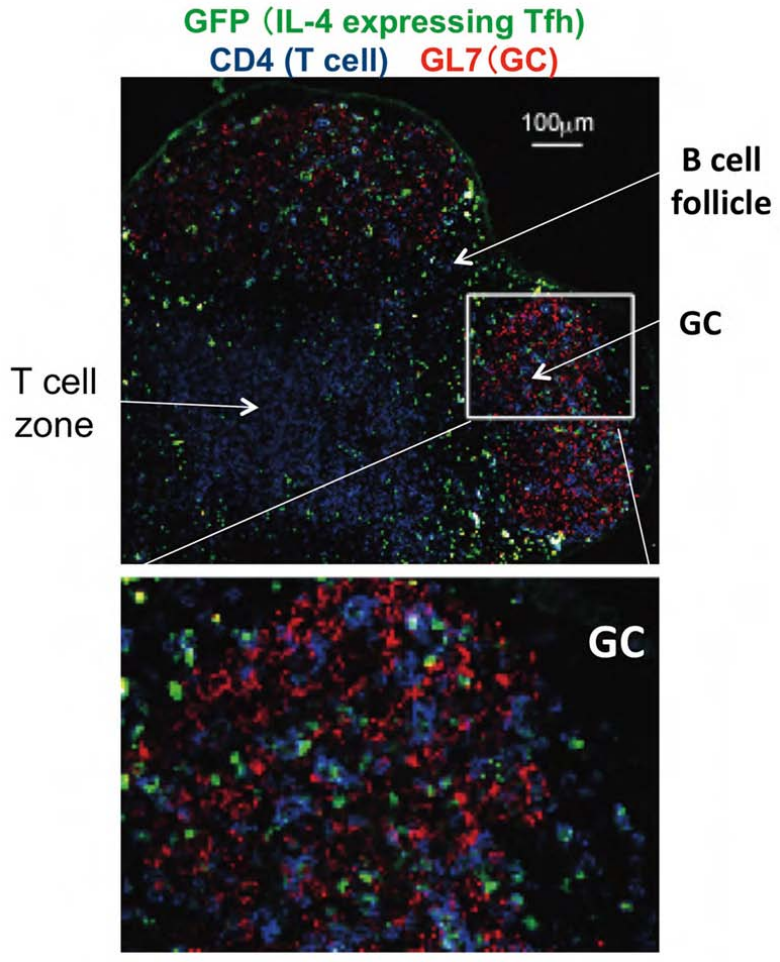


Figure: **Localization of IL-4 expressing T_{FH} cells in the Peyer's patch.** *Green* indicates GFP, a marker for IL-4 expression, *blue* indicates CD4 T cells, and *red* indicates GL-7 expressing GC-B cells.

Recent Publications

1. Harada, Y., Tanaka, S., Motomura, Y., Harada, Y., Ohno, S., Ohno, S., Yanagi, Y., Inoue, H., and Kubo, M. The 3 Enhancer CNS2 is a Critical Regulator of Interleukin-4-Mediated Humoral Immunity in Follicular Helper T Cells. ***Immunity*** 36, 88-200 (2012)

2. Sawaguchi, M., Tanaka, S., Nakatani, Y., Harada, Y., Mukai, K., Matsunaga, Y., Ishiwata, K., Oboki, K., Kambayashi, T., Watanabe, N., Karasuyama, H., Nakae, S., Inoue, H., and Kubo, M. Role of mast cells and basophils in IgE responses and in allergic airway hyperresponsiveness. ***J Immunol.*** 188, 1809-1818 (2012)

3. Hill, D.A., Siracusa, M.C., Abt, M.C., Kim, B.S., Kobuley, D., Kubo, M., Kambayashi, T., Larosa, D.F., Renner, E.D., Orange, J.S., Bushman, F.D., and Artis D. : Commensal bacterial–derived signals regulate basophil hematopoiesis and allergic inflammation. ***Nat Med.*** 18, 538-546 (2012)

4. Kurashima, Y., Amiya, T., Nochi, T., Fujisawa, K., Haraguchi, T., Iba, H., Tsutsui, H., Sato, S., Nakajima, S., Iijima, H., Kubo, M., Kunisawa, J., and Kiyono, H. : Extracellular ATP mediates mast cell–dependent intestinal inflammation through P2X7 purinoceptors. ***Nat Comm***. 3, 1034 (2012)

5. Uto-Konomi, A., Miyauchi, K., Ozaki, N., Motomura, Y., Suzuki, Y., Yoshimura, A., Suzuki, S., Cua, D., and Kubo, M. Dysregulation of suppressor of cytokine signaling 3 in keratinocytes causes skin inflammation mediated by interleukin-20 receptor-related cytokines. ***PLoS One*** 7, e40343 (2012)

Open Laboratory for

Bioenvironmental Epigenetics

Leader:
Koji Hase

Visiting Scientist: **Yukihiro Furusawa**
Technical Staff: **Yumiko Fujimura**
Student Trainee: **Yuuki Obata**

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

Humans harbor over 100 trillion bacteria in the distal intestine. These commensal bacteria have long been appreciated for the benefits they provide to the host, including their capacity to metabolize otherwise indigestible food ingredients to small metabolites that can be utilized as nutrients by host cells. Moreover, it seems that the presence of commensal bacteria eventually contributes to shape the gut immune system though promoting the development of gut-associated lymphoid tissues, the largest of the secondary lymphoid organs, which are necessary for induction of mucosal IgA responses. For instance, colonization of germ-free mice with gut-indigenous Clostridia restored colonic regulatory T cells (Treg), which play a pivotal role in host tolerance to commensal bacteria, to normal numbers. In further support of this concept, the development of Treg cells as well as IgA production are defective in germ-free mice. These previous observations led to the notion that host-microbe interactions establish immunological homeostasis in the gut, which further raises the important question of how commensal bacteria affect the host immune system.

Microbial factor (s) responsible for development of the gut immune system

The enteric microflora serve as a potent bioreactor that controls several metabolic functions. The main functions include the fermentation of indigestible food substances such as dietary fibers and resistant starch into simple sugars, absorbable nutrients, and short-chain fatty acids (SCFAs). Notably, some of the SCFAs have been reported to exert histone deacetylase (HDAC) inhibitory activity, at least *in vitro*. We recently found that intestinal microbiota-derived SCFAs may secure mucosal immune homeostasis via epigenetic mechanisms (see Fig). A high level (approximately 100 mM) of SCFAs is constitutively produced by microbial fermentation in the distal intestine of mammals. Given that down-regulation of a SCFA transporter in the colonic mucosa has been implicated in the pathogenesis of inflammatory bowel disease (IBD), SCFAs may play a non-redundant role in the maintenance of gut immune homeostasis.

Host factors responsible for maintenance of gut immune homeostasis

To determine the host molecule(s) responsive to colonization by commensal bacteria, we performed microarray-based transcriptome analysis before and after colonization of germ-free mice with commensal bacteria. We

observed that this colonization upregulated the expression levels of an adaptor protein for DNA methyl transferase (Dnap) in colonic $CD4^+$ T cells. Dnap forms a molecular complex with Dnmt and HDAC for epigenetic regulation of transcription, suggesting that colonization by the microbiota may influence the epigenetic status of intestinal $CD4^+$ T cells. To examine this possibility, we developed mice with a T-cell specific deletion of Dnap using a CD4-driven Cre/loxP system. The Dnap-deficient mice displayed a defect in colonic Treg expansion in response to bacterial colonization, and spontaneously developed severe colitis characterized by activation of T_H1 and T_H17 cells. Genome-wide gene expression profiling followed by molecular network analysis revealed that cell cycle-dependent kinase inhibitors (Cdkns) were derepressed in Dnap-deficient T cells. This change was associated with hypomethylation of the promoter region of these genes as evidenced by MeDP-Sequencing analysis. Thus, Dnap-dependent silencing of Cdkns is required for vigorous expansion of colonic Treg cells. This mechanism seems to be essential for the maintenance of gut immune homeostasis.

Recent Publications

1. Kanaya, T., Hase, K., Takahashi, D., Fukuda, S., Hoshino, K., Sasaki, I., Hemmi, H., Knoop, KA., Kumar, N., Sato, M., Katsuno, T., Yokosuka, O., Toyooka, K., Nakai, K., Sakamoto, A., Kitahara, Y., Jinnohara, T., McSorley, SJ., Kaisho, T., Williams, IR. and Ohno, H., The Ets Transcription factor Spi-B is essential for the differentiation of intestinal microfold (M) Cells. ***Nat Immunol.*** 13, 729-736 (2012)

2. Fukuda, S., Toh, H., Hase, K., Oshima, K., Nakanishi, Y., Yoshimura, K., Tobe, T., Clarke, JM., Topping, DM., Suzuki, T., Taylor, TD., Itoh, K., Kikuchi, J., Morita, H., Hattori, M., Ohno, H. Bifidobacteria protect host from enteropathogenic infection through production of acetate. ***Nature*** 469, 543-547 (2011)

3. Takahashi, D., Hase, K., Kimura, S., Nakatsu, F., Ohmae, M., Mandai, Y., Sato, T., Date, Y., Ebisawa, M., Kato, T., Obata, Y., Fukuda, S., Kawamura, YI., Dohi, T., Katsuno, T., Yokosuka, O., Waguri, S. and Ohno, H. The Epithelia-specific membrane trafficking factor AP-1B controls gut immune homeostasis in mice. ***Gastroenterology*** 141, 621-632 (2011)

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

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

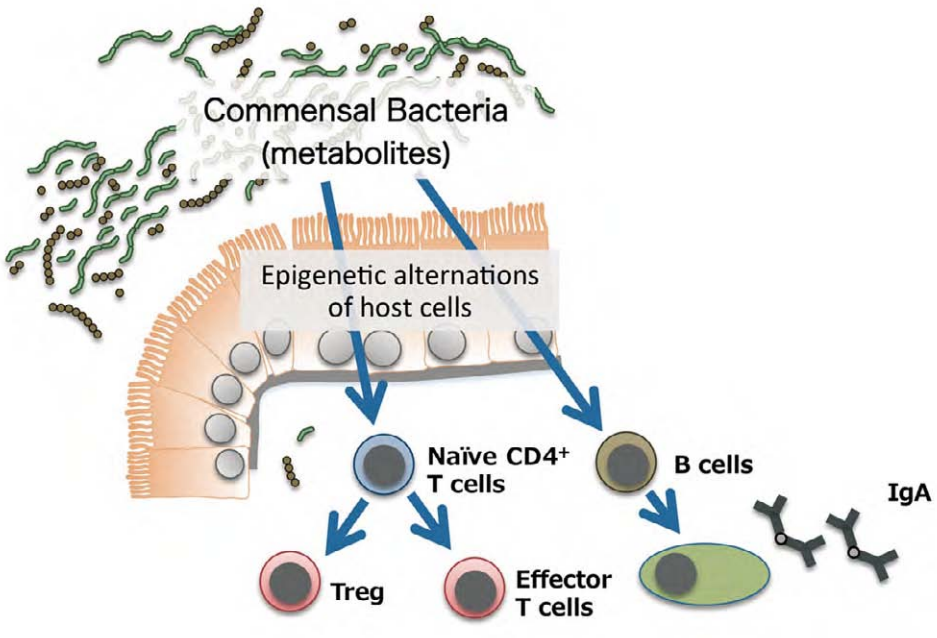
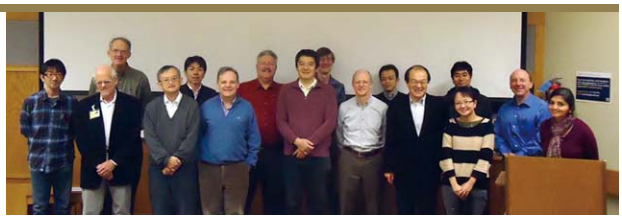


Figure : **Schematic diagram of epigenetic regulation of intestinal immunity by commensal bacteria**
Intestinal commensal bacteria actively produce small molecule metabolites. These metabolites may directly or indirectly influence cell differentiation and function of immunocompetent cells, such as T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

The 2nd RIKEN RCAI-University of Michigan Medical Center Joint Workshop



The second RIKEN RCAI-University of Michigan Medical Center Joint Workshop was held on January 16 and 17, 2013 at the medical school of the University of Michigan in Ann Arbor. This joint workshop began in 2011 to launch collaborative relationships between RCAI and the Univ. of Michigan. This year, five researchers from RCAI visited Ann Arbor to attend the joint workshop.

In the first session, "Inflammation and Pulmonary Diseases", Steven Kunkel talked about the epigenetic regulation of gene expression in $CD4^+$ T cells and Nicholas W. Lukacs, demonstrated that an epigenetic demethylase enzyme plays an important role in Respiratory Syncytial Virus infection of dendritic cells in chronic pulmonary disease.

In the second session, "Stem Cell Development and Bone Marrow Transplantation", Bethany Moore talked about the mechanisms by which alveolar macrophage (AM) function is inhibited after bone marrow transplantation and James Ferrara talked about the search for biomarkers of acute graft versus host disease (GVHD) using proteomics approaches. Tomokatsu Ikawa then described how to expand hematopoietic progenitors by simply overexpressing Id3. He also discussed their model of transcriptional networks in T cell lineage specification using their novel culture system combined with Cap Analysis of Gene Expression (CAGE).

In the third session, "Antigen-dependent B Cell Differentiation", two speakers introduced their results using two-photon imaging. Takaharu Okada showed how follicular helper T cells move into lymph nodes and get activated during the adaptive immune response and Irina Grigorova established the method for iterative application of intravital imaging and quantitative modeling approaches. Tomohiro Kurosaki showed that class-switched memory B cells and memory follicular helper T cells are the major cells responsible for rapid antibody responses and proposed the underlying mechanisms. Marilia Cascalho described the detailed characterization of TACI-deficient mice. In the fourth session, "Autoimmunity", Mariana Kaplan explained the process of neutrophil extracellular trap (NET) formation. The programmed release of chromatin fibers is thought to stimulate plasmacytoid DC to produce INFα in SLE. She described other roles of NETs in autoimmune responses, such as their direct role in vascular damage. David Markovitz discussed the oncoprotein DEK, one of the few known autoantigens associated with juvenile idiopathic arthritis (JIA), and he described the connection between DEK and NET formation. Massimo Pietropaolo described islet cell autoantigen 69 (ICA69), a protein that is predominantly expressed by pancreatic islets and neuroendocrine organs. He showed that deletion of ICA69 specifically in Aire-expressing thymus stromal cells results in spontaneous multi-organ autoimmune disorders.

In the fifth session, "Inflammation and Innate Immunity", Weiping Zou explained that human tumor infiltrating Th17 cells are polyfunctional and express multiple cytokines including IFNγ, TNFα, and GM-CSF, and that Th17 cells and Th1 cells in human autoimmune lesions coexist and work together for disease progression. Marc Peters-Golden presented a series of studies showing that two eicosanoids, LTB4 and PGE2, have activating and suppressive functions for macrophages, respectively. Yuumi Nakamura showed that a mutation in the NLRP3 inflamasome, which was known to cause a skin disease in humans, caused dysregulated expression of IL-1β by mast cells. In the sixth session, "Immune Cell Signaling and Activation", Takashi Saito described how T cell activation is dynamically and spatially differentially regulated at TCR-microclusters (TCR-MCs) and the central-supramolecular activation cluster (c-SMAC). Joel Swanson described that in murine macrophages stimulated with M-CSF, actin-rich surface ruffles transform into 1-5 mm diameter circular ruffles, or macropinocytic cups, which close at their distal margins, thereby creating endocytic vesicles called macropinosomes.

In the last session, "Genes In and Out of the Immune System", Haruhiko Koseki talked about how genes silenced by Polycomb group (PcG) proteins are reactivated during the developmental process. He identified a tissue-specific enhancer in the promoter region of the *Meis2* gene locus and showed how the enhancer, the promoter and the Polycomb binding site dynamically interact with each other to reactivate the *Meis2* gene during midbrain development. The last speaker, James Douglas Engel talked about the function of GATA3 in the T cell development. He generated GFP reporter mice whose expression is controlled by a presumptive GATA3 enhancer and showed that the enhancer specifically functions in T cells.

After the presentations, RCAI researchers had one-on-one discussions with several researchers from the Univ. of Michigan with common interests. During the workshop, researchers agreed to continue the collaborative relationships and stimulate some new personal collaborations.

2nd New Zealand - Japan Joint Immunology Workshop

On 26th and 27th February 2013, the New Zealand-Japan Joint Immunology Workshop was held at the University of Auckland campus. Eight Japanese researchers, Drs. Shin-ichiro Fujii, Shohei Hori, Takaharu Okada, Yasuyuki Ishii and Takashi Saito from RCAI and Drs. Toshinori Nakayama, Koichi Hirose and Takeshi Tokuhisa from Chiba University and thirteen researchers from New Zealand presented talks. In addition to the New Zealand delegation members who participated in the first meeting held at RCAI in 2010, sixty researchers and graduate students from the University of Auckland, University of Wellington and the University of Otago participated in this 2nd meeting.

The meeting was held at "Fale Pasifika", one of the more iconic buildings on campus. For most Pacific communities, Fale is more than just a physical structure, but it is their point of reference for activities that are central to their cultural expression and it gives them a sense of place and community. Equally, the University's Fale Pasifika is a symbol of identity for Pacific Island students and staff, and is the second largest such structure in the world. It was a sign of the warmest hospitality by the organizing committee to hold the meeting there.

In his opening remarks, Prof. Rod Dunbar, the University of Auckland said, "We were extremely pleased with the way in which the presentations at the workshop highlighted both the collaborative projects that have grown since 2010, when the New Zealand delegation visited Japan, and areas where more collaborations could be initiated. We look forward to supporting RCAI's continued collaborations. In case you are interested in contacting any of the other New Zealand workshop speakers to discuss a potential collaborative project, please don't hesitate to contact us if you would like some assistance with this."

Indeed, two collaborations had already started after the 1st workshop. One is to combine two-photon imaging data of Dr. Takaharu Okada from RIKEN RCAI with a novel computational immunology technology developed by Dr. Gib Bogle, the University of Auckland. It is quite a challenging and innovative program, and may open a new research paradigm. The other collaboration is a practical program of Dr. Yasuyuki Ishii from RIKEN RCAI with Prof. Sarah Hook, the University of Otago to establish new oral vaccines for allergic diseases. Ishii develops a liposomal allergy vaccine incorporating α-galactosylceramide, a representative ligand for invariant natural killer T (*i*NKT) cells, and Prof. Hook has a long research career and world-class technologies in oral administration of drugs. Drs. Ishii and Hook are collaborating to develop new technologies for oral drug administration, which will be applicable to asthma, atopic dermatitis, pollinosis, and food allergy. These collaborations are still ongoing.

During the workshop, there was the impression that immunologists in New Zealand communicate well with each other and were very interested in the progress of science and technologies in Japan, and there would be more possibilities for research collaborations between Japan and New Zealand. The workshop was successfully completed and all speakers were invited to the Japanese Consulate in Auckland. In the closing remarks, Prof. Tokuhisa said, "Hopefully, relationships between New Zealand and Japan will be spread much more than now. This joint Immunologist workshop is one of successful friendship and will work as a trigger for bridging."

Program

Day 1 – Tuesday 26th February 2013

Session 1: Allergy, Autoimmunity & Tolerance		
Rod Dunbar	School of Biological Sciences, Faculty of Science, The University of Auckland	Opening Remarks
Toshinori Nakayama	Department of Immunology, Graduate School of Medicine, Chiba University	CS4+ T cell memory and airway inflammation
Graham Le Gros	Malaghan Institute for Medical Research	Th2 and innate cell differentiation in allergic and parasitic disease
Koichi Hirose	Department of Allergy and Clinical Immunology, Graduate School of Medicine, Chiba University	Beta-glucan curdlan induces IL-10 producing CD4+ T cells and inhibits allergic airway inflammation
Session 2: Allergy, Autoimmunity & Tolerance		
Takeshi Tokuhisa	Department of Developmental Genetics, Graduate School of Medicine, Chiba University	Development of high affinity IgE B cells
Anne La Flamme	School of Biological Sciences, Faculty of Science, Victoria University of Wellington	Targeting innate immunity during multiple sclerosis
Yasuyuki Ishii	Laboratory for Vaccine Design, RCAI	Research for oral allergy vaccines inducing immune tolerance
Elizabeth Forbes-Blom	Malaghan Institute for Medical Research	Understanding the cellular mechanisms underlying food allergen sensitisation
Session 3: Immunotherapy		
Shinichiro Fujii	Research Unit for Cellular Immunotherapy, RCAI	iNKT cell-triggered *in vivo* DC targeting immunotherapy
Ian Hermans	Malaghan Institute of Medical Research	iNKT cells in DC-based immunotherapy
Shinichiro Motohashi	Department of Immunology, Graduate School of Medicine, Chiba University	Invariant NKT cell targeted immunotherapy for lung cancer
Gavin Painter	Callaghan Innovation	Synthetic Vaccines for Cancer Immunotherapy with NKT cells
Session 4: Immunotherapy		
Rod Dunbar	School of Biological Sciences, Faculty of Science, The University of Auckland	Targeting human APCs with synthetic peptides
Sarah Hook	School of Pharmacy, University of Otago	Development of immunogenic vaccine formulations
Sarah Young	Department of Pathology, Dunedin School of Medicine, University of Otago	The use of Virus-like particles as an immune therapy for cancer

Day 2 – Wednesday 27th February 2013

Session 5: Immune regulation & immunodynamics		
Takashi Saito	Laboratory for Cell Signaling, RCAI	Dynamic imaging analysis of T cell activation regulation
Franca Ronchese	Malaghan Institute of Medical Research	Regulating dendritic cell function
Shohei Hori	Research Unit for Immune Homeostasis, RCAI	Genetic and environmental control of regulatory T cell fitness in peripheral tissues
Session 6: Immune regulation & immunodynamics		
Takaharu Okada	Research Unit for Immunodynamics, RCAI	2-photon imaging of adaptive immune responses
Gib Bogle	Auckland Bioengineering Institute, The University of Auckland	Computational immunology: helping to elucidate lymph node processes and structure
Roslyn Kemp	Department of Microbiology and Immunology, Otago School of Medical Sciences, University of Otago	Immune Diversity in the Gut Mucosa
Session 7: Infectious immunology		
John Fraser	Faculty of Medical and Health Sciences, The University of Auckland	Microbial pathogenicity
Joanna Kirman	Department of Microbiology and Immunology, Otago School of Medical Sciences, University of Otago	The generation and maintenance of CD4+ Th1 memory
Christopher Hall	Department of Molecular Medicine and Pathology, The University of Auckland	Immunometabolism: irg1 and mitochondrial ROS production

Harvard Summer School at RCAI

Jun 11 - Aug 17, 2012

RCAI offers an undergraduate student internship program in collaboration with Harvard University's Summer School. The participants earn university credits in the Harvard Summer School Program while at RCAI. In 2011, RCAI had to cancel the program because of the aftermath of the Great East Japan Earthquake, but RCAI began accepting students again this summer. Three students, Mss. Malisa Smith, Ning Hou and Natasha M Furtado Dalomba conducted research projects in the Lab. for Immunogenomics, the Lab. for Transcriptional Regulation and the Lab. for Epithelial Immunobiology, respectively. They also participated in a series of basic immunology lectures at RCAI, the RCAI International Summer Program, and the RCAI-JSI International Symposium on Immunology, and had basic Japanese classes. Besides these studies, they enjoyed a cultural exchange with students of Yokohama Science Frontier High School (Photo), and an excursion to Kamakura.

Photo: Japanese culture visit to Yokohama Science Frontier High School (*left*) and Basic Immunology Lecture at RCAI (*right*)

RCAI-ZIBI student exchange program

RCAI International Summer School and the ZIBI Summer School in Berlin launched a student exchange program in 2011. In 2012, two Ph.D. course students from RCAI, Hisashi Wada in the Laboratory for Transcriptional Regulation and Misao Hanazato in the Laboratory for Epithelial Immunobiology participated in ZIBI summer school held on Jun 9-24, 2012, and two students from ZIBI, Kerstin Westendorf from German Rheumatism Research Center and Stefan Riebel, from Max Planck Institute for Infection Biology attended the RCAI International Summer Program.

ZIBI (Interdisciplinary Center of Infection Biology and Immunity) merges the activities of the Humboldt University and non-university research institutes in the field of infection biology and immunity in Berlin, including Robert Koch Institute, Charite (a joint institution of the Freie Universität Berlin and the Humboldt-Universität zu Berlin), The German Rheumatism Research Center Berlin (DRFZ), Leibniz Institute for Zoo and Wildlife Research, and Max Planck Institute for Infection Biology. ZIBI summer program 2012 provided a one-week lecture course, two days scientific symposium "Global Challenge of Chronic Tropical Infections" and three days of practical courses and sight-seeing in Berlin. Twenty-two master/Ph.D. students participated from Germany, Turky, Brazil, Ethiopia, South Africa, India, Indonesia, Bangladesh, Greece, Slovakia, Israel and Japan. "It was my first experience to visit abroad and I was a little worried about English. However, I could have so many discussions with various people and it was my honor to receive the poster award during the symposium," said Hanazato.

International Research Collaboration Award

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

Since the program began in 2004, 18 projects have been funded (Table) and the collaborations have resulted in several important papers. Drs. Vidal and Koseki's collaborative project on *Ring1* genes resulted in 5 papers, *Dev Cell.* (2004), *Development* (2006), *Nat Cell Biol*. (2007), *Mol Cell Biol*. (2008) and *Development* (2008). Drs. Dustin and Saito published their work on T cell microclusters in *Nat Immunol*. (2005) and *Immunity* (2006). Drs. Ewijk and Kawamoto's work on thymic progenitor cells resulted in three papers in *Development* (2006) and *Mol Immunol.* (2009 and 2010). Drs. Bix and Kubo published their work on the IL-4 repressor in *Nat Immunol.* (2009), and Drs. Ellemeier and Taniuchi published their study on transcription factor network for CD4/CD8 lineage choice in *Nat Immunol*. (2010), and Dr. Cheroutre published transcriptional reprogramming of CD4/CD8 lineage in *Nat Immunol.* (2013) with Drs. Ellemeier and Taniuchi.

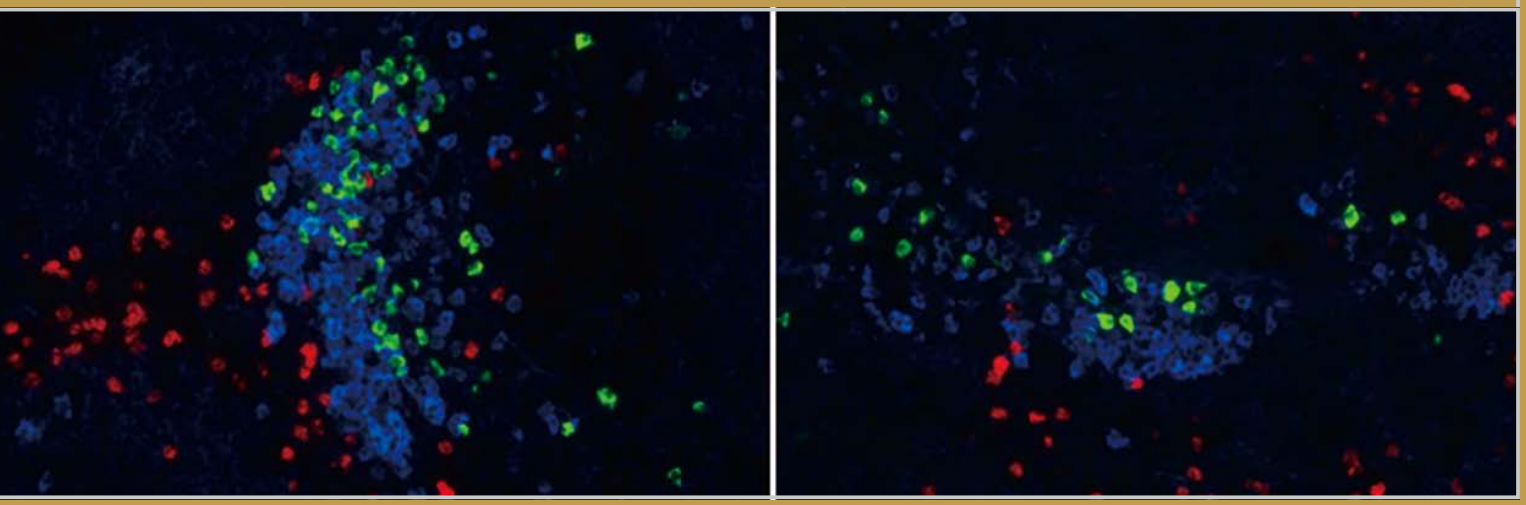
Table: Awardees of RCAI International Collaboration Award Program

Year	Host Lab.	Title of Research	Awardee
2004-2006	**Takashi Saito**	Analysis of dynamism and function of immunological synapse using planar membrane and knock-in T cells	**Dr. Michael DUSTIN** New York University School of Medicine
2004-2006	**Hiroshi Kawamoto**	Regulatory role of lymphoid progenitors during development of thymic microenvironments	**Dr. Willem van EWIJK** Leiden University Medical Center
2004-2006	**Haruhiko Koseki**	Genomic and functional analysis of the role of the Polycomb Ring1 genes in B-cell development	**Dr. Miguel VIDAL** Centro de Investigaciones Biologicas, CSIC
2004-2005	**Masaru Taniguchi**	Role of NKT cells in TSLP-mediated allergic inflammation	**Dr. Steven F. ZIEGLER** Benaroya Research Institute at Virginia Mason Medical Center
2004-2006	**Ji-Yang Wang**	Expression and function of *FcRY*-a novel Fc receptor-related gene expressed in B cells	**Dr. Peter D. BURROWS** University of Alabama at Birmingham
2005-2007	**Ichiro Taniuchi**	Study of T cell differentiation mediated by regulated expression of CD8 genes	**Dr. Wilfried ELLEMEIER** Institute of Immunology, Medical University Vienna **Dr. Hilde CHEROUTRE** La Jolla Institute for Allergy and Immunology
2005-2007	**Masato Kubo**	Understanding genetic regulation of interleukin 4 production by a CD4(+) T cell-intrinsic mechanism.	**Dr. Mark BIX** University of Washington, Seattle, Washington
2005-2006	**Yasuyuki Ishii**	Gene-array analysis and proteomics of Th2 tolerance	**Dr. Yun-Cai LIU** La Jolla Institute for Allergy and Immunology
2005-2007	**Osami Kanagawa**	Visualization of STAT protein in the cytokine mediated signaling at a single molecular level.	**Dr. Kenneth M. MURPHY** Howard Hughes Medical Institute Washington University School of Medicine
2005-2007	**Tomohiro Kurosaki**	Role of signaling molecules in B cell synapse formation and its maintenance	**Dr. Facundo Damian BATISTA** Cancer Research UK London
2006-2008	**Masato Tanaka**	Identification of Novel Necrotic Molecules from Necrotic Hepatocytes and Examination of Its Effect on the Inflammatory Response	**Dr. Sunhwa KIM** and **Dr. Michael KARIN** Department of Pharmacology, Univ. of California, San Diego, USA
2007-2008	**Takeshi Watanabe**	A study on the spleen and lymph nodes mesenchymal cells that participate in the assembly of artificial secondary lymphoid organs	**Dr. Andrea BRENDOLAN** Cornell University Medical Center, Department of Cell and Developmental Biology
2007-2009	**Ichiro Taniuchi**	Understanding of tumor suppressive mechanism of Runx complexes against leukemia and gastrointestinal cancer	**Dr. Motomi OSATO** and **Dr. Yoshiaki ITO** Institute of Molecular and Cell Biology, National University of Singapore
2007-2009	**Sidonia Fagarasan**	Nuclear reprogramming of terminally differentiated plasma cells to study the specific role of IgA in mucosal and systemic immunity and B cell development	**Dr. Stefano CASOLA** IFOM-The FIRC Institute of Molecular Oncology Foundation, Milano, Italy
2010-2012	**Mariko Okada-Hatakeyama**	Proteomics based-quantitative analysis of signal-transcriptional network	**Dr. Boris KHOLODENKO** University College Dublin, Ireland **Dr. Richard JONES** University of Chicago, USA
2011-2013	**Ichiro Taniuchi**	Understanding and Engineering of Dendritic Epidermal T Cells (DETC) development	**Dr. Florent GINHOUX** Singapore Immunology Network (SIgN), Singapore
2012-2013	**Yasuyuki Ishii**	Oral vaccine design for asthma, atopic dermatitis and pollinosis	**Dr. Sarah HOOK** University of Otago, New Zealand
2012-2013	**Hiroshi Ohno**	Identification and analysis of transcription factors directing intestinal M cell differentiation	**Dr. Ifor WILLIAMS** Emory University, USA

2012

Part 5

Outreach Activities



RCAI-Novo Science Forum

April 19, 2012

On April 19, 2012, RCAI and Novo Nordisk, a global healthcare company headquartered in Denmark, held a joint scientific forum at RCAI. Fifteen Novo Nordisk researchers and senior managers in the fields of biopharmaceuticals, immunology, pharmacology, target discovery, biotechnology, development, and disease models, visited RCAI. After the introduction of sourcing innovation at Novo Nordisk by Jørn Roland Müller, Corporate Vice President of Biopharmaceutical Sourcing in Novo Nordisk, Takashi Saito, Deputy Director of RCAI, described the structure of the Center. After these introductions, nine RCAI researchers, Toshitada Takemori, Mariko Okada, Tomohiro Kurosaki, Ji-Yang Wang, Hiroshi Ohno, Tsuneyasu Kaisho, Takashi Tanaka, Shohei Hori and Sidonia Fagarasan described their recent research discoveries. At the end of the forum, Anan Gautum, Director of Biopharmaceutical Sourcing in Novo, commented that the research in RCAI was very impressive and that Novo would continue to look for collaborative possibilities. This workshop was one of the rare opportunities for RCAI researchers to discuss science with a pharmaceutical company. Takashi Saito told that it is difficult to consider pharmaceutical or clinical applications of the research results from the Center without collaboration and support from pharmaceutical companies.

Open Seminar "When cells determine their fate"

April 21, 2012

Ichiro Taniuchi, Group Director of Laboratory for Transcriptional Regulation, spoke at the Open Seminar held in the RIKEN Wako Open Campus. The seminar "When cells determine their fate" attracted an audience of 230, which exceeded the capacity of the lecture hall, so that more than 50 people watched on a live remote screen in a separate room.

His talk started with a simple question, "What is immunity?". He effortlessly explained that immunity is a strategy that allows us to survive environmental dangers. To answer the second question, "What is the environment?", he asked the audience to close their eyes. Our eyes sense the light, and similarly, ears perceive the sound, nose perceives the smells, and tongue perceives the taste. Immune cells recognize the surrounding environment, too, but in different ways.

After he explained the basics of the immune system, he talked about helper and killer T cell fate during their development. He had discovered a mechanism that controls expression of the transcription factor ThPOK, the master regulator to choose a helper or killer fate in a cell fate decision. Interestingly, his recent study found that helper T cells could switch their function to become killer-like T cells. He compared the cell fate decision to the college preparation courses in Japanese high schools. In Japan, high school students choose natural science or humanities courses to prepare for the college entrance exams. Students who took natural science courses would major in science in college, but some students could change their major to humanities. Helper T cells can change their fate, too, he explained.

Various individuals participated in the seminar, including university students, company workers, teachers, housewives, retired folks, etc. "It was interesting that the story started from the surface and connected to a deep part. I wanted to know more about transcriptional regulation," said one man, but another said, "It was easy in the beginning but difficult at the end." Taniuchi said, "It was not easy to make the story easy-to-understand but I was glad that so many people were interested in my research."

Science Café "Gut bacterium and probiotics"

April 22, 2012

On April 22, Hiroshi Ohno, Team Leader of the Laboratory for Epithelial Immunobiology, spoke at the RIKEN Yokohama Science Café held at Kanagawa Prefectural Kawasaki Library. Among 170 applicants, 50 participants were selected by a random drawing.

Ohno first described how many bacteria live in a human body. Our body consists of 60 trillion human cells and has 30 thousand human genes. Meanwhile, one hundred trillion bacteria live in the gut, consisting of more than 1,000 species, and 3 million gut bacteria genes. Consequently, in the gut, humans have more than 100 fold more bacterial genes than human genes. Our body is actually maintained by many organisms, and this concept was termed "superorganism" by Joshua Lederberg, a Nobel laureate.

Recently, metagenomics, which means genomics on a large scale, has emerged as a powerful tool to analyze bacterial communities. Metagenomics is based on the genome sequencing of samples collected directly from the environment. It enables a survey of the different bacterial species in the environment, and metagenome analysis of human gut microbiota has been conducted mainly in Europe (MetaHIT; Metagenomics of the Human Intestinal Tract) and the US (HMP; Human Microbiome Project) as the national/international projects funded by their government.

In the latter half of the talk, Ohno explained his discovery that Bifidobacteria can protect from enteropathogenic *E. coli* O157 infection through production of acetate.

There were many questions from the participants during and after his talk, and so the café ran out of time for any more questions and answers. Most of the questions were related to health. "What is the relation between gut bacteria and colon cancer?" "Are the supplements beneficial to colon health?" "Do the gut microbiota recover after an endoscope examination?" Ohno tried to answer as much as possible, and the participants had favorable impressions of his friendly attitude.

Science Café "Do computers cure diseases?"

September 1, 2012

On September 1, 2012, Mariko Okada, Team Leader of Laboratory for Cellular Systems Modeling, spoke at the Science Café held at Yokohama City Central Library. Forty people, including three junior high and senior high students attended the event.

The talk started by defining the notion of systems biology. Okada easily introduced various pathways and components in biology. She showed multiple examples of metabolic pathways, signaling pathways, central dogma, and explained that adequate regulation of the pathways is necessary for the normal function of biological systems. She compared systems biology to a car control system. To make a car, all parts must be assembled properly and input-output pathways must be adequately controlled.

Then she described the challenges of systems biology: To understand the whole body as a system, it is necessary to transcend the different layers of body components and pathways and create an integrated network. In a human body, there are 12 kinds of organs, 4 kinds of tissues, 300 kinds of cells, 100,000 different proteins and 23,000 genes. The size and time scales of these systems are also different. Molecular or ionic changes occur in 10^{-6} seconds, signaling events occur in 10^{-3}-1 second, cell movement and cell division occur in 1-10^{4} seconds, protein turnover occurs in 10^{6} seconds and the human lifespan is about 10^{9}seconds.

Lastly, she explained how computer models could contribute to the cure of diseases. Using an American venture company, Entelos, as an example, she explained that computer model platforms are actually used for the interpretation of physiological state, disease state and therapeutic decisions. In addition, computer models could calculate uncertainties between individuals, life style differences and genetic differences.

"It was new for me to know that animals were systems, and it is composed of various networks," a participant told. "It was new, too, to know that researcher's communications and networks are important in systems biology."

RIKEN Yokohama Open Campus

September 29, 2012

The RIKEN Yokohama Institute Open Campus was held on Sep. 29, 2012. Under clear autumn skies, 1,749 people visited the campus. Haruhiko Koseki, Group Director of the Laboratory for Developmental Genetics, gave a seminar "Is the calico cat's pattern inheritable?" There were 104 people at the seminar, ranging in age from elementary school students to retired folks. He explained the genetic combinations that encode the fur color of cats and their genetic linkage to the X or Y chromosome. Then he introduced the calico cat cloning experiment. Because one of the X chromosomes was already inactivated in the somatic cell used for cloning, the cloned cat turned out to have a different fur pattern, a brown tabby. "It sounded simple in the beginning, but the epigenetic control was actually a little complicated," a participant said.

Twenty RCAI teams showed posters or movies. Highlights were the hands-on experiences provided by four teams. Ohno and his team members provided hands-on practice of Gram staining of mouse gut bacteria. Twenty-five participants each stained two mouse fecal samples, before and after the administration of antibiotics, and compared the change in the gut microbiota using a microscope. Yoshida and his team prepared stained samples of fetal mice and newborn mice at various developmental stages. Mariko Okada and her team members held "Filling a pipette tip box competitions." Participants competed for the best time for filling 100 micropipette tips into a box. The top results of the day were 1 min 6 sec for 1000μl pipette tips and 1 min 21 sec for 200μl tips. Social network sites, Facebook, Twitter and YouTube, were set up for the open campus this year. A photo of "Filling a pipette tip box competitions" gathered re-tweets and comments on Twitter. "What's this?" "Wow, I didn't have this idea." "I want to take my time!" The RIKEN Yokohama Open Campus seems to evolve year by year.

Science Café "Towards the cure for leukemia"

March 2, 2013

On March 2nd, Fumihiko Ishikawa, Group Director of the Laboratory for Human Disease Model, talked at a Science Café held at Yokohama City Central Library. Among 82 applications, forty participants were selected by a random drawing.

The first half of the café was a lecture by Dr. Ishikawa. He talked about his experience as a hematologist and how he decided to become a researcher to study the recurrence of acute myeloid leukemia. Then he introduced his current research on the development of novel therapies targeting leukemic stem cells.

"I had not heard of the humanized mouse before, but I could understand how it could contribute to the development of new therapies." "I really look forward to the anti-leukemia drug RK-20449. I felt Dr. Ishikawa's passion. His dream will come true," were some of the comments by the participants.

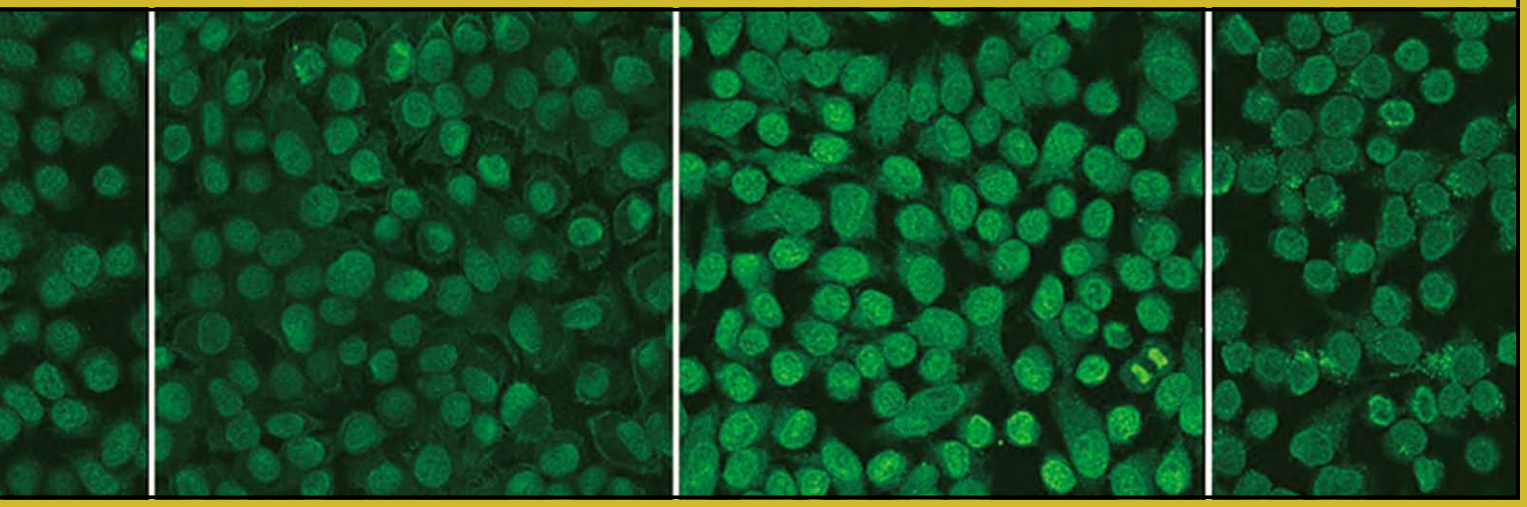
The second half of the café was a Q & A session. Participants wrote down questions on Post-it notes and Dr. Ishikawa responded to them. The 24 questions varied from the difference between hematopoietic stem cells and leukemic stem cells, characteristics of the humanized mouse, future medicine, and even the future of RIKEN. One question, "There are many questions (gene therapy, iPS therapy etc.) which cannot be explained by current basic sciences (chemistry, physics, quantum science etc.). Isn't it necessary to conduct more fundamental research to solve the questions?" induced a lively discussion from the floor. "What is the research that RIKEN can do but other institutions or universities cannot do?" "RIKEN should pursue the research irrelevant to profits." "RIKEN can do the advanced research and integrate various fields." "Dr. Ishikawa's research would be an example of what RIKEN can do."

After the café, a participant said to a RIKEN staff member, "It was like Michael Sandel's 'Justice' TV program from Harvard. You can rename this RIKEN's program to 'Passionate Science' café."

2012

Part 6

Laboratory Activities



Laboratory for

Developmental Genetics

Group Director : **Haruhiko Koseki**

Senior Research Scientist : **Kyôichi Isono**

Research Scientist : **Yûichi Fujimura, Mitsuhiro Endô, Osamu Masui, Takashi Kondô, Yixin Dong, Kit Wan Ma, Nayuta Yakushiji, Yang Li, Raul Eduardo Vizcardo Sakoda, Yuki Takada (SPDR), Shinsuke Ito (SPDR), Jafar Sharif (FPR)**

Technical Staff : **Tamie Endo, Naoko Ônaga, Rie Suzuki, Kayoko Katsuyama, Yôko Koseki, Kaori Kondô, Mami Kumon, Fuyuko Kezuka, Miho Urabe**

Student Trainee: **Misaki Ôno, Marika Shibata**

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 in 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. Particular emphasis has been put on epigenetic regulation mediated by combinatorial actions of Polycomb group (PcG) gene products and DNA methylation mechanisms.

The role of epigenetic regulators during development and differentiation

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

Regulation of large scale chromatin structures by epigenetic regulators

Accumulating evidence documents a role for PcG proteins in regulating higher order chromatin structures, but the mechanisms and impact of such structures on transcriptional regulation remain obscure. We have identified PcG bodies in mouse primary fibroblasts as distinct foci, at which PRC1 and H3K27me3 are colocalized and canonical PcG target genes are condensed. We found that PcG body formation requires Phc2-SAM polymerization, which critically contributes to condensation and repression of PcG target genes. We further showed that Phc2-SAM polymerization limits the dynamic nature of PRC1, and thereby promotes stable association of PRC1 with PcG target genes. These findings suggest a novel model by which SAM polymerization of Phc2 modulates the struc-

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2. Li X, Isono KI, Yamada D, Endo TA, Endoh M, Shinga J, Mizutani-Koseki Y, Otte AP, Casanova M, Kitamura H, Kamijo T, Sharif J, Ohara O, Toyada T, Bernstein BE, Brockdorff N, Koseki H. Mammalian Polycomblike Pcl2/Mtf2 is a novel regulatory component of PRC2 that can differentially modulate Polycomb activity at both the Hox gene cluster and at Cdkn2a genes. ***Mol Cell Biol.*** 31, 351-364 (2011)
3. Takada Y, Naruse C, Costa Y, Shirakawa T, Tachibana M, Sharif J, Kezka-Shiotani F, Kakiuchi D, Masumoto H, Shinkai Y, Ohbo K, Peters AH, Turner JM, Asano M, Koseki H. HP1γ links histone methylation marks to meiotic synapsis in mice. ***Development*** 138, 4207-4217 (2011)

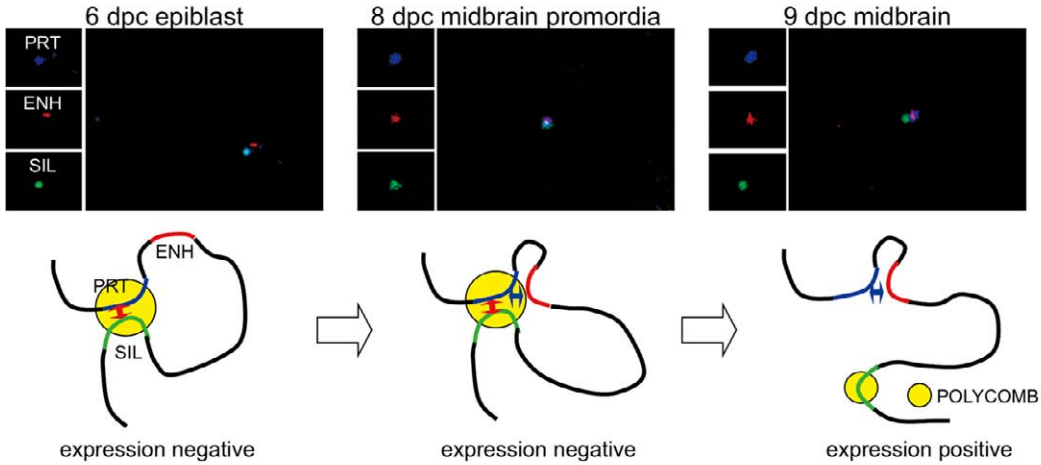

Figure : **DNA topology of promoter (PRT)/enhancer (ENH)/silencer (SIL) during midbrain development.** DNA FISH analysis indicates that PcG/PRT/SIL associate together when gene (*Meis2*) transcription is suppressed. The ENH sequence transiently associates with this complex in the 8 dpc midbrain primordium just before the onset of *Meis2* expression. SIL dissociates from the complex at the stage when *Meis2* expression begins (9 dcp midbrain). Although the tri-DNA complex formation required PcG activity, PcG is no longer necessary for maintenance of the PRT/ENH association.

tural organization of PcG complexes to enable robust yet reversible PcG-mediated repression during development.

Since *Hox* gene clusters retain a special genomic configuration, we went on to test whether PRC1 uses this mechanism also at solitary target genes. Intriguingly, by re-examining ChIP-seq data for Ring1B distributions in ESCs, we found that PRC1 is distributed to not only promoter regions but also genomic regions surrounding stop codons of repressed target genes. We hypothesized that promoter regions are associated with 3' regions of respected targets. We focused on the *Meis2* gene, which occupies a large genomic segment spanning around 200Kb in mammals. *Meis2* has complex expression patterns during development and mutations in the locus are lethal with various defects. We identified several transcriptional regulatory sequences for the *Meis2* gene, which are located at large distances from the promoter in the linear DNA sequence, and analyzed their topological relationships at transcription activity transitions during development using knockout mice, 3C, transgenic analysis and histological FISH. In the transcription repressive state, such as in early embryonic stages, the promoter/silencer (*Polycomb* binding sequence) associate with each other in a *Polycomb*-dependent manner, while the promoter/enhancer are in close proximity without silencer association in the active state (Figure). Detailed observation of midbrain expression during midbrain organogenesis revealed that all three sequences-promoter/enhancer/silencer transiently associate into close proximity in the preparative state just prior to *Meis2* expression in midbrain primordium neural plate, then the silencer dissociates from the complex to activate gene expression. This three-region-association is also dependent on *Polycomb* activity, while maintenance of the active state of expression does not require *Polycomb* activity.

A novel role for tubulins to organize transcriptionally repressive domains at the nuclear periphery

In metazoans, the inner surface of nuclear envelope is lined by nuclear lamina (NL), a filamentous protein network primarily consist of lamins. Physical interactions of NL and intranuclear molecules are engaged in regulation of chromosome status and of transcriptional activity. Most of the genes associating with the NL are transcriptionally repressed. Consistent with this, the NL also associates with a variety of chromatin modifying factors that correlate with gene silencing such as histone deacetylases (HDACs). However, the molecular mechanisms which link the NL and its binding factors in living cells are poorly understood. We have shown that tubulin proteins persistently associate with the NL and are indispensable for association of NL-chromatin, and of NL-HDACs. Loss of tubulin from the NL results in the disengagement of these factors, and up-regulation of histone acetylation at the nuclear periphery. These findings suggest novel functions of tubulins as a scaffold for molecular assembly of genomic DNA and determinants for chromatin status at the nuclear periphery.

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

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Laboratory for

Lymphocyte Development

Team Leader:
Hiroshi Kawamoto

Research Scientist: **Kiyokazu Kakugawa**
Kyoko Masuda
Research Associate: **Rumi Satoh**
Technical Staff: **Midori Kawauchi**
Visiting Scientist: **Nagahiro Minato**
Yoshimoto Katsura
Toshio Kitamura
Takeshi Watanabe

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

Individual hematopoietic stem cells in human bone marrow stably give rise to limited cell lineages

To sustain hematopoiesis, hematopoietic stem cells (HSCs) must, on the one hand, replenish themselves by self-renewal and, on the other hand, produce differentiating progenitor cells. It is also known that most HSCs remain dormant and are only rarely and randomly activated. It has been estimated that each human possesses a total of 10^4 HSCs, and that ~400 HSCs actively contribute to hematopoiesis, replicating once per year[1,2]. However, the actual dynamics of hematopoiesis by HSCs remains uncertain. For example, it is unclear how long an individual HSC maintains hematopoiesis and whether all major lineages of cells are produced from a single HSC. These issues have been difficult to address due to the lack of appropriate experimental systems, regardless of animal species.

GPI-APs$^-$ cells were seen in limited lineages of blood cells from most BM failure patients

Mutation of the *PIG-A* gene in hematopoietic stem cells (HSCs) results in the loss of glycosylphosphatidylinositol-anchored proteins (GPI-APs) on HSCs, but minimally affects their development and thus can be used as a clonal marker of HSCs. We analyzed GPI-AP expression on six major lineage cells in a total of 574 patients with bone marrow (BM) failure in which the microenvironment itself is thought to be unaffected, including aplastic anemia (AA) and myelodysplastic syndrome (MDS). We determined the proportion of GPI-AP-deficient (GPI-APs$^-$) cells in six major lineages, namely granulocytes (G), monocytes, erythrocytes, T cells, NK cells and B cells, in peripheral blood (PB) cells from BM failure patients using a highly sensitivity flow cytometric analysis.

GPI-APs$^-$ cells were detected in 250 patients. Whereas the GPI-APs$^-$ cells were seen in all six lineages in a majority of patients who had a higher proportion (>3%) of GPI-APs$^-$ cells, they were detected in only limited lineages in 92.9% of cases in the lower proportion (<3%) group. In

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1. Katagiri T*, Kawamoto H*, Nakakuki T*, Ishiyama K, , Okada-Hatakeyama Ohtake S, Seiki S, Hosokawa K and Shinji Nakao. (*equal contribution) Individual hematopoietic stem cells in human bone marrow of patients with aplastic anemia or myelodysplastic syndrome stably give rise to limited cell lineages. ***Stem Cells*** 31, 536-546, 2013 (*equal contribution)
2. Vizcardo R, Masuda K, Yamada D, Ikawa T, Shimizu K, Fujii S-I, Koseki H, Kawamoto H. Regeneration of human tumor antigen-specific T cells from iPS cells derived from mature CD8$^+$ T cells. ***Cell Stem Cell*** 12, 31-36, (2013).
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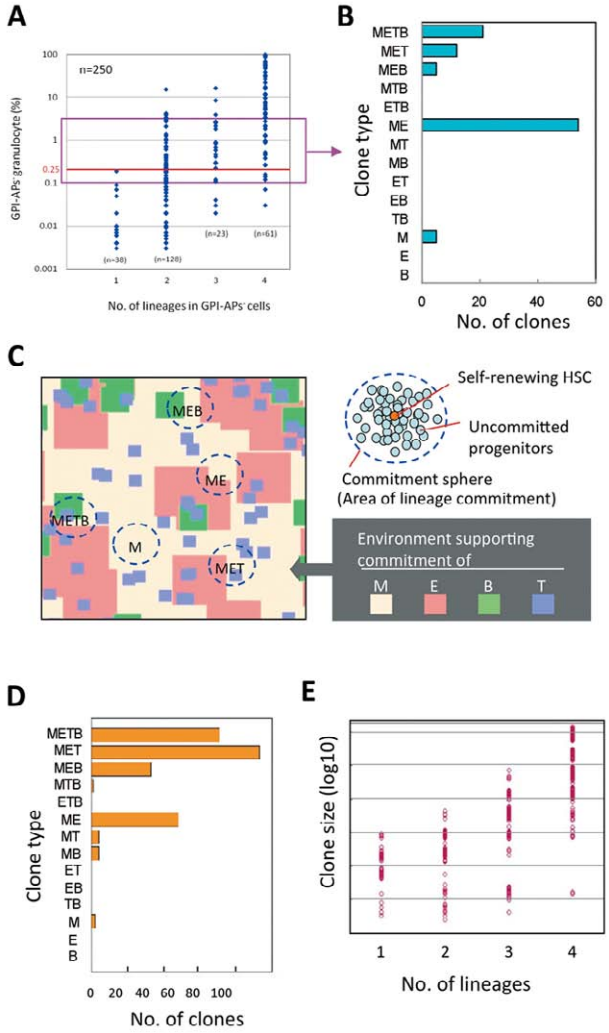

Figure : Simulation of HSC differentiation recapitulates the clinical observations.

A. The percentage of GPI-APs⁻ granulocytes vs. the number of GPI-APs⁻ cell lineages in individual patients. For simplicity, granulocytes and monocytes are placed into the myeloid lineage while T and NK cells are placed into the T lineage, consequently all lineages can be classified as M, E, T, or B lineages. The red line at 0.25% indicates the expected value for an average size of a single HSC clone.

B. Frequency of clone types among cases where the percentage of GPI-APs⁻ granulocytes is between 0.3 to 3 %.

C. A model assuming that the BM microenvironment is heterogeneous in its capacity to support the commitment of progenitors. A certain range where the commitment of progenitors mainly occurs (with a probability of >97%) is termed the "commitment sphere". Areas colored beige, pink, light green, or blue represent environments supporting commitment only towards M, E, T or B cells, respectively. If a HSC happens to reside in a location in which the commitment sphere contains only M and E regions, then this HSC can eventually produce only M and E cells, even if committed progenitors expand enormously and become widely scattered. The depicted simulation goes as follows: a virtual HSC undergoes several cell divisions at fixed intervals while randomly migrating. The uncommitted progenitors then undergo a fate decision based on their ultimate location in the commitment sphere. Committed progenitors can also randomly move around, but can proliferate only in the lineage-specific supporting environment. After several additional fixed time cell divisions as committed progenitors, cells become mature and lineage restricted.

D. One example illustrating the frequency of clone types simulated as shown in (C). A total of 100 virtual HSCs were simulated to form hematopoietic clones, and the numbers of the resulting clone types are illustrated.

E. Simulation of individual HCSs for size and number of progeny lineages. The simulation well recapitulated the clinical observations shown in (A).

all 250 cases, the same lineages of GPI-APs⁻ cells were detected even after 6-18 month intervals, indicating that the GPI-APs⁻ cells reflect hematopoiesis maintained by a self-renewing HSC in most cases. The frequency of clones with limited lineages seen in mild cases of AA was similar to that in severe cases, and clones with limited lineages were seen even in two healthy volunteer cases. These results strongly suggest that most individual HSCs produce only a restricted set of lineages even in the normal steady state.

A model for the hematopoietic microenvironment to explain the production of limited cell lineages from a single HSC

While the observed restriction could reflect heterogeneity in the developmental potential of HSCs, we propose an alternative model in which the BM microenvironment is mosaic in its capacity to support commitment of progenitors towards distinct lineages. For simplicity, we reclassified the six lineages into four lineages (Fig. A, B). In the model, we assume that a self-renewing HSC is located in a particular site in BM and that uncommitted progenitors derived from this HSC can reach to a certain defined area (Fig. C), which we term here the "commitment sphere". If the BM microenvironment is mosaic in terms of function in inducing the commitment of progenitors towards a particular lineage, and the size of such a mosaic is as large as the commitment sphere, then production of progenitors of limited lineages can occur. We have named our model "mosaic commitment-inducing microenvironment model".

To test whether our model can explain the *in vivo* findings, we applied a computer simulation approach, in collaboration with Mariko Okada (RCAI) and Takashi Nakakuki (Kougakuin University). Simulations based on this model nicely recapitulated the observed *in vivo* findings (Fig. D, E).

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Laboratory for

Transcriptional Regulation

Group Director:

Ichiro Taniuchi

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Mari Tenno
Hirokazu Tanaka (SPR)
Wooseok Seo (JSPS)

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Chizuko Miyamoto
Risa Chihara
Mayu Okoshi
Mika Ikegaya

Student Trainee : **Sebastian Nieke (IPA)**
Hisashi Wada (JRA)

One of fundamental questions in developmental biology is how the fate of progenitor/precursor cells differentiating into opposing lineages is determined upon exposure to environmental cues. Even as we learn more about the link between cellular signaling and cell fate determination in the nucleus, other questions arise about how genetic programming leads to establishment of cell identity, which is maintained in differentiated cells in many cases. Research in my laboratory is directed toward understanding (a) how precursor cells sense external differentiation stimuli and then integrate them into a genetic program for regulating fate decisions and (b) how the lineage-specific gene expression pattern established during the commitment process is stably inherited in differentiated cells. We have been addressing these questions by studying the mechanisms that govern lineage choice by $CD4^+CD8^+$ double-positive (DP) thymocytes that differentiate into either $CD4^+$ helper- or $CD8^+$ cytotoxic-lineage T cells after positive selection. The specificity of the TCR to the MHC has been documented to correlate well with the outcome of the CD4/CD8 lineage decision. In addition, recent genetic studies in mice demonstrated that expression of the ThPOK transcription factor with accurate kinetics and lineage-specificity is essential to link TCR/MHC specificity with CD4/CD8 lineage choice. Thus *Thpok* gene regulation can serve as an ideal model to study how the analog differences in TCR signals are converted into digital outcomes of cell fate, i.e. either $CD4^+$ helper or $CD8^+$ cytotoxic lineage cells.

Another research focus in my laboratory is on the function of Runx/Cbfβ transcription factor complexes in the development and homeostasis of the immune system. By analyzing several mouse strains harboring mutations in Runx family genes, we have reported an essential requirement for Runx complexes in the development of a variety of hematopoietic cells such as B-lymphocytes, lymphoid tissue inducer (LTi) cells and basophils. To gain a better understanding of Runx/Cbfβ complex functions, we are exploring how these complexes are involved in regulation of immune cells and immune responses.

Timing of lineage choice after positive selection in the thymus

Our previous studies have shown that a transcriptional silencer in the *Thpok* locus is essential to repress *Thpok* expression specifically in cells developing into the cytotoxic-lineage, while a transcriptional enhancer in proximity to the *Thpok* gene is essential to upregulate ThPOK expression with correct kinetics in cells developing into the helper-lineage. Given that the silencer-mediated *Thpok* repres-

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2. Mucida D, Husain M.M, Muroi S, van Wijk F, Shinnakasu R, Naoe Y, Reis B, Huang Y, Lambolez F, Docherty M, Attinger A, Shui J.W, Kim G, Lena C, Sakaguchi S, Miyamoto C, Wang P, Atarashi K, Park Y, Nakayama T, Honda K, Ellmeier W, Kronenberg M, Taniuchi I* and Cheroutre H*. Transcriptional Reprogramming of Mature CD4 T helper Cells generates distinct MHC class II restricted Cytotoxic T Lymphocytes. ***Nat Immunol.*** 14, 281-9 (2013) *corresponding author

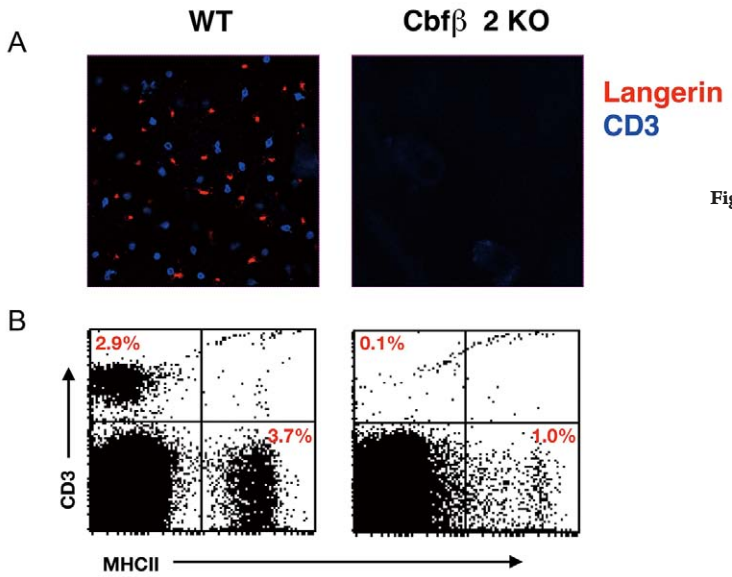


Figure: **Essential requirement for Runx3/Cbfβ2 in development of DETC and Langerhans cells**
Our body is continuously exposed to a variety of pathogenic microorganisms at the interface to the outer environment. Primary immune soldiers are placed at such a frontier of the immune battlefield during embryogenesis. In mice, Langerhans cells (LC) and dendritic epidermal T cells (DETC) are present for primary defense and provide a link between the frontiers and central systemic immunity. We found that Runx3/Cbfβ2 complexes are essential for development of both cell types. In mice deficient for Cbfβ2, one of the RNA splicing variants of Cbfβ, both LC and DETC are undetectable in skin epidermis by immune histochemical analyses. Immuno-histochemistry (A) and flow cytometer analyses (B) demonstrating the absence of Langerin expressing LC and CD3 expressing DETC cells in the epidermis of Cbfβ2-deficient mice.

sion already operates in precursors prior to exposure to differentiation cues, i.e. positive selection signals by TCR engagement, reversal of the *Thpok* silencer is required for efficient induction of the *Thpok* gene. This also suggests that the *Thpok* silencer would function as a nuclear sensor and converter of TCR signals into regulatory cues to control the expression status of the *Thpok* gene, a major factor that discriminates these two lineages. These unique characteristics of the *Thpok* gene have prompted us to extensively study mechanisms regulating its expression.

In the last few years, we have examined whether epigenetic mechanisms are involved in *Thpok* gene regulation. By using a genetic strategy that allows conditional removal of the *Thpok* silencer, we obtained evidence showing that an epigenetic silent state, which is established at the *Thpok* locus during commitment to the cytotoxic-lineage in thymus, can be then maintained without the silencer in differentiated $CD8^+$ T cells. Interestingly, two promoters in the *Thpok* gene are repressed through distinct repressive epigenetic mechanisms. Strikingly, when the silencer activity is enhanced by increasing its copy number, epigenetic sealing of the *Thpok* locus can even occur in helper-lineage cells. Thus, the epigenetic machinery that confers crucial epigenetic marks onto the *Thpok* locus can work independently of commitment to the cytotoxic-lineage. Collectively, our results provide a novel epigenetic insight into the timing of the helper/cytotoxic lineage decision. Even once signals through an MHC class II-specific TCR open a gate to the developmental path toward the helper-lineage, the gate is closed again if such TCR signals are perturbed. Thus the decision to become a helper-lineage cell must be made within a particular time window, during which TCR signals must be sustained to keep the gate open. Our on-going studies aim to identify molecule(s) that work as the gate-keepers that switch the gate from close to open upon receiving TCR signals to initiate ThPOK expression. We are also working to provide evolutionary insights to understand how such a regulatory mechanism that finely converts environmental cues into a correct fate decision process is acquired during evolution.

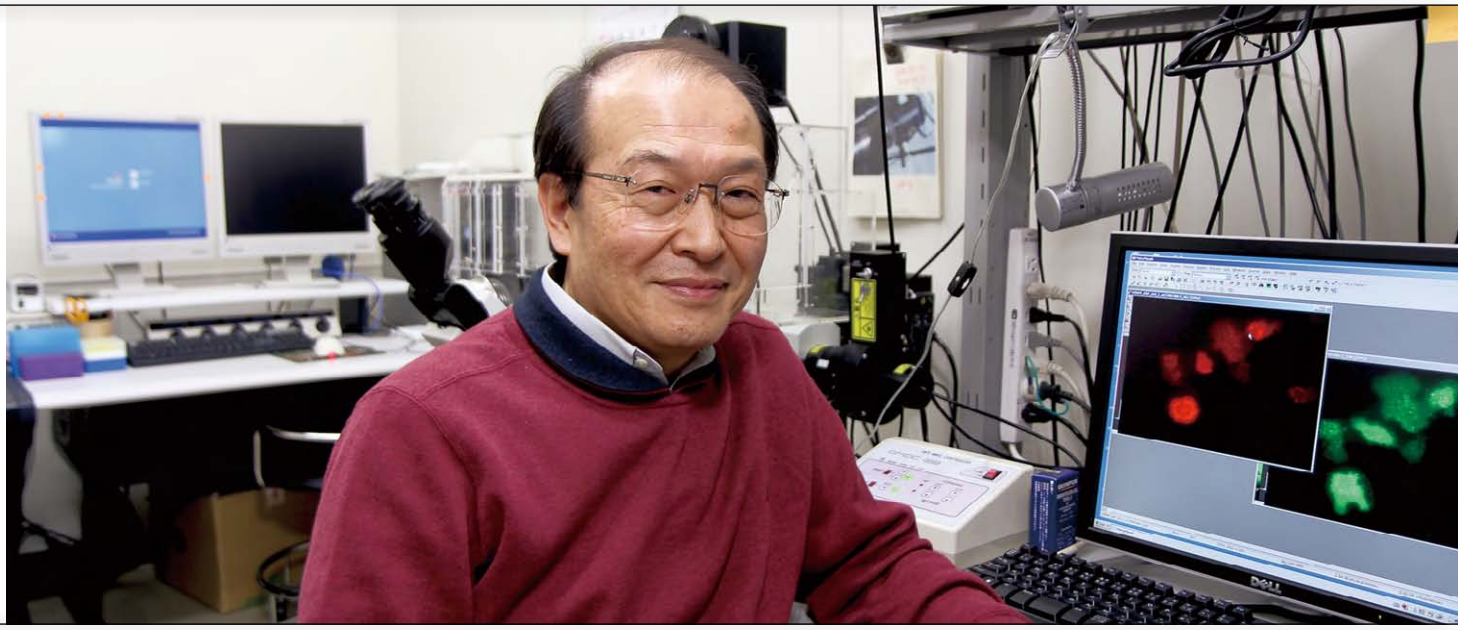
Role of Runx complexes for placing immune cells at the interface to the environment

Our body is continuously exposed to microorganisms at the interfaces between body and environment. The skin is one of such interface tissue, and inhibits invasion of microorganism mainly by forming a mechanistic and primitive biological barrier of tightly connected keratinocytes layers. However, immune cells are present beneath this skin barrier to sense invaders and initiate systemic immune responses, if necessary, for eliminating them. In mice, two types of immune cells are placed at the front of the immune battle before the birth: Langerhans cells (LC) and dendritic epidermal T cells (DETC). Interestingly, we found that both of these cells are missing in a mutant mouse strain that lacks Cbfβ2 protein (Fig.), encoded by one of the RNA splicing variants of the obligatory Runx partner protein Cbfβ. Our ongoing analyses of these Cbfβ2-deficient mice will provide valuable insights to understand the developmental pathway of these primary immune soldiers and their roles in defense against pathogenic microorganisms at one of the immunologic frontiers.

3. Muroi S, Tanaka H, Miyamoto C and Taniuchi I. Fine-tuning of Thpok gene activation by an enhancer in close proximity to its own silencer. ***J Immunol.*** 190, 1397-401 (2013)
4. Seo W., Ikawa T., Kawamoto H., Taniuchi I. Runx1/Cbfβ is essential for early B lymphocyte development through regulation of Ebf1 expression. ***J Exp Med.*** 209, 1255-62 (2012)
5. Setoguchi R., Tachibana M., Naoe Y., Muroi S., Akiyama K., Tezuka C., Okuda T., Taniuchi I. Repression of the Transcription Factor Th-POK by Runx Complexes in Cytotoxic T Cell Development. ***Science*** 319, 816-19 (2008)

Laboratory for

Cell Signaling



Group Director: **Takashi Saito**

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Student Trainee: **Mohamed El Sherif Gadelhaq Gadlehaq Badr (IPA), Yuya Anai**
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RCAI Central Facility Confocal Laboratory: **Yasutaka Wakabayashi** (Leica Microsystems**), Ikuo Ishige** (BM Equipment)
RCAI Central Facility mAb Laboratory: **Tomomi Aoyama, Kazuyo Uchida** (Program for Drug discovery and Medical technology platform)

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

Dynamic regulation of T cell activation by co-stimulation

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

Recent Publications

1. Yokosuka T., Takamatsu M., Kobayashi-Imanishi W., Hashimoto-Tane A., Azuma M., Saito T.: Programmed cell death 1 forms negative costimulatory microclusters that directly inhibit T cell receptor signaling by recruiting phosphatase SHP2. ***J Exp Med.*** 209, 1201-1217 (2012)
2. Hashimoto-Tane A., Yokosuka T., Sakata-Sogawa K., Sakuma M., Ishihara C., Tokunaga M., Saito T. Dynein-driven transport of T cell receptor microclusters regulates immune synapse formation and T cell activation. ***Immunity*** 34, 919-931(2011)
3. Kong K-F., Yokosuka T., Canonigo-Balancio A J., Isakov N., Saito T., Altman, A. A motif in the V3 domain of the kinase PKC-θ determines its localization in the immunological synapse and functions in T cells via association with CD28. ***Nat Immunol.*** 12, 1105 -1112 (2011)

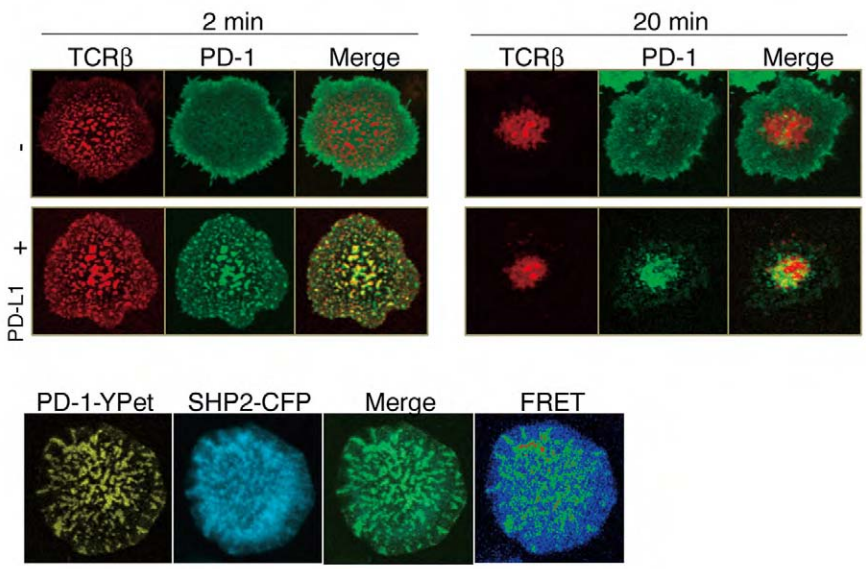


Figure: **Dynamic regulation of PD-1-mediated inhibition of T cell activation through PD-1-microclusters**
(*Top*) AND-TCR transgenic T cells expressing PD-1-GFP and pre-stained DyLight-anti-TCRβ Fab were stimulated on a planar bilayer containing PD-L1. Real time imaging analysis revealed that PD-1 (*green*) was initially (2 min) co-localized with TCR-microclusters (*red*), and moves to the cSMAC at 20 min where PD-1 accumulates in the TCR^{lo} area, where CD28 and CTLA4 also accumulate. For PD-1-mediated suppression, it is critical to be within the same TCR-microclusters. (*Bottom*) T cells expressing both PD-1-YPet and SHP2-CFP were stimulated on the planar membrane with specific peptide-MHC and analyzed for specific FRET between PD-1 and SHP2. SHP2 was transiently recruited to TCR-microclusters upon stimulation in a PD-L1-dependent manner and dephosphorylated upstream signaling molecules.

semble signaling molecules and induce activation signals, which then translocate into the center of the IS and form the central supramolecular activation cluster (cSMAC).

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

Regulation of T cell activation and differentiation

We have analyzed genes up-regulated during T cell development. To this end, we found that the putatively B cell-specific transcription factor Bach2 is well-expressed even in T cells and up-regulated during T cell differentiation. Bach2 deficiency resulted in the reduction of naïve T cells and up-regulation of genes related to effector-memory T cells, particularly to Th2-type cells in naïve T cells. Bach2-deficient T cells induce rapid secretion of Th2 cytokines and memory-effector related molecules. We identified the direct Bach2 target genes, which are also regulated in effector-memory cells. Collectively, Bach2 is a critical repressor that maintains the naïve state of T cells by suppressing effector-memory related genes.

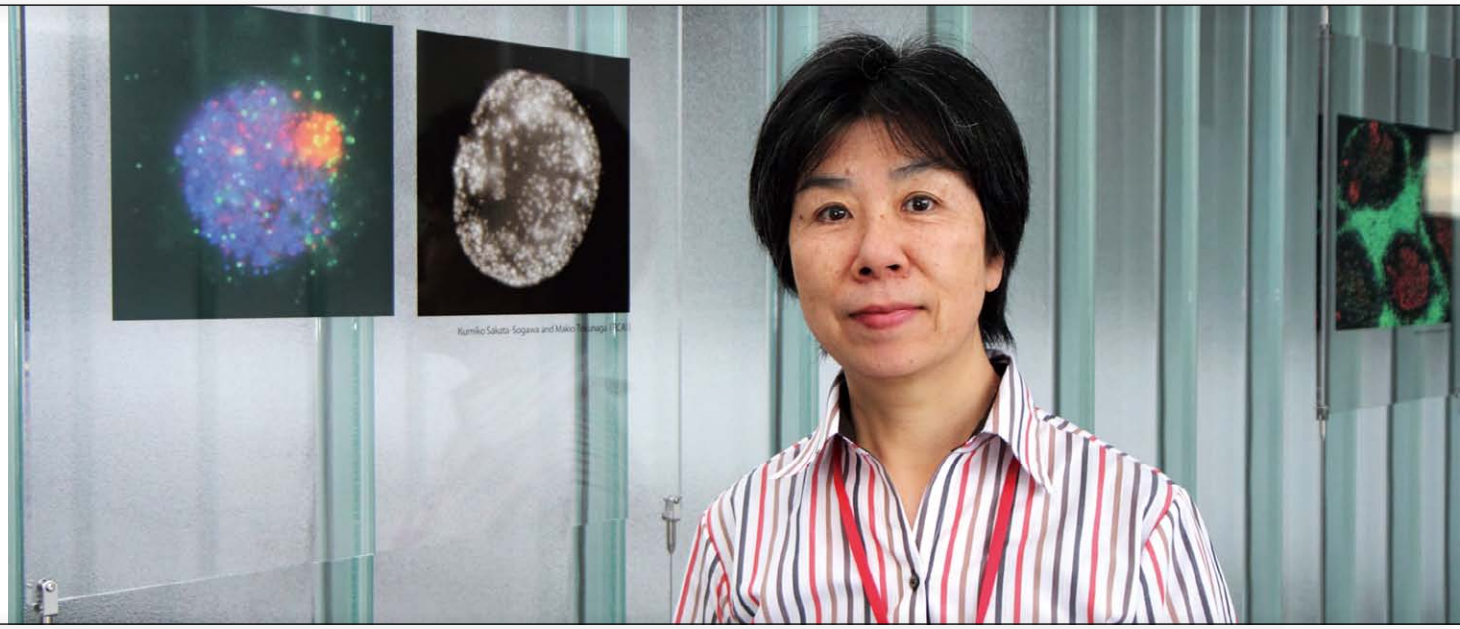
We have also analyzed the regulation of T cell activation by signals important in innate immunity. After we identified the critical roles of IRAK4 in T cell activation, we analyzed the function of innate signal molecules, particularly TLRs and RLRs expressed in T cells. Initial analysis revealed that TLR2 directly activates Th1 cells to induce cytokine production independently of TCR stimulation. Whereas T cell co-stimulation through TLR2 is TLR/MyD88-dependent, surprisingly, the co-stimulation of T cells by nucleic acids was found to be independent of MyD88/TRIF. We found that for the optimal T cell co-stimulatory activity, nucleic acids have to be aggregated by DNA-binding histones or other proteins. The direct co-stimulation of T cells by nucleic acids independently of the recognition by dendritic cells/macrophages provides new insights into *in vivo* induction of inflammation and related immunological diseases.

4. Yokosuka T., Kobayashi W., Takamatsu M., Sakata-Sogawa K., Zeng H., Hashimoto-Tane A., Yagita H., Tokunaga M., Saito T. Spatiotemporal basis of CTLA-4 costimulatory molecule-mediated negative regulation of T cell activation. ***Immunity*** 33, 326-339 (2010)

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

Research Unit for

Single Molecule Imaging



Unit Leader: **Kumiko Sakata-Sogawa**

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

The main target 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 (HILO) for use in single cell/single molecule studies. HILO has an advantage when it comes to observations inside cells with 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 a quantitative approach 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.

Spatial-temporal regulation of NF-κB inactivation by PDLIM2

NF-κB is an important transcription factor that activates expression of inflammatory genes in response to various stimuli. Although the inflammatory response is an essential reaction against invaders, overreaction could damage the host. Accordingly, both activation and suppression are inevitable.

In resting cells, NF-κB is sequestered in the cytoplasm by binding to IκBα. Stimulation leads to phosphorylation of Ser residues of IκBα and the subsequent ubiquitination results in degradation by the proteasome. As a result of IκBα degradation, liberated NF-κB translocates to the nucleus and activates expression of target genes, including the IκBα gene. The nascent IκBα binds to nuclear NF-κB and exports it to the cytoplasm, resulting in negative feedback loop. In addition to this canonical IκBα regulatory pathway, PDLIM2 was found to work as an E3 ubiquitin

Recent Publications

1. Hotta K, Nashimoto A, Yasumura E, Suzuki M, Azuma M, Iizumi Y, Shima D, Nabeshima R, Hiramoto M, Okada A, Sakata-Sogawa K, Tokunaga M, Ito T, Ando H, Sakamoto S, Kabe Y, Aizawa S, Imai T, Yamaguchi Y, Watanabe H, Handa H.: Vesnarinone Suppresses TNFa mRNA Expression by Inhibiting Valosin-containing Protein. ***Mol Pharmacol.*** 83, 930-938 (2013)
2. Hashimoto-Tane-A. ,Yokosuka T., Sakata-Sogawa K., Sakuma M., Ishihara C., Tokunaga M., Saito T.: Dynein-driven transport of T cell receptor microclusters regulates immune synapse formation and T cell activation. ***Immunity*** 34, 919-931 (2011)
3. Yokosuka T., Kobayashi W., Takamatsu M., Sakata-Sogawa K., Zeng H., Hashimoto-Tane A., Yagita H., Tokunaga M., Saito T.: Spatiotemporal basis of CTLA-4-mediated negative regulation of T-cell activation. ***Immunity*** 33, 326-339 (2010)

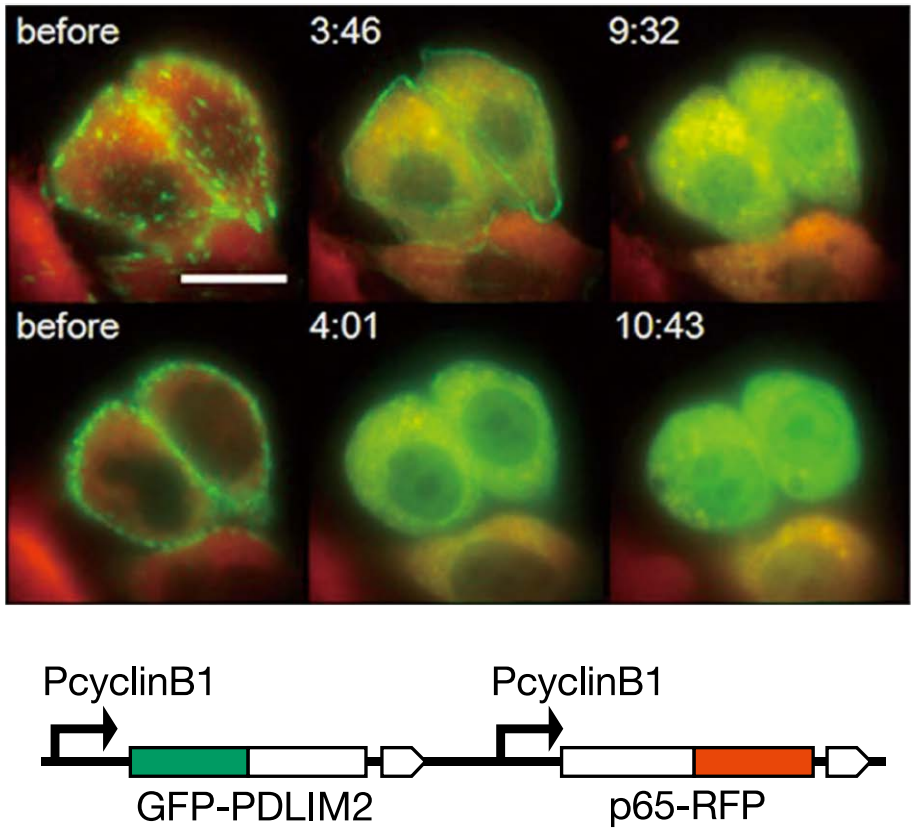


Figure : **PDLIM2 translocates into the nucleus upon stimulation with PMA and ionomycin.** CHO cells expressing GFP-PDLIM2 and p65-RFP are imaged at the indicated time points after stimulation. The upper images are focused on the cell membrane. The lower images are focused on the nucleus. Bar: 10 μm

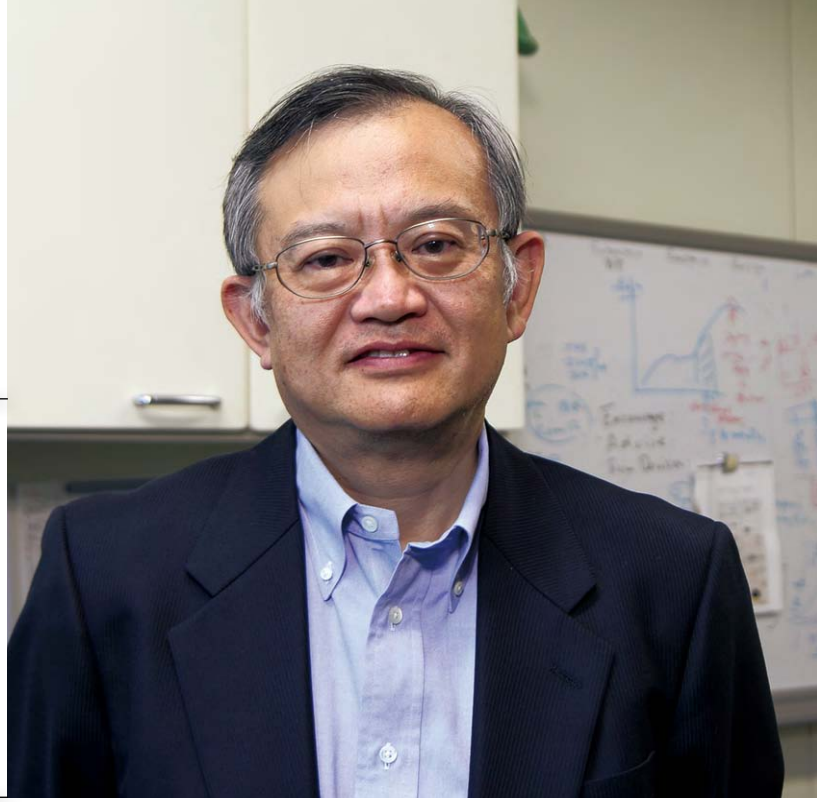
ligase responsible for the regulation of NF-κB activation (Tanaka, T. *et al. Nat Immunol.*, 2007). Despite the important role of PDLIM2 as a regulatory protein of inflammation, little is known about its regulation mechanism.

Aiming to investigate why PDLIM2 works only after stimulation, we performed a series of imaging analyses. When a GFP-PDLIM2 fusion protein was expressed in cultured cells, the protein was localized, not in the nucleus, but at the stress fibers near the plasma membrane. In fact, PDLIM2 has been reported to bind α-Actinin1, an actin binding protein, and to play an important role in cell adhesion and cell migration. Recent studies of monocytes indicates that PDLIM2, located in the nucleus in the immature state, is accumulated in the cytoplasm after PMA-induced differentiation and nuclear activity of NF-κB is increased. When we stimulated the GFP-PDLIM2 expressing cells with PMA and ionomycin, PDLIM2 was translocated into the nucleus within 10 min (Fig). These findings suggest that the function of PDLIM2 is in tight relation to its localization in the cell. This finding clearly explains why PDLIM2 degrades NF-κB only after stimulation. If PDLIM2 was free in the cytoplasm, it might ubiquitinate NF-κB there.

The PDLIM2 molecule consists of PDZ and LIM domains. We expressed the GFP fusion protein of the mutant PDLIM2 protein (ΔPDZ) in HeLa cells and observed a homogenous distribution including the nucleus. This indicates that the PDZ domain is responsible to the sequestration at the stress fibers. Next we tested the effect of mutation of serine phosphorylation sites in the PDZ domain. We mutated 5 serine residues into alanine or aspartic acid. Although the alanine substitutions showed no difference from WT, mutation into aspartic acid resulted in a homogenous distribution of PDLIM2. This result suggests that PDLIM2 localization is regulated by the phosphorylation of serine residue(s) in the PDZ domain.

Laboratory for

Lymphocyte Differentiation



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Ryo Shinnakasu

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Takeshi Inoue, Masanori Matsumoto
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Technical Staff : **Yoko Fujii, Shiori Maeda, Miwako Tochigi**

B cells play an essential role in the regulation of immune responses. Upon first encountering their cognate antigen, B cells exert multiple functions including antibody production, antigen-presentation, and induction of T cell differentiation. When B cells recognize the same antigen the second time, memory antibody responses can be induced. These are typically seen in the response to T-cell-dependent antigens and are characterized by the rapid production of high-titers of high-affinity antigen-specific antibody. Classical studies have focused on isotype-switched memory B cells (mainly of the IgG isotype). However, recent advances in monitoring antigen-experienced B cells have revealed the existence of un-switched IgM type memory B cells, suggesting the functional heterogeneity of memory B cells. Our laboratory has now focused on clarifying the functional differences between IgM and IgG type memory B cells and on revealing the mechanisms underlying the robustness of memory antibody responses.

Unique function of IgM memory B cells

Fate mapping-methods have allowed us to monitor both un-switched (IgM-type) and switched (IgG-type) memory B cells after injection of NP-CGG into mice. When B cells encounter antigens *in vivo*, almost all the cells induce AID at the transcriptional level. Thus, we can genetically label the antigen-experienced B cells by using AID expression. Employing this system, we have isolated two subsets of IgM type memory B cells (IgM^+IgD^+ and IgM^+IgD^-) and clarified that IgM^+IgD^+ memory B cells undergo somatic mutation, whereas the IgM^+IgD^- cells do not. These observations suggest that IgM type memory B cells are generated through both GC-dependent (IgM^+IgD^+) and independent (IgM^+IgD^-) pathways.

When IgM type memory B cells were adoptively transferred together with activated T cells, these B cells re-initiate the GC reaction. In contrast to IgM type memory B cells, IgG type memory B cells showed less expansion activity and have a high propensity to differentiate into plasma cells (Figs. 1 and 2). Thus, IgM type memory B cells can be considered as a more central type of memory cells.

Furthermore, activation of IgM type, but not IgG type, memory B cells was severely inhibited by addition of anti-NP specific IgG antibodies, suggesting that only IgM type memory B cells are negatively regulated by generated Abs. Thus, the following scenario can be envisaged; once titers of antigen-specific IgG Abs that are produced in the primary response are sufficiently reduced, then IgM type memory B cells could get activated. Together, the above results suggest that IgM and IgG type memory B cells have distinct features.

Contribution of the transcription factor Bach2 to generation and function of IgG type memory B cells

Two-non-mutually exclusive models (BCR-intrinsic and

Recent Publications

1. Yasuda T., Kometani, K., Takahashi, N., Imai, Y., Aiba, Y. and Kurosaki, T. Erk kinases control plasma cell differentiation by regulating expression of Blimp-1. ***Sci Signal.*** 4, (169) ra25 (2011)
2. Kometani, K., Yamada, T., Sasaki, Y., Yokosuka, T., Saito, T., Rajewsky, K., Ishiai, M., Hikida, M. and Kurosaki, T. CIN85 drives B cell responses by linking BCR signals to the canonical NF-κB pathway. ***J Exp Med.*** 208, 1447-1457 (2011)
3. Onodera, T., Takahashi, Y., Yokoi, Y., Ato, M., Kodama, Y., Hachiman, S., Kurosaki, T. and Kobayashi, K. Memory B cells in the lung participate in protective humoral immune responses against recurrent pulmonary influenza virus infection. ***Proc Natl Acad Sci USA.*** 109, 2485-2490 (2012)

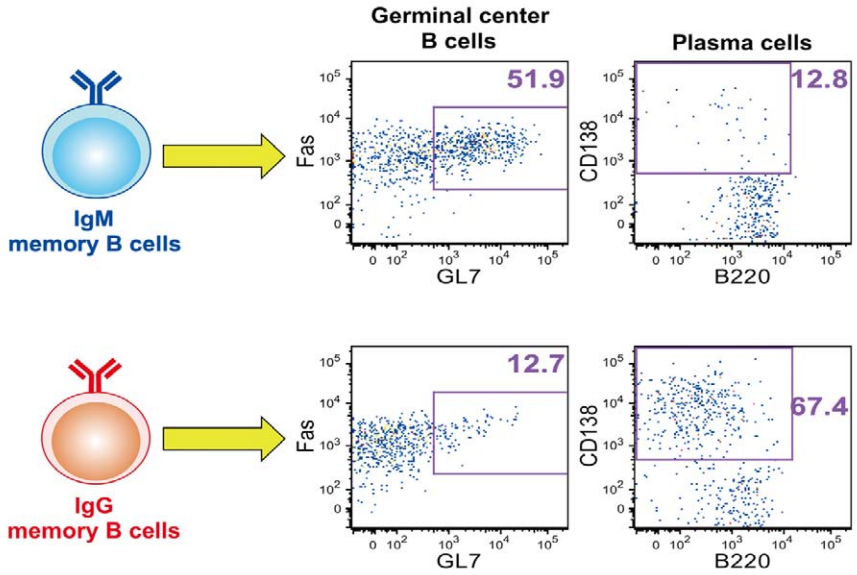

Figure 1: Differentiation of IgM and IgG memory B cells after antigen re-exposure
IgM type memory B cells highly re-enter the germinal center (GC) reaction, but are less able to differentiate into plasma cells after re-exposure to the antigen. IgG type memory B cells show less expansion and preferentially differentiate into plasma cells.

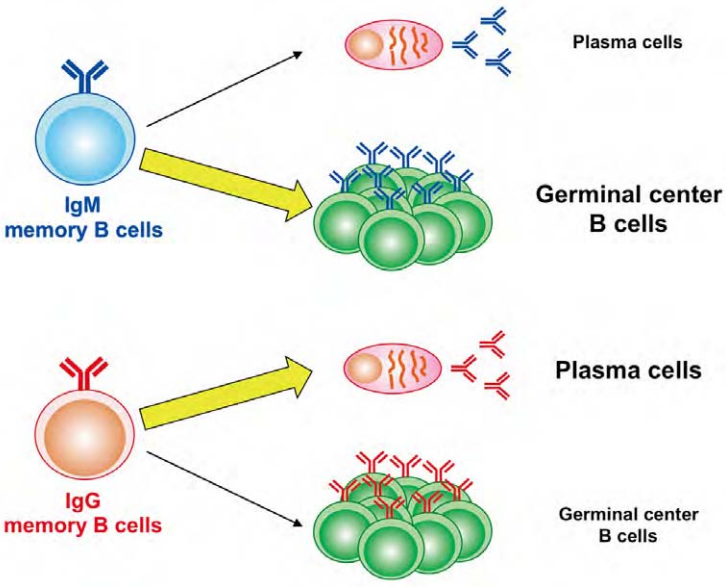

Figure 2: IgM and IgG memory B cells differentiate via distinct pathways.
IgM type memory B cells mainly differentiate into germinal center B cells and generate descendent memory B cells. On the contrary, IgG type memory B cells preferentially differentiate into plasma cells and protect our body from pathogens.

BCR-extrinsic) to explain the robust antibody responses of IgG type memory B cells have been long debated. By establishing a mouse model in which antigen-non-experienced B cells express an IgG1 BCR, we previously addressed this question, and showed that a BCR-intrinsic mechanism alone is not sufficient to explain the heightened differentiation activity of IgG type memory B cells. As an extrinsic model, we proposed that reorganization of transcription factors such as Bach2 takes place during generation of IgG1 type memory B cells after primary antigen exposure, and is critical for rapid responsiveness of IgG1 memory B cells (BCR-extrinsic model).

To directly test our model, we first demonstrated that the level of Bach2 was repressed after antigen encounter. Then we have established mice in which Bach2 could be overexpressed or knocked out in an inducible manner. When Bach2 was overexpressed after IgG1 class-switching, more IgG1 type memory B cells were generated, but the secondary Ab responses were decreased. These results clearly suggest that the expression level of Bach2 is involved in generation of memory B cells as well as in their activation.

4. Dong, Z., Davidson, D., Perez-Quintero, L.A., Kurosaki, T., Swat, W., and Veillette, A. The Adaptor SAP Controls NK Cell Activation by Regulating the Enzymes Vav-1 and SHIP-1 and by Enhancing Conjugates with Target Cells. ***Immunity*** 36, 974-985 (2012)

5. Takahashi, Y., Onodera, T., Kobayashi, K., and Kurosaki, T. Primary and Secondary B-Cell Responses to Pulmonary Virus Infection. ***Infect Disord Drug Targets.*** 12, 232-240 (2012)

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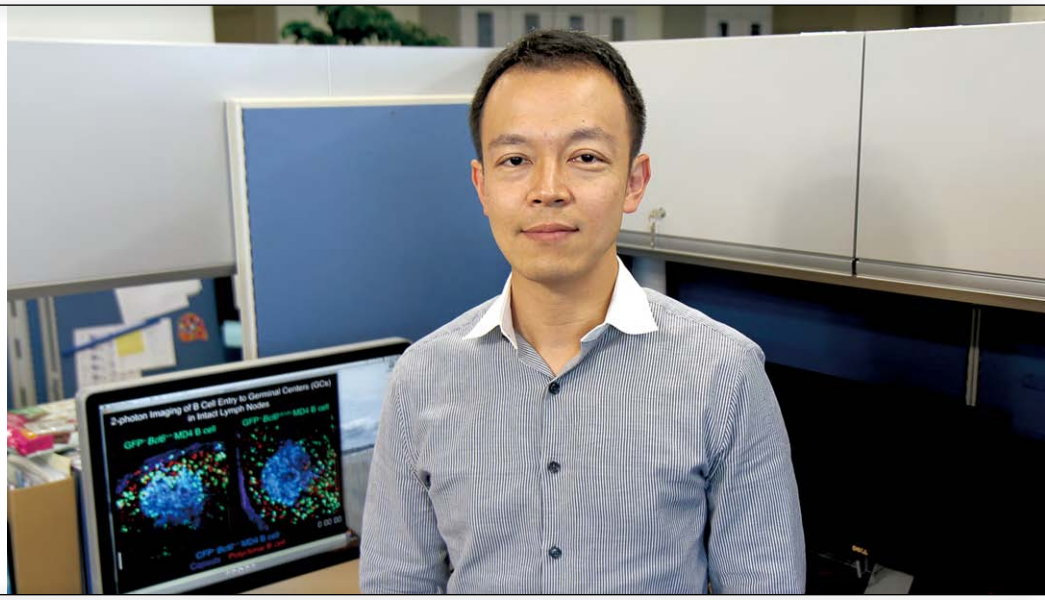

The goal of our research is to understand the mechanisms regulating cell migration and interactions in the tissues that shape immune responses. For this purpose we use real time imaging, in particular two-photon microscopy, to analyze *in vivo* cellular migration and interactions. This microscopy method, which was introduced recently to the field of immunology, has been revealing striking dynamics of immune cells in the lymphoid organs, underlining the importance of this approach to resolve the complexity of the immune system. Last year, we started new projects to understand mechanisms controlling dynamics of two different T cell types, follicular helper T (Tfh) cells and cytotoxic T cells, which play pivotal roles in humoral and cellular immunity, respectively. This year, we have continued to work on these two topics.

Dynamics of GC Tfh cells

Tfh cells are essential for initiation and maintenance of T-dependent B cell responses, particularly germinal center (GC) reactions, which ultimately lead to long-term, high affinity antibody production. A subpopulation of Tfh cells that has physical access to the GC is believed to be responsible for controlling GC reactions. However, little has been known about mechanisms that regulate dynamics of GC Tfh cells. Last year, we performed two-photon imaging analysis of the migration of Tfh cells within the GC area and the surrounding follicular mantle (FM). The analysis clearly showed that Tfh cells in the GC and those in the FM tended to be retained in the respective regions, suggesting differential expression of molecules responsible for their localization. Then, we performed gene expression analysis of Tfh cells harboring hypomorphic mutations in the gene encoding Bcl6, which is the essential transcription factor for Tfh cells, because the Bcl6 hypomorphism led to a selective reduction of GC Tfh cells while leaving FM Tfh cells relatively intact. As a result of this gene expression analysis, we found that expression of a G coupled-receptor gene might be enriched in GC Tfh cells. Then, we generated a fluorescent reporter animal for this gene. By using the reporter mouse, we could show by histology that a Tfh cell subset highly expressing this G protein-coupled receptor was localized in the GC but not in the FM. Furthermore, deficiency of the receptor led to a reduction of the Tfh cell number within GCs. Two-photon imaging analysis revealed that the reduction of GC Tfh cells was due to impaired retention of Tfh cells in the GC. We are currently investigating the impact of this impaired Tfh cell retention in the GC on long-term antibody responses.

Overall, our work during the last several years contributed to understanding of the relationship between Bcl6

Recent Publications

1. Okada T., Moriyama S., Kitano M. Differentiation of germinal center B cells and follicular helper T cells as viewed by tracking Bcl6 expression dynamics. ***Immunol Rev.*** 247, 120-132 (2012)
2. Hirata E., Yukinaga H., Kamioka Y., Arakawa Y., Miyamoto S., Okada T., Sahai E., Matsuda M. In vivo fluorescence resonance energy transfer imaging reveals differential activation of Rho-family GTPases in glioblastoma cell invasion. ***J Cell Sci.*** 125, 858-868 (2012)
3. Kitano M., Okada T. Four-dimensional tracking of lymphocyte migration and interactions in lymph nodes by two-photon microscopy. ***Method Enzymol.*** 506, 437-454 (2012)

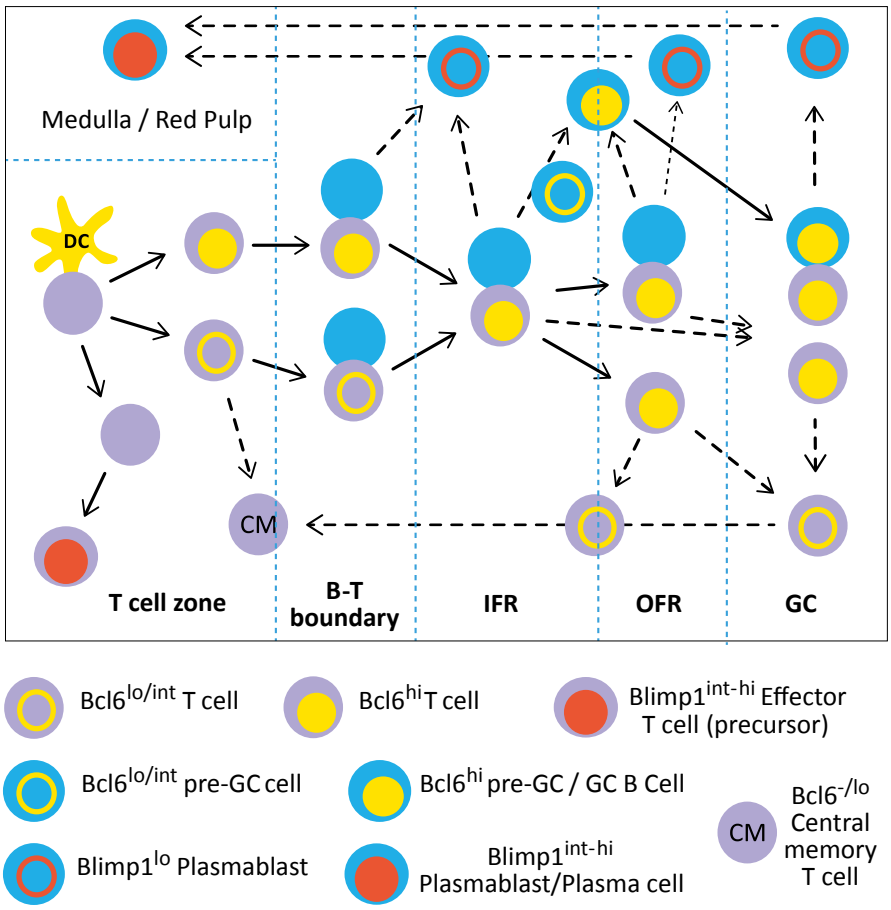

Figure : **Change label: Bcl6$^{lo/int}$pre-GC B cell**
A model for the relationship between antigen-specific interactions and differentiation of CD4$^+$ T cells and B cells in various microenvironments. Solid arrows indicate the connections supported by relatively direct evidence. Dashed arrows indicate possible connections suggested by circumstantial evidence. IFR: inter-follicular region, OFR: outer follicular region.

expression dynamics and Tfh cell differentiation. It is now becoming clear that Tfh cells contain diverse subsets with different localization and different roles in B cell responses. Furthermore modulation of Bcl6 expression is proposed to be an important event for development of memory helper T cells from Tfh cells (Fig.).

Cellular interaction dynamics during cytotoxic T cell differentiation

Cytotoxic T cells are known to be generated through interactions of antigen-specific CD8$^+$ T cells with dendritic cells (DCs) that cross-present antigens. In skin-draining lymph nodes, there are two subsets of cross-presenting DCs, namely CD103$^+$ migratory DCs immigrating from the skin and CD8$^+$ lymph node-resident DCs. However, the spatio-temporal regulation of their interactions with CD8$^+$ T cells is poorly understood. In collaboration with Dr. Kaisho's group (Lab for Host Defense, RIKEN RCAI; and Immunology Frontier Research Center, Osaka University), we developed novel methods that allow visualization of these two subsets of cross-presenting DCs in the lymph node. Two-photon microscopy analysis suggested differential contribution of the cross-presenting DC subsets to activation of antigen-specific CD8$^+$ T cells. Furthermore, our results have begun to reveal a previously unknown compartmentalization of the DC subsets within the T cell zone of the lymph node. The physiological significance of this compartmentalization is currently under investigation.

4. Baumjohann D, Okada T, Ansel KM. Cutting Edge: Distinct waves of BCL6 expression during T follicular helper cell development. ***J Immunol.*** 187, 2089-2092 (2011)

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

Single Molecule Imaging and Molecular Quantification in Cells

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

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

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

Reduction of the background intensity of images yields clear images. The background intensity of images depends on the volume of the illuminated region in specimens. To examine the thickness of the illuminated region, we obtained intensity profiles of illumination along the z-direction, that is, the depth direction in specimens. We devised a method to decrease the illumination thickness by

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3. Yokosuka T., Kobayashi W., Takamatsu M., Sakata-Sogawa K., Zeng H., Yagita H., Tokunaga M., Saito T.: Spatiotemporal basis of CTLA-4-mediated negative regulation of T-cell activation. ***Immunity*** 33, 326-339 (2010)

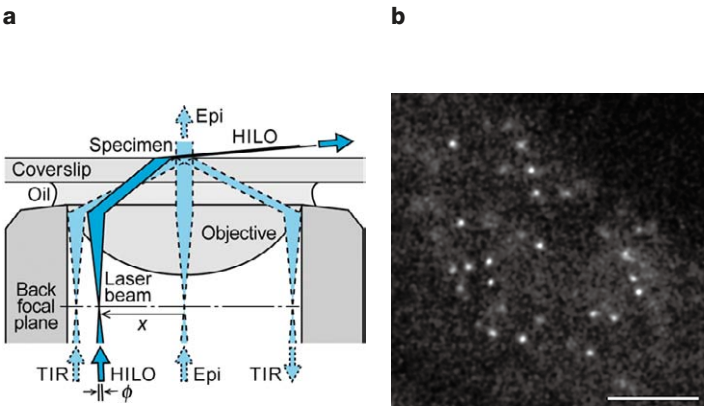

Figure 1: Molecular imaging enables one to visualize and quantify molecular dynamics, interactions, and kinetics in cells for molecular systems biology. (a) HILO microscopy for molecular imaging in cells. Illumination by a highly-inclined and thin beam increases image intensity and decreases background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

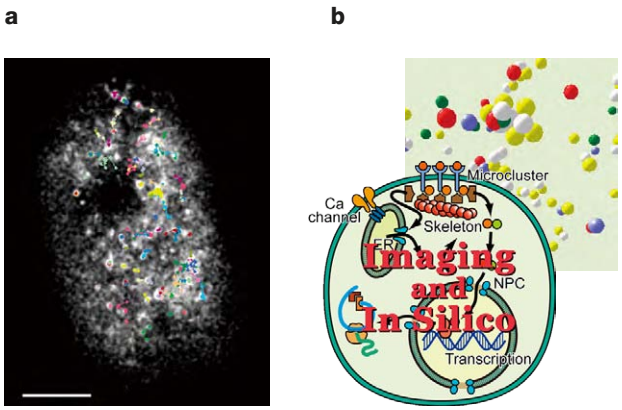

Figure 2: The combination of single molecule quantification and *"in silico"* modeling opens new approaches for developing molecular systems biology. (a) Automatic tracking of single molecules of the Sp1 transcription factor visualized in a living cell. Molecular dynamics and interactions can be quantified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

narrowing the illumination area using a field stop. Notably, we have achieved the reduction of the illumination thickness to less than 10 μm (for example, 7 μm of thickness with a field stop for 20 μm-diameter illumination).

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

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

"*In silico*" Modeling and Simulation

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

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

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

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The immune system plays a critical role in host defense against invading microbes. Immune reactions are also involved in the onset and pathophysiology of many diseases including allergic and autoimmune diseases. Different types of both innate and adaptive immune cells interact with each other in order to induce complex immune reactions. Recent studies have identified multiple subsets among innate lymphocyte corresponding to Th subsets of the adaptive immune system. NK cells, LTi cells and NH cells play distinct roles in innate immune responses by producing Th1, Th17 and Th2 cytokines, respectively. Cooperation between these innate lymphocytes and antigen-specific T and B cells are likely important in various inflammatory responses including protective immunity against various microbes and allergic diseases such as asthma. We are elucidating the immune cell network in order to understand how immune cells induce complex immune reactions in our body.

Studies on natural helper (NH) cells

We currently focus on an innate lymphocyte population that we first identified in a new lymphoid tissue in mouse and human mesentery that we termed fat-associated lymphoid cluster (FALC). We discovered in the FALC a new innate lymphocyte subset producing Th2 cytokines and named this new lymphocyte “Natural Helper (NH) Cell”. NH cells constitutively produce IL-5, IL-6 and IL-13 and support the proliferation of B1 cells in the peritoneal cavity and IgA production. Upon helminth infection or allergic response, NH cells respond to a combination of IL-2 and IL-25 or IL-33 to produce large amounts of Th2 cytokines, most notably IL-5 and IL-13, which induce eosinophilia and goblet cell hyperplasia and play an important role in anti-helminth immunity and pathogenesis of allergic diseases in the lung such as asthma (Fig. 1). After we reported NH cells, other groups also reported novel Th2 cytokine producing innate cells, such as MPP^{type2} cells, nuocytes, and innate helper type 2 (Ih2) cells. There are similarities and differences among these newly identified cell populations and NH cells, e.g., MPP^{type2} cells can differentiate into other myeloid cells, making this cell type distinct from the others. MPP^{type2} cells, nuocytes, and Ih2 cells were reported to respond to IL-25 alone, but NH cells do not respond to IL-25 without IL-2 but respond strongly to IL-33. Localization of NH cells in FALC is also one of intriguing differences from the other cell types, which are found in lymph node and/or spleen.

Studies on Th17 cells

In the adaptive immune system, we have been interested in the role and differentiation of IL-17-producing helper T (Th), Th17. We have shown that the phosphoinositide 3-kinase (PI3K)-Akt-mammalian target of rapamycin complex 1 (mTORC1) axis plays a critical role in the differentiation of Th17 cells. We demonstrated that the suppression of the PI3K-Akt-mTORC1 axis by deletion of p85α or with PI3K/

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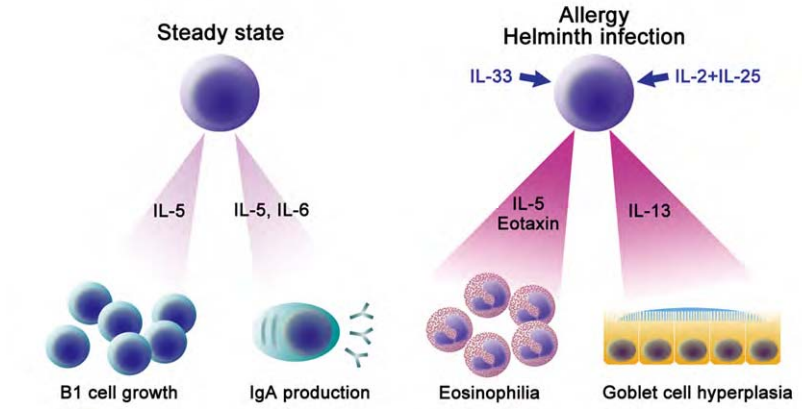

Figure 1: Functions of NH cells
(a) NH cells constitutively produce IL-5 and IL-6 and support IgA production by B cells. In addition, IL-5 supports the growth of B1 cells in the peritoneal cavity. (b) Upon helminth infection, IL-33 produced by epithelial cells, endothelial cells and/or adipocytes induces high levels of IL-5, eotaxin and IL-13 production by NH cells, resulting in eosinophilia and goblet cell hyperplasia, which play important roles in the early phase of anti-helminth immune responses. Similarly, a combination of IL-2 and IL-25 also induces large amounts of IL-5 and IL-13 from NH cells.

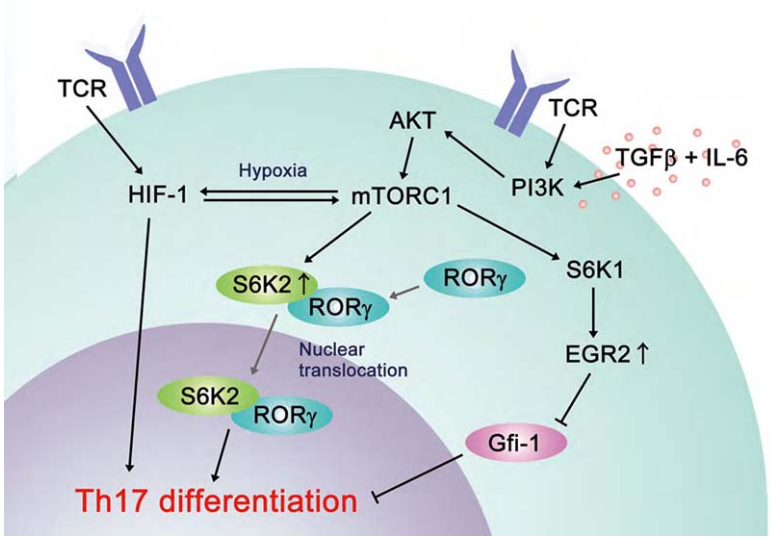

Figure 2 : The PI3K-Akt-mTORC1 axis and HIF-1 play critical roles in Th17 induction.
Downstream of the PI3K-Akt-mTORC1 axis, S6K1 downregulates Gfi1, a negative regulator of Th17 differentiation, and S6K2, a nuclear counterpart of S6K1, binds RORγ, a critical transcription factor for Th17 differentiation, and carries it to the nucleus. In addition, a positive feedback loop between HIF-1 and mTORC1 accelerates Th17 cell differentiation.

mTORC1 inhibitors, as well as T cell-specific deletion of raptor, an essential component of mTORC1, impairs Th17 differentiation *in vitro* and *in vivo* in a S6K1/2-dependent fashion. Inhibition of the PI3K-Akt-mTORC1-S6K1 axis impairs the downregulation of Gfi1, a negative regulator of Th17 differentiation. Furthermore, S6K2, a nuclear counterpart of S6K1, is induced by the PI3K-Akt-mTORC1 axis, binds RORγ, and carries RORγ to the nucleus. These results point toward a pivotal role of the PI3K-Akt-mTORC1-S6K1/2 axis in Th17 differentiation. During this study, we have noticed that, in addition to mTORC1, hypoxia inducible factor-1 (HIF-1, consisting of HIF-1α and HIF-1β) regulates Th17 differentiation positively as well. Oxygen (O_2) tension is generally low in these secondary lymphoid tissues compared to the bloodstream or atmosphere. However, the effect of changes in O_2 concentration on the differentiation of Th cells remains unclear. We primed naïve $CD4^+$ T cells under 5% O_2, which has been observed in the lymph node and spleen, and then reoxygenated the cultures under normoxia that mimicked the O_2 concentration in blood. In this model, the differentiation of Th17 cells, but not Th1 or iTreg cells, was enhanced. At 5% O_2, mTORC1 was activated and this led to the stabilization of HIF-1α in Th17 cells. The activation of mTORC1 and the acceleration of Th17 cell differentiation, which occurred when cells were primed under 5% O_2, were not observed in the absence of HIF-1α but were accelerated in the absence of von Hippel-Lindau tumor suppressor protein (vHL), a factor critical for HIF-1α degradation. Thus, a positive feedback loop between HIF-1α and mTORC1 induced by hypoxia followed by reoxygenation accelerates Th17 cell differentiation (Fig. 2).

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5. Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, Wei D, Goldfarb KC, Santee CA, Lynch SV, Tanoue T, Imaoka A, Itoh K, Takeda K, Umesaki Y, Honda K, Littman DR. Induction of intestinal Th17 cells by segmented filamentous bacteria. ***Cell*** 139, 485-98 (2009)

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

Understanding of M-cell differentiation steps

M cells are atypical epithelial cells specialized for uptake of luminal antigens, including the phagocytosis of viruses and bacteria, to deliver them to dendritic cells that accumulate beneath the FAE. Recently, Dr. Ifor I. Williams' group discovered that RANKL expressed by the stromal cells underneath the FAE is essential for the induction of M cells. They have further shown that systemically administered exogenous recombinant RANKL induces ectopic M-cell differentiation in the small intestinal villi. In collaboration with Dr. Williams, we employed RANKL-induced *in vivo* M-cell differentiation and identified Spi-B as an M-cell specific transcription factor highly induced within a few hours upon RANKL treatment. In collaboration with Dr. Tsuneyasu Kaisho, who has established Spi-B KO mice, we have found that there are no mature M cells in FAE of the KO mice. Accordingly, translocation of *Salmonella enterica* serovar Typhimurium and *Yersinia enterocolitica* into PP is markedly diminished in Spi-B KO mice compared to wild-type mice. As a consequence, *S. enterica* Typhimurium-specific T-cell activation in PP is also strongly sup-

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2. Nakato G, Hase K, Suzuki M, Kimura M, Ato M, Hanazato M, Tobiume M, Horiuchi M, Atarashi R, Nishida N, Watarai,M, Imaoka K, Ohno H *Brucella abortus* exploits a cellular prion protein on intestinal M cells as an invasive receptor. ***J Immunol.*** 189, 1540-1544 (2012)
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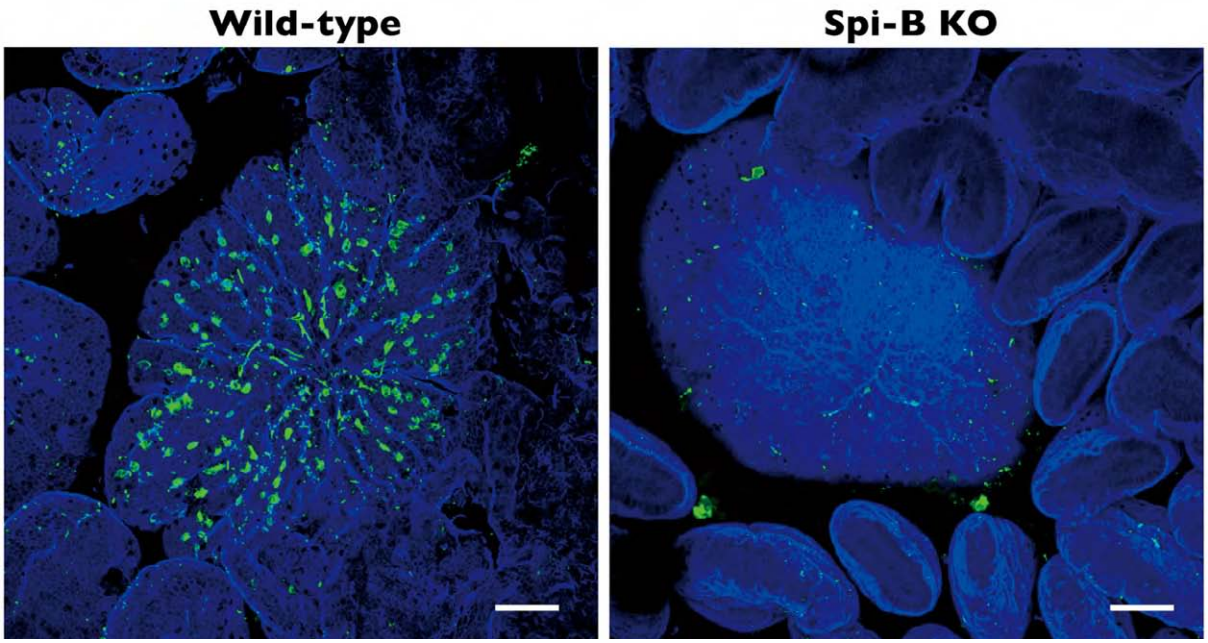

Figure: **Lack of M cells in Spi-B KO mice**
Whole mount GP2 staining of PPs from wild-type and Spi-B KO mice was performed. The FAE of Spi-B KO mice is devoid of GP2$^+$ mature M cells. GP2 is shown in green, while F-actin is stained in blue. Scale bar: 80μm.

pressed. These observations clearly indicate that Spi-B serves as a key transcription factor intrinsic to intestinal epithelial cells for M-cell differentiation, and that Spi-B KO mice provide a useful animal model for elucidating *in vivo* roles of M cells.

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

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

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The gut is colonized by trillions of bacteria that perform vital metabolic and defensive functions for the host. These bacteria, generally called indigenous bacteria or the microbiota, coevolved with mammals and for this reason are integral in digestive and immune system function and homeostasis. Besides serving as a natural defense against invading pathogens, indigenous bacteria make important contributions to nutrient processing, generation of immunomodulatory products (vitamins, short-chain fatty acids etc.) and to the development of the immune system. In spite of having available a wealth of immune sensing and effector mechanisms capable of triggering inflammation in response to microbial intrusion, we can live together with our body's bacteria without any adverse effects. This is made possible by a continuous "dialog" between bacteria and host cells that generates finely tuned signaling programs ensuring a state of non-responsiveness against dietary antigens and controlled responses against indigenous bacteria. In turn, the immune system maintains diverse and healthy bacterial communities in the gut, facilitated by innate and adaptive immunity, by mechanisms that remained to be clarified.

Our long-term goal is to understand the host-microbial relationship in the gut and to apply such knowledge for health maintenance and disease prevention/treatment.

Biology of gut germinal centers: role of PD-1 in selection of IgAs in germinal centers of Peyer's patches

Immunoglobulin (Ig) A, the most abundantly produced antibody isotype in mammals, is secreted into the gut lumen as dimeric molecules linked by a 'joining' chain [also called secretory (S)IgA]. The protective role of SIgA was elucidated in the context of mucosal infections. However, it is increasingly clear that IgA was selected and maintained throughout evolution, not just to stop pathogen entry through the epithelium, but rather to maintain the complex interplay between microbiota, epithelium and the immune system.

Diversification of the IgA repertoire by somatic hypermutation (SHM) appears critical to maintain intestinal homeostasis. SHM takes place mostly in the specialized microenvironment of the germinal center (GC), in the presence of activation-induced cytidine deaminase (AID) and a subset of CD4 T cells named T follicular helper (T_{FH}) cells. T_{FH} cells are defined as T cells expressing high levels of the chemokine receptor CXCR5 and the inhibitory co-receptor programmed cell death-1 (PD-1). We sought to elucidate the role of PD-1 on T_{FH} cells and GC biology.

We discovered that, in gut, PD-1 is an essential component of the program that regulates IgA selection re-

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2. Wei M., Shinkura R., Doi Y., Maruya M., Fagarasan S., Honjo T. Mice carrying a knock-in mutation of Aicda resulting in a defect in somatic hypermutation have impaired gut homeostasis and compromised mucosal defense. ***Nat Immunol.*** 12, 264-70 (2011)
3. Maruya M., Suzuki K., Fujimoto H., Miyajima M., Kanagawa O., Wakayama T., Fagarasan S. Vitamin A-dependent transcriptional activation of the nuclear factor of activated T cells c1 (NFATc1) is critical for the development and survival of B1 cells. ***Proc Natl Acad Sci U S A***. 108, 722-727 (2011)

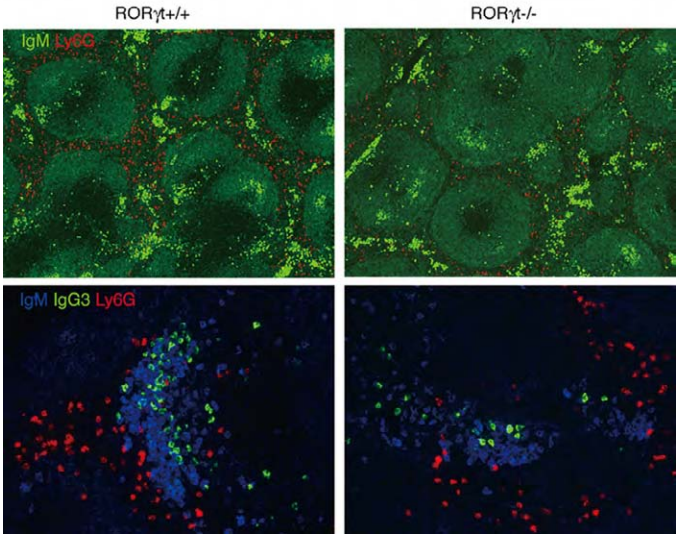

Figure : **Splenic ILCs are bridging innate with adaptive responses via neutrophils and marginal zone B cells by facilitating T-independent antibody production.** Representative sections of spleen stained as indicated, revealing reduced numbers of neutrophils associated with impaired IgG3-producing plasmablasts or plasma cells in RORγgt$^{-/-}$ mice.

quired for the maintenance of a fit microbiota. We found that PD-1 affects the dynamics of GC B cells by controlling the number and the nature of T helper cells in the PPs. The deregulation of the T cell compartment impacts on the selection of IgA plasma cells leading to gut dysbiosis. Defects in IgA selection, by perturbing the microbial composition and gut microenvironment, have the potential to increase autoimmune susceptibility. In several mouse models of autoimmune diseases, the development of the disease required intestinal bacteria and expansion of specific subsets of T helper cells (e.g., SFB and Th17). Indeed, PD-1-deficient mice develop autoimmune diseases only in the presence of microbiota and AID, suggesting that the autoreactive antibodies in PD-1-deficient mice may arise after AID-induced genetic alterations in GCs, which are driven by stimulation from the gut microbiota.

These findings suggest that the maintenance of appropriate composition and density of microbiota by dynamic IgA production in the gut confers resistance to autoimmunity in peripheral compartments (See Fig. on p. 2, *Creation of New Paradigms*). Our analysis of PD-1$^{-/-}$ mice opens new perspectives for studies aimed at understanding the contribution of gut microbiota to the development of autoimmune diseases.

Linking innate and adaptive responses: The role of innate lymphoid cells (ILCs) in conditioning marginal zone B cell antibody responses through granulocyte recruitment

Collaborative project with the laboratory of Andrea Cerutti, Institut Municipal d'Investigació Mèdica-Hospital del Mar, Barcelona, Spain

ILCs are innate effector cells whose development depends on the transcriptional repressor Id2 and the common γ-chain of the IL-2 receptor. However ILCs are rather heterogeneous and different ILC subsets show distinct transcription factor requirements and discrete cytokine secretion patterns, which mirror those of conventional T helper cells. ILC1 release interferon-γ and require the transcription factor T-bet, ILC2 secrete IL-5 and IL-13 and require the transcription factor GATA3. ILC3 represent cells that induce development of lymphoid structures (LTi) or secrete IL-22 and require the transcription factors RORγt and aryl hydrocarbon receptor (AhR).

We studied the spleen in RORγt-deficient mice lacking ILC3. RORγt-deficient mice have an overrepresented B cell compartment, yet they produce reduced homeostatic IgG3 antibodies and post-immune IgG3 responses to a TI antigen mimicking bacterial polysaccharides. This humoral deficiency depended on the lack of maturation and/or survival signals provided by splenic ILCs to IgG3-secreting plasmablasts and plasma cells (Fig.), because IgG3 class-switched B cells were conserved in RORγt-deficient mice. Indeed, splenic ILCs normally expressed APRIL, a CD40L (and BAFF)-related molecule that supports plasma cell survival.

Interestingly, in RORγt-deficient mice the impairment of IgG3 responses to TI antigens was associated with a deficiency not only of ILCs but also of neutrophils (Fig.). RORγt-deficient mice showed fewer neutrophils than either WT or T cell-deficient mice, suggesting that ILC3 contribute to recruitment, activation or survival of neutrophils in spleen. Certainly, ILCs produce the neutrophil-attracting chemokine IL-8 and the neutrophil-stimulating cytokine GM-CSF. In turn, the stimulated neutrophils release various cytokines including the proliferation/survival factor BAFF. Thus our study revealed that the deficiency of RORγt and ILCs impairs marginal zone (MZ) B cell responses in spleen, indicating that ILCs orchestrate an innate antibody-inducing network at the MZ-blood interface.

4. Suzuki K., Maruya M., Kawamoto S., Sitnik K., Kitamura H., Agace W.W., Fagarasan S. The sensing of environmental stimuli by follicular dendritic cells promotes immunoglobulin A generation in the gut. ***Immunity*** 33, 71-83 (2010)

5. 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 (2009)

Laboratory for

Immune Diversity

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

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

With the tools provided by completion of human and mouse genome projects, the availability of microarray data, and the technical advancements in generation of gene-manipulated mice, we have taken a molecular genetic approach to study B cell activation and differentiation. We performed comprehensive searches of the RCAI RefDIC and public microarray databases, and have identified several uncharacterized genes that are exclusively expressed in B cells, including the germinal center (GC) subset of B cells and other subsets. We have established knockout mice for these genes and obtained interesting preliminary results. For example, deficiency of a B-cell-specific transcription elongation factor caused a decrease of B cell survival after antigen stimulation and resulted in immune deficiency. Further analysis of these and additional mice with defective B cell-specific genes should allow us to uncover new pathways that regulate various aspects of B cell survival, activation, differentiation and antibody production.

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

High-affinity antibodies are central to humoral immunity. Somatic hypermutation (SHM) of Ig genes in GC B cells is an essential process for generating high-affinity B cells. SHM is initiated by the activation-induced cytidine deaminase (AID), which is thought to convert cytosine (C) to uracil (U) and generate U:G lesions on DNA. Consistent with its substrate specificity, ectopic expression of AID in fibroblasts induces mutations predominantly at C/G bases. However, remarkably, half of the mutations in the Ig genes of GC B cells are induced at non-targeted A/T bases and thus their origin is difficult to explain. We found that induction of A/T mutations is dependent on the GC B cell environment but independent of the target gene. We also found that high levels of AID caused the strand-bias of A/T mutations in Burkitt's lymphoma cells and established a model system to study this unsolved mystery in the hypermutation field.

Recent Publications

1. Kano, C. and Wang, J.-Y. High levels of AID cause strand bias of mutations at A versus T in Burkitt's lymphoma cells. ***Mol Immunol.*** 54, 397-402 (2013)
2. Li, S., Zhao, Y. and Wang, J.-Y. Analysis of Ig gene hypermutation in *Ung*$^{-/-}$*Polh*$^{-/-}$ mice suggests that UNG and A:T mutagenesis pathway target different U:G lesions. ***Mol Immunol.*** 53, 214-217 (2013)
3. Ouchida, R., Mori, H., Hase, K., Takatsu, H., Kurosaki, T., Tokuhisa, T., Ohno, H. and Wang, J.-Y. Critical role of the IgM Fc receptor in IgM homeostasis, B cell survival and humoral immune responses. ***Proc Natl Acad Sci U S A.*** 109, E2699-2706 (2012)

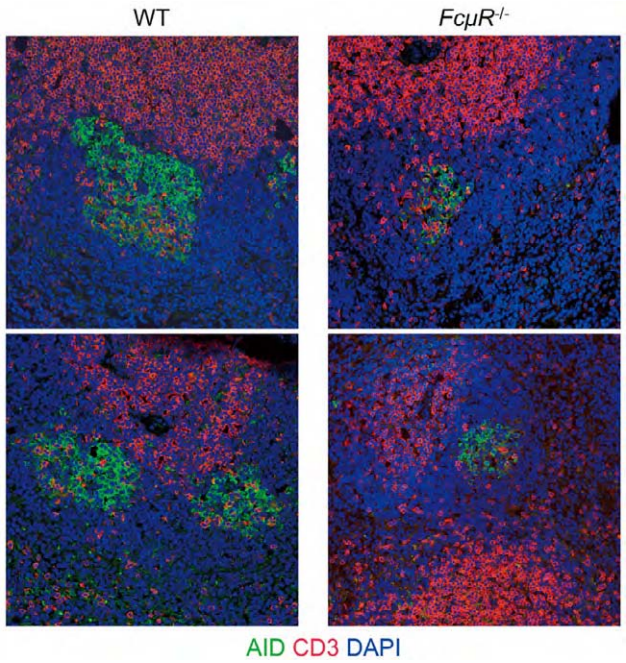

Figure 1: Impaired GC formation in *FcμR*$^{-/-}$ mice. WT and *FcμR*$^{-/-}$ mice were immunized with 10 μg of NP-chicken gamma globulin in alum and their spleen sections were stained with anti-AID to detect GC B cells and anti-CD3 to detect T cells. Representative staining results of 4 WT and 5 *FcμR*$^{-/-}$ mice are shown.

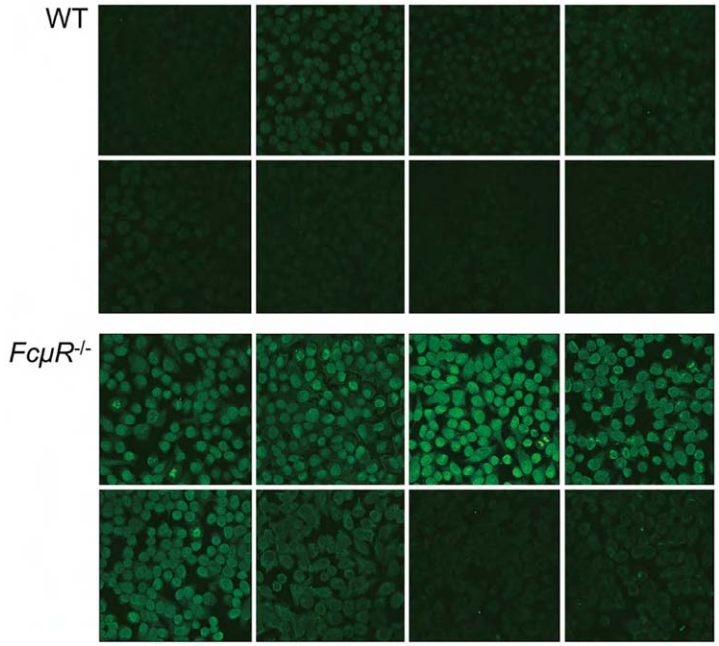

Figure 2: *FcμR*$^{-/-}$ mice produce autoantibodies. Hep2 cells were stained with sera (100-fold dilution) from WT and *FcμR*$^{-/-}$ mice, followed by FITC-anti-mouse IgG. Results of 8 WT (1 male and 7 females) and 8 *FcμR*$^{-/-}$ (2 males and 6 females) mice are shown.

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

The existence of a receptor for IgM (FcμR) was suggested since 40 years ago, but the gene encoding FcμR was identified only recently. FcμR is expressed exclusively in B cells in mice and in B and T cells in humans. In collaboration with the Laboratory for Epithelial Immunobiology of RCAI and the University of Alabama at Birmingham, we have shown that FcμR positively regulates B cell activation and germinal center formation, and is required for efficient humoral immune responses (Fig. 1). Intriguingly, *FcμR*$^{-/-}$ mice produced autoantibodies as they age (Fig. 2), suggesting that FcμR is also required for maintaining tolerance to self-antigens.

4. Li, Y., Li, S., Hoshino, M., Ishikawa, R., Kajiwara, C., Gao, X., Zhao, Y., Ishido, S., Udono, H. and Wang, J.-Y. HSP90α deficiency does not affect Ig gene hypermutation and class switch but causes enhanced MHC class II antigen presentation. ***Int Immunol***. 24, 751-758 (2012)

5. Kano, C., Hanaoka, F. and Wang, J.-Y. Analysis of mice deficient in both REV1 catalytic activity and POLH reveals an unexpected role for POLH in the generation of C to G and G to C transversions during Ig gene hypermutation. ***Int Immunol.*** 24, 169-174 (2012)

Laboratory for

Immunological Memory

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Immunological memory is an important element of the mammalian immune system. We have known about immunological memory for centuries, but critical aspects of its development and maintenance remain poorly defined. A minimal definition of memory cells would be the population of lymphocytes that persists long term in a resting state after antigen clearance. The critical feature of memory cells is their ability to undergo rapid differentiation into secondary effectors with potent functional activity.

We have studied the development of antigen-specific memory B and T cells in response to soluble protein antigens or influenza virus and characterized their properties, genetic signature, and functional activity.

Two pathways of memory B cell development

The germinal center reaction is a critical event in T cell dependent responses, during which antigen-activated B cells accumulate somatic hypermutation and undergo isotype class switching of their immunoglobulin genes. Classically, memory B cells have been considered to be high-affinity isotype-switched variants of precursors that had been generated from the germinal center reaction. However, this simplistic view is not consistent with many observations that have recently been made in mice and humans, for example some memory B cells express unmutated V_H genes. This finding raised the idea that memory B cells may also develop in the absence of GC.

We have recently presented experimental findings that clearly encapsulate the previous predictions with very convincing data using novel mouse models of TD immune responses, and which reveal that immunological memory is established early during an immune response, prior to GC formation. The GC-dependent and -independent memory B cells differ from one another with respect to turnover and gene expression and also in their dependence on different subsets of T helper cells, whereas they share the important function of protecting the host against pathogens. In this context, we have observed that the GC-independent memory pathway functions in mice in response to influenza viruses and assists the GC-dependent memory pathway to protect the host against viral infections.

We observed that both GC-independent and -dependent memory cells accumulate mutations optimally adapted to the recall challenge. Therefore, it seems likely that both unmutated and mutated memory B cells can enter the GC upon secondary challenge and undergo accumulation of mutations by SHM in the GC reaction and create a diverse antibody repertoire in response to infections with pathogens, thus providing effective and efficient protection against mutated pathogens.

Recent Publications

1. Kishi Y, Aiba Y, Higuchi T, Furukawa K, Tokuhisa T, Takemori T, Tsubata T. Augmented antibody response with premature germinal center regression in CD40L-transgenic mice. ***J Immunol.*** 185, 211-219 (2010)
2. Fujii H, Ato M, Takahashi Y, Otake K, Hashimoto S, Kaji T, Tsunetsugu-Yokota Y, Fujita M, Adachi A, Nakayama T, Taniguchi M, Koyasu S, Takemori T. HIV-1 Nef impairs multiple T-cell functions in antigen-specific immune response in mice. ***Int Immunol.*** 23, 433-441 (2011)
3. Kuwahara, K., Nakaya, T., Phimsen, S., Kitabatake, M., Kaji, T., Takemori, T., Watanabe, T., and Sakaguchi, N. Lyn signaling to upregulate GANP is critical for the survival of high-affinity B-cells in germinal centers of lymphoid organs. ***J Immunol.*** 189, 3472-3479 (2012)

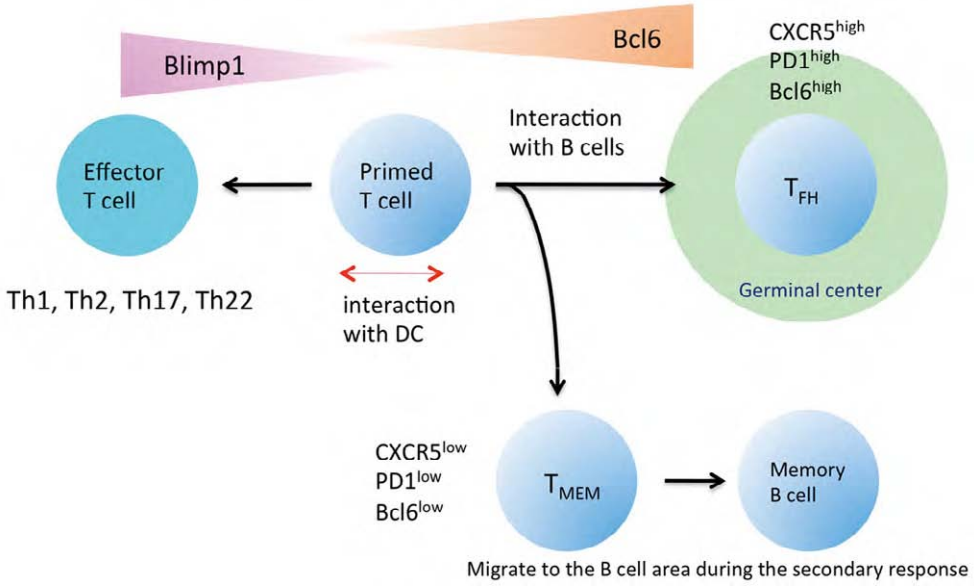

Figure: Bcl6 is required for CD4⁺ memory T cell development

Bcl6 is required for CD4⁺ T cell development to memory T cells that function to help memory B cell terminal differentiation.

The transcriptional repressors Bcl-6 and Blimp-1 counteract each other in many types of cells and play roles in T cell polarization to different lineages. Expression of high levels of Bcl-6 represses the expression or function of transcription factors necessary for effector CD4⁺ T cell differentiation pathways and leads to T follicular helper (T_{FH}) cell development (Fig.). We observed that conditional Bcl6 deletion in CD4 T cells abrogated T_{FH} development and the secondary memory response with its high affinity antibodies. Therefore, we decided to determine whether Bcl6 plays a role in memory T cell development.

We chose to evaluate memory CD4⁺ T cell function by measuring help for the secondary antibody response, which better reflects those functions of memory T cells that protect the host. We observed that conditional Bcl6-deletion in OVA-specific CD4⁺ T cells abrogated their development to memory T cells with the ability to initiate an anti-NP memory B cell response upon stimulation with NP-OVA.

It has been predicted that, after the GC reaction subsides, some of the T_{FH} cells within the GC may become quiescent memory cells while retaining low expression of some T_{FH} cell markers. However, our analysis suggests that the GC is required for development of T_{FH} cells but not for CD4⁺ memory T cells, suggesting that memory T cells are not derived from T_{FH} at their terminal differentiation in the GC.

We observed that CD4 memory T cells consist of central (cm) and effector (em) memory subsets, which are distinguishable by surface markers and genetic signature, whereas both displayed the activity to help in a memory B cell response. Tcm attains functional activity by interaction with antigen-specific B cells (cognate interaction), whereas Tem does not require cognate interaction for their functional development. These results support the idea that T cells upregulate Bcl6 expression during dendritic cell (DC) priming and then polarize to the Tem pathway or to Tcm or T_{FH} pathway upon cognate B-cell interaction. Whether Tcm precursors are derived from cells that undergo differentiation along the pathways to T_{FH} remains unknown.

Thus the immune system utilizes a different set of pathways for immunological memory in both B cell and T cell compartments. Memory B cells localize in the B cell area for a long period of time, whereas memory T cells localize in the T cell follicle. Memory B cells require memory T cells for their terminal differentiation, thus they are quiescent during the primary response. Once reactivated, memory T cells enter the B cell follicle, initiating memory B cell terminal differentiation.

Acknowledgement

We are grateful to Klaus Rajewsky for discussion, help and encouragement and Masaru Taniguchi for reviewing our activity and support. We thank Peter Burrows, Yoshimasa Takahashi, Masaki Hikida, Atsushi Hijikata, Osamu Ohara, Tomo Kurosaki and Masato Kubo for help.

4. Kaji T, Ishige A, Hikida M, Taka J, Hijikata A, Kubo A, Nagashima T, Takahashi Y, Okada M, Kurosaki, T., Ohara O, Rajewsky K and Takemori T. Distinct cellular pathways select germ-line encoded and somatically mutated antibodies into immunological memory. ***J Exp Med.*** 209, 2079-2097 (2012)

5. Ato, M., Takahashi, Y., Fujii H., Hashimoto, S.-I., Kaji, T., Yamamoto, K., Itamura, S., Horiuchi, Y., Arakawa, Y., Tashiro, M., Takemori, T. Influenza A whole virion vaccine induces a rapid reduction of peripheral blood leukocytes via interferon-α-dependent apoptosis. ***Vaccine*** 31, 2184-2190 (2013)

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Antigen-presentation is a critical process for the initiation of immunity and tolerance. Invading pathogens are phagocytosed by antigen-presenting cells (APCs) and their protein constituents are processed and presented through MHC class II (MHC II). The MHC II-peptide complex stimulates CD4 T cells through its interaction with peptide-MHC-specific T cell receptors. With the maturation signals for APCs, peptide-bound MHC II (pMHC II) can initiate immunity against the pathogens. Thus, it is important to reveal how the MHC II-mediated antigen presentation is regulated *in vivo*. In this regard, we and other groups found that the expression level of pMHC II is regulated on the surface of APCs by ubiquitination. In addition, we used genetically modified mice to identify MARCH-I as a physiological E3 ubiquitin ligase for MHC II.

To examine the role of MARCH-I-mediated pMHC II ubiquitination, several groups including ours examined how MARCH-I and pMHC II ubiquitination are regulated in the context of immune responses. At present, it has been demonstrated that several signals inducing the activation/maturation of dendritic cells (e.g. TLR stimuli) stabilize the pMHC II though inhibition of its ubiquitination by down-regulation of MARCH-I. Thus, loss of pMHC II ubiquitination is one of the mechanisms for activation of dendritic cells. Based on these findings, most researches in this field believe that loss of pMHC II promotes pathogen immunity through enhancement of antigen-presentation. However, at present, there are no findings supporting such an attractive hypothesis. On the contrary, we have reported that splenic DCs in mice whose pMHC II is not ubiquitinated have impaired functions, suggesting that loss of pMHC II ubiquitinaiton provides negative feedback to immune responses. Therefore, we are examining how loss of pMHC II ubiquitination influences the function of cDCs in a cell-intrinsic manner by using *in vitro* generated bone marrow-derived DCs.

Since our results suggest that stabilization of pMHC II by loss of MARCH-I inhibits immune responses, we propose that loss of pMHC II ubiquitination induces a negative feedback signal to dendritic cells. At present, we are investigating the mechanism for MARCH-I regulation. Once we discover this regulatory mechanism, we will test our hypothesis by perturbing regulation of MARCH-I.

Inhibition of CD4 T cell activation by loss of pMHC II ubiquitination

In order to examine the role of loss of MHC II ubiquitination on CD4 T cell activation, we generated cDCs from bone marrow cells (BM cells) of MARCH-I KO mice, in which pMHC II is not ubiquitinated. During the differentiation of BM cells into DCs with Flt3-L, DCs were loaded with OVA as a model antigen. OVA-presenting BM-derived DCs were positively selected from Flt3-L-treated BM cells on the basis of CD11c expression and cDCs were purified from selected BM-derived DCs by depletion of pDC with B220. Naïve CD4 T cells isolated from OT-II transgenic mice were

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1. Furuta, K., Ishido, S. and Roche, PA. Encounter with antigen-specific promed CD4 cells promotes MHC class II degradation in dendritic cells. ***Proc Natl Acad Sci USA.*** 109, 19380-19385 (2012)
2. Hunt, D., Wilson, JE., Weih, KA., Ishido, S., Harton, JA., Roche, PA. and Drake, JR. *Francisella tularensis* elicits IL-10 via a PGE_2-inducible factor, to drive macrophage MARCH1 expression and class II down-regulation. ***PLoS One*** 7(5):e37330 (2012)
3. Galbas, T., Steimle, V., Lapointe, R., Ishido, S. and Thibodeau J. MARCH1 down-regulation in IL-10-activated B cells increases MHC class II expression. ***Cytokine*** 59, 27-30 (2012)

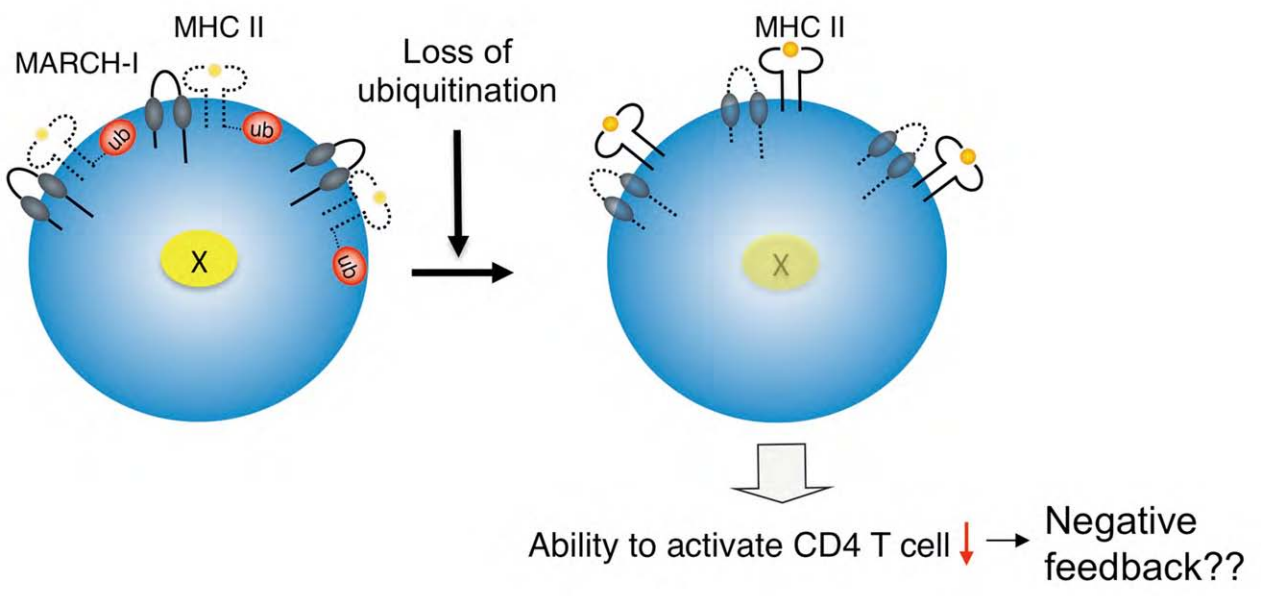

Figure: **Inhibition of DC function by stabilization of pMHC class II**
In immature DCs, MHC class II-peptide complexes (pMHC II) are continuously degraded by MARCH-I-mediated ubiquitination (*left*). During the process of maturation, the function of MARCH-I is inhibited and pMHC II is stabilized through loss of ubiquitination. Stabilized pMHC II suppresses one signaling molecule (shown as "X"), and this regulation may be critical for negative feedback.

co-cultured with OVA-presenting MARCH-I-deficient cDCs or OVA-presenting wild type cDCs. This experiment revealed that MARCH-I-deficient cDCs had a lower capacity to stimulate naïve CD4 T cells than wild type cDCs, since IL-2 secretion was lower in cultures with MARCH-I-deficient cDCs than with wild type cDCs. This was due to the low production of IL-2, because the amount of IL-2 mRNA was low in the MARCH-I-deficient cDCs. Finally, we confirmed that activation of ERK was low in the mixture of OT-II CD4 T cells and MARCH-I-deficient cDCs. These results clearly demonstrate that MARCH-I-deficient cDCs have the poor ability to activate CD4 T cells, and suggest that loss of pMHC II ubiquitination suppresses the function of cDCs in a cell intrinsic manner.

Inhibition of stimulatory molecule expression by loss of pMHC II ubiquitination

Previously, we showed that loss of pMHC II ubiquitination enhances antigen-presentation. Therefore, we expected that loss of pMHC II ubiquitination would enhance the activation of CD4 T cells. However, as mentioned above, we found the unexpected result that loss of pMHC II ubiquitination inhibits CD4 T cell activation. This result suggests that loss of pMHC II ubiquitination might induce a negative signal to CD4 T cells, or suppress an activating signal. At present, several cDC molecules have been reported as activating or inhibitory molecules; therefore the candidate molecules were examined. Among them, we found that one important signaling molecule was significantly inhibited in MARCH-I-deficient cDCs or MHC II-KI-derived cDCs. At present, we are examining how this molecule contributes to inhibition of DC functions.

4. Tanno, H., Yamaguchi, T., Goto, E., Ishido, S. and Komada, M. The Ankrd 13 family of UIM-bearing proteins regulates EGF receptor endocytosis from the plasma membrane. ***Mol Biol Cell.*** 23, 1343-1353 (2012)

5. Kajikawa, M., Li PC., Goto, E., Miyashita, N, Aoki-Kawasumi, M, Mito-Yoshida, M, Ikegaya, M., Sugita,, Y. and Ishido, S*. The inter-transmembrane region of KSHV MIR2 contributes to B7-2 downregulation. ***J Virol.*** 86, 5288-5296 (2012)

Research Unit for

Inflammatory Regulation

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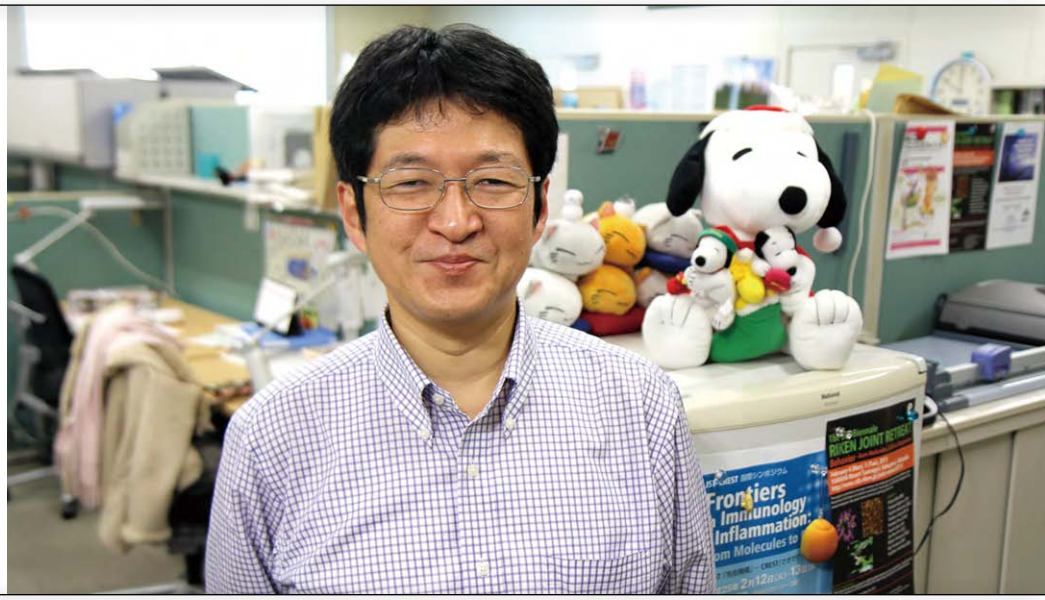
Student Trainee: **Masanaka Sugiyama (JRA)**
Rumiko Ono



The inflammatory response is an important host defense mechanism to sense and eliminate invading microbial pathogens. Dendritic cells first detect pathogens using their pathogen sensors (e.g. Toll-like receptors [TLR]). TLR signaling then leads to the activation of the transcription factor NF-κB, which enters the nucleus and induces the expression of a series of inflammation-related genes, including those encoding proinflammatory cytokines such as interleukin-6 (IL-6) and IL-12. These inflammatory responses then direct T-helper (Th) lymphocyte differentiation into distinct effector T cell subsets, such as Th1, Th2 and Th17, through the activation of STAT4, STAT6 and STAT3 transcription factors, respectively, to combat various pathogens. These initially helpful inflammatory responses must be terminated at the appropriate time point, however, otherwise, excessive responses can damage normal tissue and may cause autoimmune or allergic diseases. Our research goal is to identify key regulators of inflammation-related signal transduction pathways and to clarify the molecular mechanisms for regulating inflammatory responses. These studies should contribute to the development of new therapeutic tools to control the exaggerated inflammation seen in certain human diseases. Our research now focuses on the role of PDLIM2 (PDZ and LIM-domain protein-2) and related LIM proteins in the negative regulation of inflammatory responses.

The role of LIM proteins in the regulation of T-helper cell differentiation

The LIM protein family is classified into several groups. LIM proteins containing a PDZ domain at the N-terminus and LIM domains at the C-terminus, including PDLIM2, are designated as the PDZ-LIM protein subfamily. To date, seven proteins in this subfamily have been identified. It is well known that both PDZ and LIM domains are involved in protein-protein interactions, but the functions of PDZ-LIM proteins in the immune system remain unclear. PDLIM2, also known as SLIM (STAT-interacting protein), has been shown to be a nuclear ubiquitin E3 ligase for the STAT4 and STAT3 transcription factors in $CD4^+$ T cells, suppressing Th1 and Th17 cell differentiation (Tanaka *et al.*, *Immunity*, 2005, Tanaka *et al.*, *Sci. Signal.*, 2011). We recently found that PDLIM4, another PDZ-LIM protein family member, is also a negative regulator of STAT-mediated signaling. In contrast to PDLIM2, however, PDLIM4 is located in the cytoplasm. PDLIM4 bound to STAT3, STAT4 and STAT6, and suppressed their STAT-mediated gene activation. Interestingly, PDLIM4 did not promote polyubiquitination and degradation of these STAT molecules, but instead inhibited the phosphorylation of a tyrosine residue essential for

Recent Publications

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

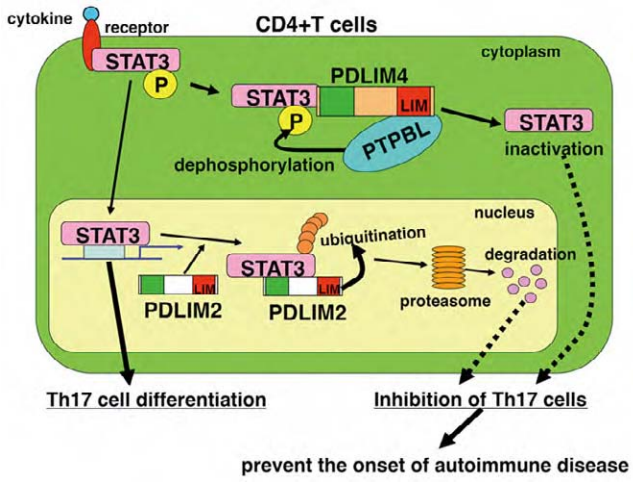

Figure 1: PDLIM2 and PDLIM4 regulate Th17 cell-mediated inflammatory responses through different mechanisms. PDLIM2 interacts with STAT3 in the nucleus and promotes its ubiquitin/proteasome-dependent degradation, whereas PDLIM4 binds to STAT3, recruits PTP and enhances its dephosphorylation and inactivation in the cytoplasm.

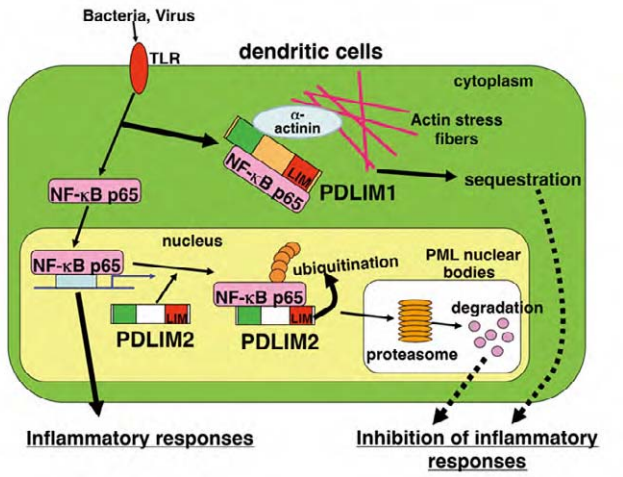

Figure 2: PDLIM2 and PDLIM1 negatively regulate NF-κB signaling through different mechanisms. PDLIM2 binds to and promotes polyubiquitination of the p65 subunit of NF-κB in the nucleus, and then targets p65 into discrete intranuclear compartments, called PML nuclear bodies, for degradation. By contrast, PDLIM1 sequesters the p65 subunit of NF-κB in the cytoplasm and inhibits its nuclear translocation.

cytokine-induced STAT activation. We further demonstrated that PDLIM4 bound to and recruited PTP-BL, a protein tyrosine phosphatase, and facilitated dephosphorylation of STAT proteins. In $CD4^+$ T cells, PDLIM4-deficiency resulted in augmented tyrosine phosphorylation of these STAT proteins and consequently enhanced Th1, Th2 and Th17 cell differentiation. Our findings delineate an essential role of PDLIM4 in negatively regulating STAT-mediated effector T cell differentiation through a mechanism quite distinct from PDLIM2. Moreover, we demonstrated that a single nucleotide polymorphism (SNP) in the LIM domain of PDLIM4 is associated with susceptibility to rheumatoid arthritis, a Th17-related autoimmune disease, suggesting that PDLIM4 may prevent the onset of human autoimmune diseases by negatively regulating Th17 responses.

The role of LIM proteins in the regulation of inflammatory responses

We previously demonstrated that PDLIM2 negatively regulates TLR-mediated NF-κB activity in dendritic cells and subsequent inflammatory responses, acting as a nuclear ubiquitin E3 ligase targeting the p65 subunit of NF-κB. (Tanaka *et al.*, *Nat. Immunol.*, 2007). PDLIM2 binds to p65 and promotes its polyubiquitination. In addition, PDLIM2 targeted p65 to discrete intranuclear compartments, called PML nuclear bodies, where polyubiquitinated p65 was degraded by the proteasome. We recently found that PDLIM1, another LIM protein family member, is also a negative regulator of NF-κB-mediated signaling. PDLIM1 is located in the cytoplasm and inhibits NF-κB p65-mediated gene activation. PDLIM1 did not promote polyubiquitination of NF-κB, but instead sequestrated p65 in the cytoplasm, possibly by interaction with actin stress fibers through binding to α-actinin, an actin binding protein, and suppressed nuclear translocation of p65 protein. Consistently, PDLIM1 deficiency lead to decreased cytoplasmic and increased nuclear p65 protein levels, and thus enhanced proinflammatory cytokine expression in response to TLR stimuli. These data delineate an essential role of PDLIM1 in suppressing NF-κB activation through mechanisms different from PDLIM2. Collectively, PDZ-LIM proteins appear to be a new family of adaptors that can negatively regulate signal transduction pathways in the immune system.

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

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

Research Unit for

Immunotherapeutic Model

Unit Leader:
Kanako Shimizu

Technical staff: **Miki Asakura**

The goal of our laboratory is to develop immunotherapeutic models for cancer. We have been focusing on the biological role of dendritic cells (DCs) *in vivo* as a link between innate and adaptive immunity. NKT cells have unique immunoregulatory features that include the ability to rapidly produce large quantities of cytokines. We have attempted to generate an approach for inducing adaptive immunity based on the adjuvant effect of NKT cell ligands and using *in vivo* DC maturation, which we have found to be more effective than *ex vivo* manipulation of DCs. Previously, we have established a unique strategy using α-galactosylceramide (α-GalCer) loaded on tumor cells (Tumor/Gal) for enhancing innate and adaptive immunity. This strategy induces antigen-specific T cell immunity via *in vivo* DC maturation and we have been optimizing systems for tumor antigen delivery to DCs. One of the remarkable points is that this strategy also induces memory T cells. Thus, we have been evaluating the memory T cells in the immunized animals in detail in order to develop a more effective immunotherapeutic strategy.

We have recently established an immunotherapeutic strategy using an artificial adjuvant vector cells (aAVCs), composed of glycolipid-loaded, antigen-encoding mRNA-transfected allogeneic fibroblasts for enhancing both innate (NKT and NK cells) as well as adaptive immunity (T cells). We focused on the mechanism of DCs in detail. Based on these findings, we have attempted to launch preclinical studies with human aAVCs, in collaboration with Dr. Fujii (Cellular Immunotherapy Research Unit).

The strategy of using *in vivo* DC maturation by NKT Cells to generate adaptive immunity and memory T cells

Although $CD8^+$ memory T cells have been intensely studied in a range of acute infectious pathogen models, the generation of memory T cells reactive with cell-associated antigens, such as those found in cancer, by vaccination is less well understood and has not been established. With the “Tumor/Gal” vaccine, we found that T cells responding to the specific tumor antigen can be maintained as memory T cells, resulting in long-term antigen-specific antitumor protection even 12 months after vaccination (Fig.). These memory T cells had a central memory phenotype and a multifunctional cytokine response to antigen. We have been elucidating the mechanism of how to most effectively generate this memory T cell response.

Recent Publications

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2. Fujii, S., Shimizu, K. DC-based immunotherapy targeting NKT cells. In Terabe, M. and Berzofsky, JA. (eds), ***Natural killer T cells: Balancing the regulation of tumor immunity.*** Springer New York Dordrecht Heidelberg London, 95-110. 2011
3. Shimizu, K., Asakura, M., Fujii, S. Prolonged antitumor NK cell reactivity elicited by CXCL-10-expressing dendritic cells licensed by $CD40L^+CD4^+$ memory T cells. ***J Immunol.*** 186, 5927-5937. 2011

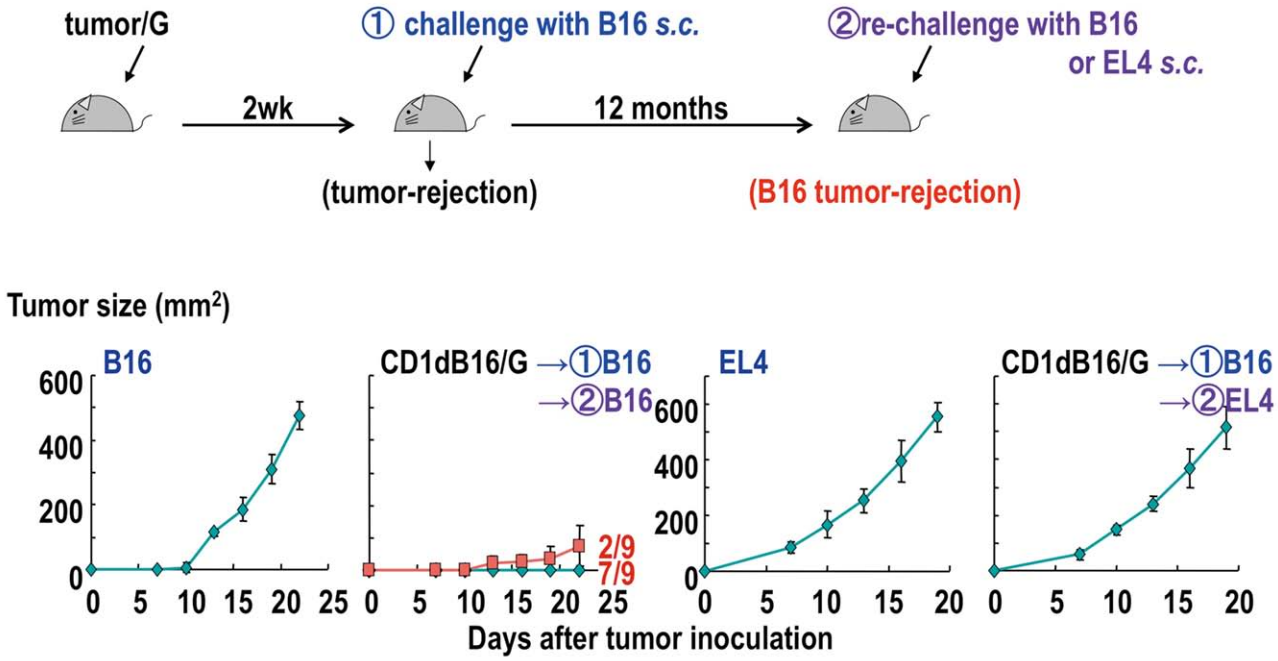

Figure: **Efficacy of adjuvant vector cells for induction of long-term antitumor responses**
C57BL/6 mice were injected i.v. with the CD1d^{hi}-B16 cell line loaded with α-GalCer (tumor/Gal). The mice were challenged s.c. with parental B16 tumor cells 4 wk later. Moreover, when the mice were re-challenged with B16 one year later, they rejected the B16 tumor cells, but not EL4 tumor cells, demonstrating long-term immunity.

Vaccination with antigen-transfected, NKT cell ligand-loaded, human cells elicits a robust *in situ* immune responses by dendritic cells

We previously demonstrated in mice that α-GalCer-loaded, tumor antigen-expressing allogeneic cells can act as cellular adjuvants, linking the innate and adaptive immune systems. We have now established human "artificial adjuvant vector cells (aAVC)" consisting of human HEK293 embryonic kidney cells stably transfected with CD1d, loaded with α-GalCer and then transfected with antigen-encoding mRNA. When administered to mice or dogs, these aAVCs activated invariant NKT (*i*NKT) cells and elicited antigen-specific T cell responses with no adverse events. By harnessing the innate immune system and generating an adaptive immune response to a variety of antigens, this unique tool could prove clinically beneficial in the development of immunotherapies for malignant and infectious diseases.

4. Watarai, H., Fujii, S., Yamada, D., Rybouchkin, A., Sakata, S., Nagata, Y., Iida-Kobayashi, M., Sekine-Kondo, E., Shimizu, K., Shozaki, Y., Sharif, J., Matsuda, M., Mochiduki, S., Hasegawa, T., Kitahara, G., Endo, TA., Toyoda, T., Ohara, O., Harigaya, K., Koseki, H., Taniguchi, M. Murine induced pluripotent stem cells can be derived from and differentiate into natural killer T cells. ***J Clin Invest.*** 120, 2610-2618. 2010
5. Shimizu, K., Hidaka, M., Bickham, K., Moriwaki, M., Fujimoto, K., Fujii, S. Human leukemic cells loaded with α-GalCer activate murine NKT cells in situ. ***Int J Hematol.*** 92, 152-160. 2010
6. Fujii, S., Goto, A., Shimizu, K. Antigen mRNA-transfected, allogeneic fibroblasts loaded with NKT-cell ligand confer antitumor immunity. ***Blood*** 113, 4262-4272. 2009

Research Unit for

Immune Homeostasis

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

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

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

The emerging notion of environment-induced Treg cell reprogramming remained controversial. We and others have previously shown that at least some of Foxp3$^+$ T cells can differentiate into Foxp3$^-$ helper T (Th) cells in response to environmental changes such as lymphopenia or inflammation, whereas other recent reports argue against the plasticity phenomena. We have recently proposed that this controversy can be resolved by considering the "heterogeneity model" of plasticity, which hypothesizes that the observed plasticity of Foxp3$^+$ T cells does not reflect lineage reprogramming of Treg cells but stems from a minor population of uncommitted Foxp3$^+$ T cells. By genetic fate mapping and adoptive transfers, we have addressed the nature and origin of such uncommitted Foxp3$^+$ T cells and identified them as a minor population of non-regulatory cells exhibiting promiscuous and transient Foxp3 expression (Fig.). Such cells give rise to Foxp3$^-$ Th cells and selectively accumulate in inflammatory cytokine milieus or in lymphopenic environments including those in early ontogeny. By contrast, Treg cells did not undergo reprogramming under those conditions irrespective of their thymic or peripheral origins. Moreover, although a few Treg cells transiently lose Foxp3 expression, such "latent" Treg cells retain phenotypic memory and robustly re-express Foxp3 and suppressive function upon activation. The sta-

Recent Publications

1. Miyao T., Floess S., Setoguchi R., Luche H., Fehling H.J., Waldmann H., Huehn J., Hori S. Plasticity of Foxp3$^+$ T cells reflects promiscuous Foxp3 expression in conventional T cells but not reprogramming of regulatory T cells. ***Immunity*** 36, 262-275 (2012)
2. Hori S: Stability of regulatory T-cell lineage. ***Adv Immunol.*** 112, 1-24 (2011)
3. Kendal AR, Chen Y, Regateiro FS, Ma J, Adams E, Cobbold SP, Hori S, Waldmann H: Sustained suppression by Foxp3$^+$ regulatory T cells is vital for infectious transplantation tolerance. ***J Exp Med.*** 208, 2043-2053 (2011)

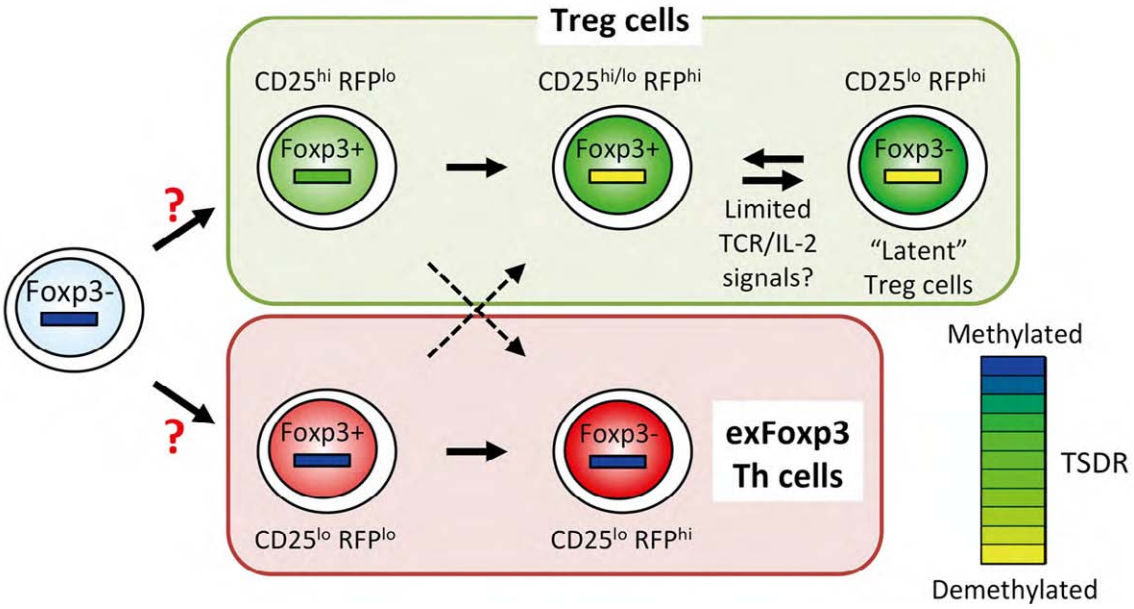

Figure: **A model of Treg cell lineage commitment**
During thymic or peripheral Treg cell differentiation, TCR and other signals lead to Foxp3 up-regulation. (The RFP reporter visualizes Foxp3$^+$ cells and their progeny, independently of continuous or transient Foxp3 expression, see Miyao *et al. Immunity*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

ble differentiated state of Treg cells was associated with complete DNA demethylation of a conserved non-coding region termed Treg-specific demethylation region (TSDR) within the *Foxp3* locus. This study established that Treg cells constitute a stable cell lineage, whose committed state in a changing environment is ensured by an epigenetic mechanism, irrespective of ongoing Foxp3 expression. Our next focus is to discover the signals that lead to irreversible commitment to the Treg cell fate.

Genetic and environmental control of Treg cell fitness in peripheral tissues

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

4. Hori S: Regulatory T cell plasticity: beyond the controversies. ***Trends Immunol.*** 32, 295-300 (2011)

5. Atarashi K, Tanoue T, Shima T, Imaoka A, Kuwahara T, Momose Y, Cheng G, Yamasaki S, Saito T, Ohba Y, Taniguchi T, Takeda K, Hori S, Ivanov II, Umesaki Y, Itoh K, Honda K: Induction of colonic regulatory T cells by indigenous *Clostridium* species. ***Science*** 331, 337-341 (2011)

Laboratory for

Immune Regulation

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Student Trainee: **Yue Ren (IPA)**
Sabrina Hamouche (Intern until Jun 2012)

T cells are a diverse group of immune cells involved in cell-mediated acquired immunity. One unique type of T cell is the innate-like invariant natural killer T (*i*NKT) cell, which recognizes glycolipid ligands instead of peptides on target cells.

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

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

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

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

Recent Publications

1. Watarai H, Yamada D, Fujii S, Taniguchi M, Koseki H. Induced pluripotency as a potential path towards *i*NKT cell-mediated cancer immunotherapy. ***Int J Hematol.*** 95, 624-631 (2012)
2. Nagato K, Motohashi S, Ishibashi F, Okita K, Yamasaki K, Moriya Y, Hoshino H, Yoshida S, Hanaoka H, Fujii SI, Taniguchi M, Yoshino I, Nakayama T. Accumulation of activated invariant natural killer T cells in the tumor microenvironment after α-Galactosylceramide-pulsed antigen presenting cells. ***J Clin Immunol.*** 32, 1071-1081 (2012)
3. Watarai H, Sekine-Kondo E, Shigeura T, Motomura Y, Yasuda T, Satoh R, Yoshida H, Kubo M Kawamoto H, Koseki H, Taniguchi M. Development and Function of Invariant Natural Killer T cells Producing T_H2- and T_H17-cytokines. ***PLoS Biol.*** 10, e1001255 (2012)

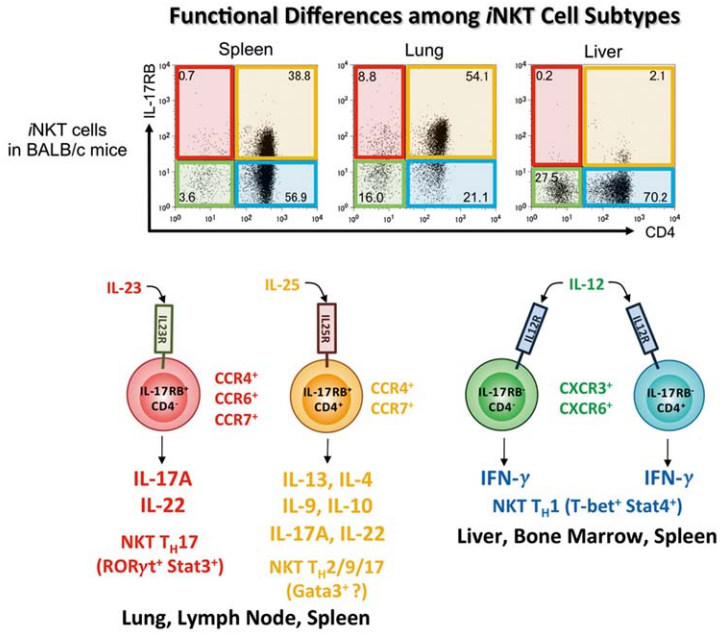

Figure : **The NKT cell subsets**
NKT cells can be divided into four subsets based on the expression of IL-17RB and CD4. The newly identified IL-17RB$^+$ CD4$^-$ and IL-17RB$^+$ CD4$^+$ NKT cells respond to IL-23 and IL-25, respectively, triggering inflammation by the production of TH2/9/17 cytokines,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

approved by the government to qualify for the advanced medical care assessment system and was started beginning in Jan 2012 at Chiba University.

Identification of NKT cell precursors in a subset of CD4/CD8 double-negative (DN) thymocytes

It is still under intense debate whether the developmental pathway of the NKT cell lineage is as unique as their nature and behavior suggests, or whether it is similar to that of conventional β T cells. Currently, the majority opinion holds that NKT cells are derived from the same precursor cells as conventional T cells after CD1d selection at the CD4/8 double-positive (DP) thymocyte stage. However, we have previously shown the presence of NKT cell precursors in DN4 stage thymocytes, that is before reaching the DP stage, in both wild-type and CD1d-deficient mice. These results provided new insights into the early development of NKT cells prior to surface expression of their invariant Vα14 antigen receptor and suggested a unique differentiation pathway of NKT cells, different from that of conventional T cells. Our current goal is to characterize further these NKT cell precursors with the aim of elucidating the factors and the mechanisms underlying this unique developmental pathway of NKT cells from DN precursors. A better understanding of NKT cell biology should provide us with theoretical foundations for future clinical interventions to treat various immune-compromised situations.

Development and function of NKT cell subtypes

We know that functionally distinct subtypes of NKT cells are involved in specific pathologies, yet the development, phenotypes, and functions of these cells are not well understood. We have determined the relationship between various mouse NKT cell subsets, identified reliable molecular markers for these subsets, and shown that these contribute to their functional differences. We identified four NKT cell subsets that arise via different developmental pathways and exhibit different cytokine profiles. Importantly, we these subsets can be isolated from the thymus, as well as from peripheral tissues such as spleen, liver, lung, and lymph nodes. Contrary to the general understanding that NKT cells mature after their exit from the thymus and their migration into peripheral tissues, we could show that distinct phenotypic and functional NKT cell subsets can be distinguished in the thymus by virtue of the presence or absence of the cytokine receptor IL-17RB and another cell surface molecule CD4, and that these subsets then migrate to peripheral tissues where they retain their phenotypic and functional characteristics. Regarding functional significance, we have shown that the NKT cell subsets that lead to airway hyper-responsiveness to respiratory viruses are different to those that lead to allergen-induced airway hyperreactivity. These findings should enable researchers to focus on specific subsets as potential targets for therapeutic intervention.

4. Motomura Y, Kitamura H, Hijikata A, Matsunaga Y, Matsumoto K, Inoue H, Atarashi K, Hori S, Watarai H, Zhu J, Taniguchi M, Kubo M. E4bp4, a mammalian basic leucine zipper transcriptinal factor, is a critical regulator of IL-10 and IL-13 production by CD4 T cells. ***Nat Immunol.*** 12, 450-459 (2011)

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Laboratory for

Dendritic Cell Immunobiology

Team Leader :

Katsuaki Sato

Technical Staff: **Kaori Sato**
Yumiko Sato
Haruna Otsuka

Dendritic cells (DCs) are essential antigen-presenting cells (APCs) that initiate primary immune responses. DCs are heterogeneous, and are mainly classified as 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 or virus-infected cells through various pattern-recognition receptors. Subsequently, under inflammatory conditions they become mature DCs (mDCs), with up-regulated expression of MHC and costimulatory molecules. 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 (T_{eff}) cells, including T helper type (T_H)1 cells, T_H2 cells, and T_H17 cells, depending on environmental cues. DCs thereby play a crucial role in the link between innate and adaptive immunity. Conversely, iDCs are also crucial for the induction of immunological tolerance under steady-state conditions. Their tolerogenic mechanisms include recessive tolerance mediated by clonal deletion and anergy as well as dominant tolerance involving active immune suppression by $CD4^+Foxp3^+$ regulatory T (T_{reg}) cells in the periphery, a function of likely importance in self-tolerance as well as in immune disorders and transplant rejection. However, the precise functional role of each DC subset in immune responses remains unclear. Our goal is to clarify the role of DC subsets in the immune system *in vivo* and to identify the molecular basis for the regulation of their function.

Plasmacytoid dendritic cells are crucial for the initiation of inflammation and T cell immunity *in vivo*

Plasmacytoid dendritic cells (pDCs) are specialized immune cells that are capable of producing large amounts of type I interferon (IFN) by sensing single-stranded RNA or unmethylated CpG DNA through endosomal toll-like receptors (TLRs). Sialic acid binding Ig-like lectin (Siglec)-H is exclusively expressed on murine pDCs and is unique among Siglec proteins in that it associates with the ITAM-bearing adaptor molecule, DAP12. However, the role of pDCs and their Siglec-H-mediated regulation in the control of immune responses *in vivo* remains unclear. Here we report a critical role for pDCs in the regulation of inflammation and T cell immunity *in vivo* using gene-targeted mice with a deficiency of Siglec-H and conditional ablation of pDCs. Siglec-H controlled the threshold of responsiveness to TLR ligands for the production of type I IFN and proinflammatory cytokines and this was mediated through the regulation of a MyD88 downstream signaling pathway. pDCs were required for inflammation triggered by a TLR ligand as well as by bacterial and viral infections. pDCs also controlled homeostasis of $CD4^+$ T_{eff} cells and $CD4^+$ $Foxp3^+$ T_{reg} cells. Upon antigenic stimulation and microbial infection, pDCs suppressed the induction of $CD4^+$ T-cell responses and participated in the initiation of $CD8^+$ T-cell

Recent Publications

1. Fukaya T, Murakami R, Takagi H, Sato K, Sato Y, Otsuka H, Ohno M, Hijikata A, Ohara O, Hikida M, Malissen B, Sato K. Conditional ablation of $CD205^+$ conventional dendritic cells impacts the regulation of T cell immunity and homeostasis *in vivo*. ***Proc Natl Acad Sci USA.*** 109, 11288-11293 (2012)
2. Takagi H., Fukaya T., Eizumi K., Sato Y., Sato K., Shibazaki A., Otsuka H., Hijikata A., Watanabe T., Ohara O., Kaisho T., Malissen B., Sato, K. Plasmacytoid dendritic cells are crucial for the initiation of inflammation and T cell immunity *in vivo*. ***Immunity*** 35, 1-14 (2011)
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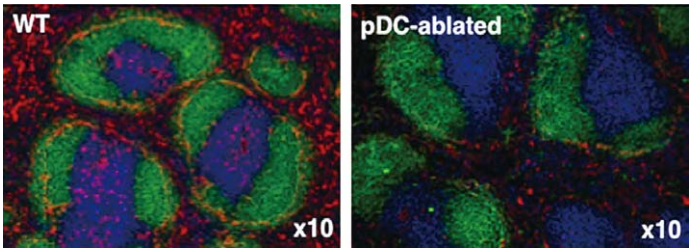

Figure 1: Splenic localization of pDCs is disrupted in pDC-ablated mice.
Immunofluorescent microscopic analysis was performed on frozen horizontal sections of spleen in WT mice and pDC-ablated mice. Sections were stained for CD19 (*green*), CD3ε (*blue*) and BST2 (*red*). Original magnification; 10x.

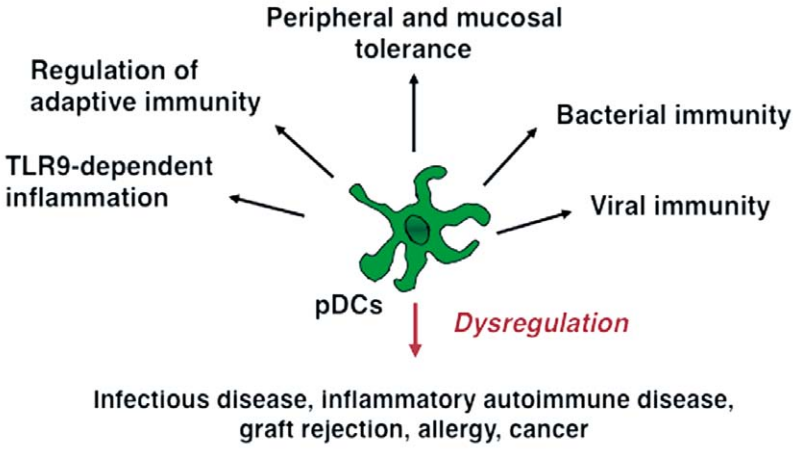

Figure 2: The Multiple roles of pDCs in immune responses *in vivo*
pDCs secrete massive amounts of type I IFNs after activation with viral or bacterial nucleic acids via TLR7 and/or TLR9. Type I IFNs are key cytokines that link innate and adaptive immune responses during viral or bacterial infection. The pDC-ablated mouse model demonstrates the following *in vivo* roles of pDCs: (1) TLR9-mediated inflammation: pDCs were shown to initiate TLR-mediated inflammation triggered by CpG DNA; (2) regulation of adaptive immunity: pDCs were shown to control antigen-specific $CD4^+$ T cells responses by generating $CD4^+Foxp3^+$ T_{reg} cells and to induce differentiation of $CD8^+$ T cells after exposure to exogenous antigens through the cross-presentation pathway ; (3) mucosal tolerance: pDCs maintained mucosal tolerance by generating $CD4^+Foxp3^+$ T_{reg} cells; (4) bacterial immunity: pDCs induced bacterial septic shock and further exacerbated bacteria infection by impairing bacterial clearance; and (5) viral immunity: pDCs controlled viral infection by producing type I IFNs and by supporting antiviral $CD8^+$ T cell differentiation through the cross-presentation pathway. Dysregulation of these pDC functions could be involved in the pathogenesis of infectious disease, inflammatory autoimmune disease, graft rejection, allergy and cancer.

responses. Furthermore, Siglec-H appeared to modulate the function of pDCs *in vivo*. Thus, our findings highlight previously unidentified roles of pDCs and the regulation of their function for the control of innate and adaptive immunity.

Conditional ablation of $CD205^+$ conventional dendritic cells impacts the regulation of T cell immunity and homeostasis *in vivo.*

Dendritic cells (DCs) are composed of multiple subsets that play a dual role in inducing immunity and tolerance. However it is unclear how $CD205^+$ conventional DCs (cDCs) control immune responses *in vivo*. Here we generated novel knock-in mice that allowed the selective conditional ablation of $CD205^+$ cDCs. $CD205^+$ cDCs contributed to antigen-specific priming of $CD4^+$ T cells under steady-state conditions, whereas they were dispensable for antigen-specific $CD4^+$ T cell-responses under inflammatory conditions. By contrast, $CD205^+$ cDCs were required for antigen-specific priming of $CD8^+$ T cells to generate cytotoxic T lymphocytes (CTLs) mediated through cross-presentation. While $CD205^+$ cDCs were involved in the thymic generation of $CD4^+Foxp3^+$ T_{reg} cells, they maintained the homeostasis of $CD4^+Foxp3^+$ T_{reg} cells and $CD4^+$ T_{eff} cells in peripheral and mucosal tissues. On the other hand, $CD205^+$ cDCs were involved in the inflammation triggered by TLR ligands as well as by bacterial and viral infections. Upon microbial infections, $CD205^+$ cDCs contributed to the cross-priming of $CD8^+$ T cells for generating antimicrobial CTLs to efficiently eliminate pathogens, whereas they suppressed antimicrobial $CD4^+$ T cell-responses. Thus, these findings reveal a critical role for $CD205^+$ cDCs in the regulation of T cell immunity and homeostasis *in vivo*.

5. Fukaya T., Takagi H., Taya H., Sato K. DCs in immune tolerance in steady-state conditions. ***Methods Mol. Biol.*** 677, 113-126 (2010)

Laboratory for
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Despite their increasing prevalence in developed countries, the molecular mechanisms leading to allergy and autoimmune diseases remain poorly understood. The ultimate goal of the Cytokine Signaling Research Group is to help elucidate the underlying molecular and immunological mechanisms of allergy and autoimmune diseases from the viewpoint of signal transduction within the immune system. We have shown that heavy metal cations such as Zinc (Zn) can 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.

Molecular mechanisms of the mast cell-dependent allergic reaction

We are investigating the role of Zinc (Zn) in mast cells in allergy. We previously showed that stimulation of the high-affinity IgE receptor (FcεRI) rapidly released intracellular Zn^{2+} from the ER in mast cells and we named this phenomenon the "Zn wave" (Yamasaki *et al.*, *J Cell Biol*, 2007). However, the gatekeeper for the Zn wave and the roles of the Zn wave in allergic reactions remain unclear. We showed that the a1 subunit of the Cav1.3 L-type calcium channel (LTCC, α_{1D}) is localized on the ER membrane rather than on the plasma membrane in mast cells and has a novel function as a gatekeeper for the Zn wave without affecting calcium influx. These results not only showed that the α_{1D} subunit is an authentic gatekeeper for the Zn wave but also made it possible for us to answer the question of what are the roles of the Zn wave. Furthermore, we showed that the Zn wave was involved in cytokine production without affecting degranulation of mast cells through acting on the FcεRI-mediated NF-κB signaling pathway. Consistent with this finding, an LTCC antagonist inhibited the cytokine-mediated delayed-type allergic reaction in mice without affecting the immediate-type allergic reaction. Thus, the Zn wave regulates FcεRI-mediated cytokine production and is required for cytokine-dependent allergic reaction (Fig. 1, Yamasaki *et al.*, *PLoS ONE*. 2012).

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 demonstrated that LPS-induced maturation of DCs is partly mediated through lowering the intracellular concentration of free Zn^{2+} by down regulating Zn transporters, including Slc39a6 (Kitamura *et al.*, *Nat Immunol.* 2006), suggesting involvement of Zn^{2+} in MHC class II cell surface expression through regulating endocytosis and membrane trafficking. In order to understand the role and function of Zn^{2+} and Zn transporters *in vivo*, we have generated mice deficient in the Slc39/Zip family of zinc transporters. We found that Slc39a13/Zip13 knockout mice (*Slc39a13*-KO) show changes in connective tissues reminiscent of the human disease Ehlers-Danlos syndrome (EDS), which is characterized by 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. A homozygous *SLC39A13* loss of function mutation was identified in sibs affected with a unique variant of EDS that recapitulates the pheno-

Recent Publications

1. Yamasaki S., A. Hasegawa, S. Hojyo, W. Ohashi, T. Fukada, K. Nishida, and T. Hirano. A Novel Role of the L-Type Calcium Channel α(1D) Subunit as a Gatekeeper for Intracellular Zinc Signaling: Zinc Wave. ***PLoS ONE*** 7(6): e39654, (2012)
2. Bin, B-H., T. Fukada, T. Hosaka, S. Yamasaki, W. Ohashi, S. Hojyo, T. Miyai, K. Nishida, S. Yokoyama and T. Hirano. Biochemical characterization of human ZIP13 protein: a homo-dimerized zinc transporter involved in the Spondylocheiro dysplastic Ehlers-Danlos syndrome. ***J Biol Chem.*** 286, 40255-40265 (2011)
3. Nishida K., S. Yamasaki, A. Hasegawa, A. Iwamatsu, H. Koseki, and T. Hirano. Gab2, via PI-3K, Regulates ARF1 in Fc ε RI-Mediated Granule Translocation and Mast Cell Degranulation. ***J Immunol.*** 187, 932-941 (2011)

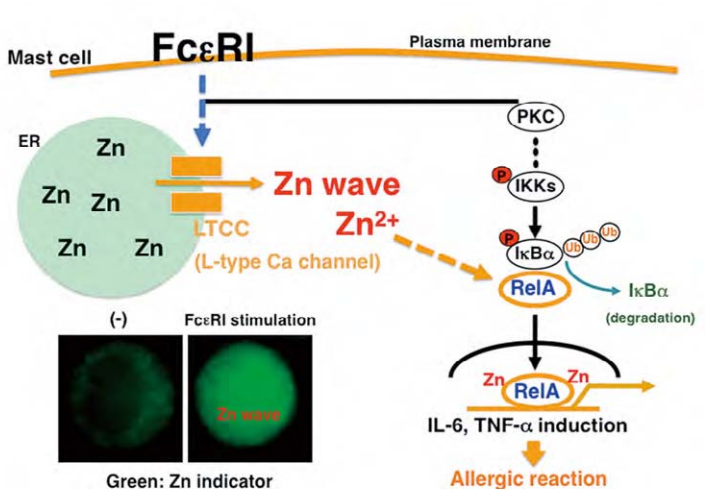

Figure 1: The Zn wave participates in FcεRI-mediated mast cell activation.
FcεRI stimulation induces the rapid elevation of intracellular Zn^{2+} from the perinuclear region, around the ER area, and we named this phenomenon the "Zn wave". The α1D subunit of the LTCC is expressed on the ER membrane and acts as the Zn wave gatekeeper in mast cells. In addition, the LTCC-mediated Zn wave is a positive regulator of the DNA-binding activity of NF-κB and is involved in regulation of the allergic reaction.

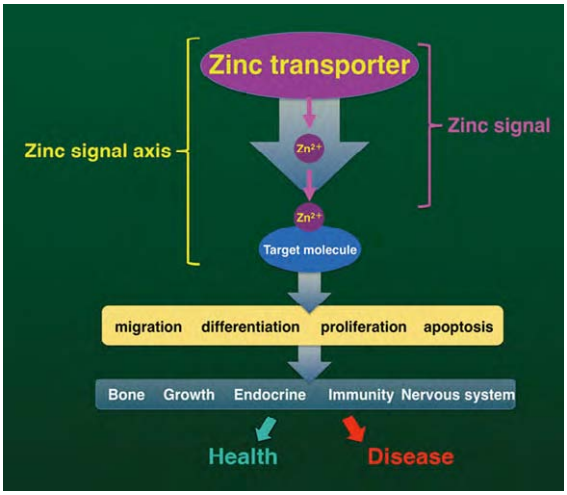

Figure 2: "Zn signal axes" affect distinct signaling pathways to promote and control cellular functions
Each Zn signal axis targets a specific molecule. This mode of Zn action affects a variety of cellular functions by selectively regulating distinct signaling pathways. Therefore, the disruption of a Zn signal axis can be a pathogenesis factor for disease in the absence of redundant systems.

type observed in *Slc39a13*-KO mice. Hence, our results have revealed a crucial role of SLC39A13 in connective tissue development, at least in part due to its involvement in BMP/TGF-β signaling pathways (Fukada *et al.*, *PLoS ONE*, 2008). We have found that the human ZIP13 protein forms homo-dimers (Bin *et al.*, *J. Biol. Chem*, 2011), and its further characterization by crystallography is under investigation. In addition, we have investigated the role of Slc39a14 by generating deficient mice. The *Slc39a14*-KO mice showed progressive systemic growth defects and impaired signaling of a G-protein -coupled receptor (GPCR) in the growth plate, pituitary grand, and liver. We found that Slc39a14-mediated Zn^{2+} plays a role in control of GPCR-cAMP response element-binding protein (CREB) signaling via regulating cAMP levels by suppressing phosphodiestarase (PDE) activity (Hojyo *et al.*, *PLoS ONE*, 2011), suggesting a crucial role of Slc39a14 in control of mammalian systemic growth. We also showed that Zn^{2+} suppresses T_h17-mediated autoimmune diseases by inhibiting the development of T_h17 cells via attenuating STAT3 activation. In mice injected with type II collagen to induce arthritis, Zn^{2+} treatment inhibited T_h17 cell development. IL-6-mediated activation of STAT3 and *in vitro* T_h17 cell development were all suppressed by Zn^{2+}. Importantly, Zn^{2+} binding changed the α-helical secondary structure of STAT3, disrupting its association with JAK2 kinase and with a phospho-peptide that contained a STAT3-binding motif from the IL-6 signal transducer gp130. Thus, we conclude that Zn^{2+} suppresses STAT3 activation, which is a critical step for T_h17 development (Kitabayashi *et al.*, *Intnl Immunol.,* 2010). Together these results support the idea that Zn^{2+} has roles in mediating and controlling intracellular signaling events. We propose the term "Late Zn signaling" for this type of 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). We also proposed that a "Zn signal axis" composed of Zn transporters and target molecules determines the biological specificity of Zn signaling (Fig. 2, Fukada *et al.*, *J Bone Miner Metab.*, 2012).

4. Fukada, T., S. Yamasaki, K. Nishida, M, Murakami, and Hirano, T. Zinc homeostasis and signaling in health and diseases: Zinc signaling. ***J Biol Inorg Chem.*** 16, 1123-1134 (2011)

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

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

ENU mutant panel study

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

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. We also examined tissue sections from animals at 16 weeks of age. We have thus analyzed the mutant phenotypes by pathological, cytological and molecular biological evaluations of affected tissues, lymphoid organs and blood cells.

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

Allergic disease mutant mouse model

By ENU recessive mutant screening, we identified and established a mutant mouse line with phenotypic features reminiscent of a common human skin disease, atopic dermatitis, a Th2-mediated condition. The ear skin became thicker and red and, as the mice aged, they started to scratch the ear skin and face. The serum IgG1 and IgE levels 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. Ultimately the mice developed

Recent Publications

1. Sugiyama M, Nakato G, Jinnohara T, Akiba H, Okumura K, Ohno H, Yoshida H. Expression pattern changes and function of RANKL during mouse lymph node microarchitecture development. ***Int Immunol.*** 24, 369-378 (2012)
2. Watarai H, Sekine-Kondo E, Shigeura T, Motomura Y, Yasuda T, Satoh R, Yoshida H, Kubo M, Kawamoto H, Koseki H, Taniguchi M. Development and function of invariant natural killer T cells producing T(h)2- and T(h)17-cytokines. ***PLoS Biol.*** 10(2):e1001255 (2012)
3. Tachibana M, Tenno M, Tezuka C, Sugiyama M, Yoshida H, Taniuchi I. Runx1/Cbf β2 complexes are required for lymphoid tissue inducer cell differentiation at two developmental stages. ***J Immunol***. 186, 1450-1457 (2011)

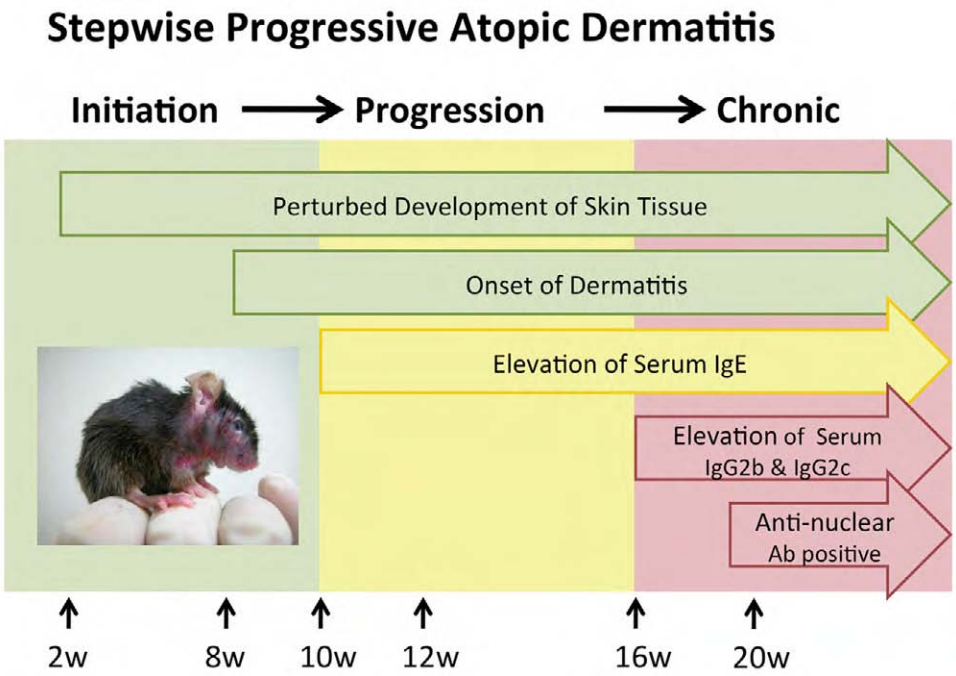

Figure : **An atopic dermatitis-like mutant mouse was established from the ENU screen.**
The above scheme illustrates the stepwise progression of AD disease in the mutant animals. The responsible point mutation in a single gene and the developmental mechanism(s) of this dermatitis have been clarified (manuscript under submission).

chronic facial and ear skin inflammation and not only Th2 serum immunoglobulin but also Th1 serum immunoglobulin levels increased. These stepwise symptoms and findings are compatible with the diagnostic criteria for human infantile atopic dermatitis (AD). We have mapped the phenotype-causing point mutation to a distinct gene and directly confirmed this causality by genetic manipulation to introduce the same mutation into the gene of wild type mice. A bone marrow transplantation study demonstrated that the disease is induced by mutant skin tissue and not by mutant bone marrow cells. We have clarified the disease onset mechanism and succeeded in disease onset prevention based on the results of our studies. Interestingly, the causative mutation was found in a ubiquitously expressed signal transduction gene and the responsible tissue for dermatitis development was skin but not bone marrow. In accordance with our findings, we have found several ways to prevent the dermatitis onset (manuscript under submission). We are now further analyzing the stepwise progression of this AD-like disease in the mutant mouse to better understand the allergic disease progression in skin and the immune system.

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 had phenotypes related to allergic diseases. From these mutant lines, we are now analyzing a mutant line with features of experimental allergic asthma, rhinitis and conjunctivitis by OVA immunization.

We are now backcrossing some of the mice to the C3H/HeJ strain in order to map the responsible loci. As of December 2012, we have mapped thirty-two independent mutant-causing loci to distinct regions, and nine of them have been identified to be point mutations in independent genes. Along with allergic disease model mutant screening, we have identified and characterized a few other interesting phenotypes in mutant lines with/without allergic defects. As listed in the publications section, we have reported two independent mutant lines in which there are point mutations in distinct genes. One mutation is found in the *Pla2g6* gene and may be a good model for the human neurodegenerative disease infantile neuroaxonal dystrophy (INAD) (Wada *et al*., *Am J Pathol.* 2009). The other is caused by a point mutation in the *Themis* gene, which plays an important role in thymocyte development, and this mutant line may be a good model for a primary immunodeficiency disease (Kakugawa *et al*., *Mol Cell Biol.* 2009).

4. 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 U S A***. 107,6436-6441 (2010)

5. Yoshibumi M., Yoshiaki K., Toyoyuki T., Kunie M., Yuta S., Hisahiro Y., Yoshiyuki M., Hajime K., and Hiromichi Y. An Atopic Dermatitis-Like Skin Disease with Hyper-IgE–Emia Develops in Mice Carrying a Spontaneous Recessive Point Mutation in the *Traf3ip2* (*Act1/CIKS*) Gene. ***J Immunol***. 185,2340-2349 (2010)

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Histamine-releasing factor in allergy

Factors that can induce the release of histamine from basophils and mast cells have been studied for more than 30 years. A protein termed histamine-releasing factor (HRF) was purified and molecularly cloned in 1995. HRF can stimulate histamine release and IL-4 and IL-13 production from IgE-sensitized basophils and mast cells. HRF-like activities were found in body fluids during the late phase of allergic reactions, implicating HRF in allergic diseases. However, definitive evidence for the role of HRF in allergic diseases has been elusive. On the other hand, we found effects of monomeric IgE on the survival and activation of mast cells without any involvement of specific antigen, as well as heterogeneity of IgE molecules in their ability to cause such IgE effects. The latter property of IgE molecules seemed to be similar to the heterogeneity of IgEs in their ability to prime basophils in response to HRF. This similarity led to our recent finding that ~30% of IgE and IgG molecules can bind to HRF via interactions of their Fab with two binding sites within the HRF molecule (Fig. 1). Given the existence of a disulphide-linked dimeric form of HRF, this result can explain how co-stimulation with HRF-reactive IgE and recombinant HRF can activate mast cells and basophils *in vitro* (Fig. 2). Use of short IgE-binding HRF-derived peptides that can block HRF-IgE interactions in a competitive fashion revealed an essential role of HRF in promoting skin hypersensitivity (i.e., passive cutaneous anaphylaxis) and airway inflammation. Intranasal injection of recombinant HRF induced macrophage-dominant lung inflammation in naïve mice, similar to that observed in transgenic mice expressing HRF in a Clara cell-specific manner. We have begun to study the possible involvement of HRF in human allergic diseases. In atopic dermatitis (AD), our data thus far indicate that HRF levels are increased in sera of AD patients and some (~10 percent) AD patients have measurable levels of HRF-reactive IgE as measured by ELISA. By contrast, normal subjects have undetectable levels of serum HRF-reactive IgE. Overall, our published studies strongly support the notion that HRF secreted during allergic reactions promotes or amplifies allergic reactions by activating mast cells (and basophils) through cross-linking of HRF-reactive IgE bound to FcεRI on these cells (as well as through cross-linking of HRF-reactive IgG-bound to FcγRs). Our unpublished results also suggest that HRF plays a proallergic role in a mouse model of food allergy. We are currently measuring serum HRF and HRF-reactive IgG and IgE in food allergy patients who have or have not benefited from rapid oral

Recent Publications

1. Kashiwakura, J.*, Ando, T.*, Matsumoto, K., Kimura, M., Zajonc, D.M., Ozeki, T., Siraganian, R.P., Broide, D., Kawakami, Y., and Kawakami, T. Histamine-releasing factor has a proinflammatory role in mouse models of asthma and allergy. ***J Clin Invest.*** 122, 218-228 (2012) *equal contributions
2. Kawakami, T. Human FcγRIIA at the center stage of anaphylaxis. ***Blood*** 119, 2432-2433 (2012)
3. Kawakami, T., Ando, T., and Kawakami, Y. HRF-Interacting Molecules. ***Open Allergy J.*** 5, 41-46 (2012)

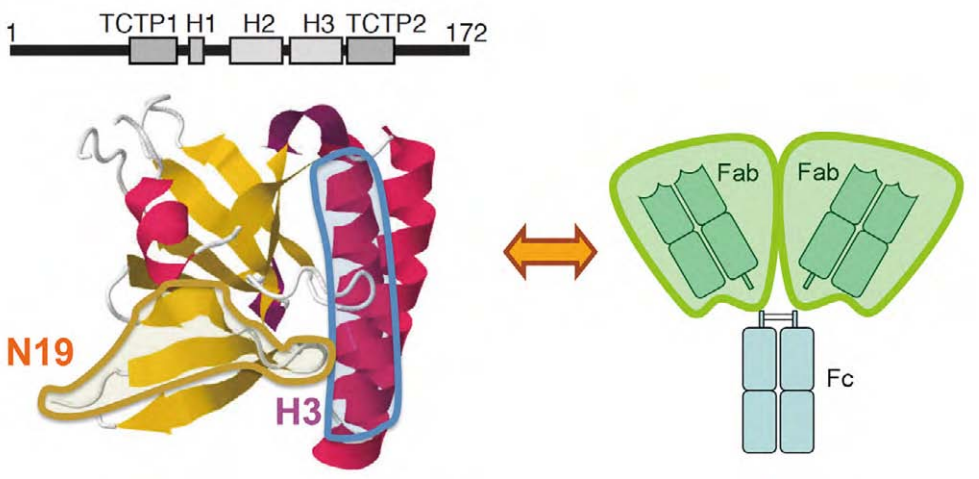

Figure 1: Interaction sites between HRF and IgG. Ig-binding sites were mapped to the N-terminal 19 residues (N19) and the H3 (residues 107-135) portion of HRF, while HRF binds to the Fab portion of Igs. The top left and bottom left panels show the domain structure and the 3-D structure of human HRF (Protein Data Bank, 1YZ1), respectively. The N19 and H3 portions are highlighted. The TCTP1 portion is omitted from the model because it does not form interpretable electron density in the crystal structure. The right panel shows a cartoon of an IgG molecule.

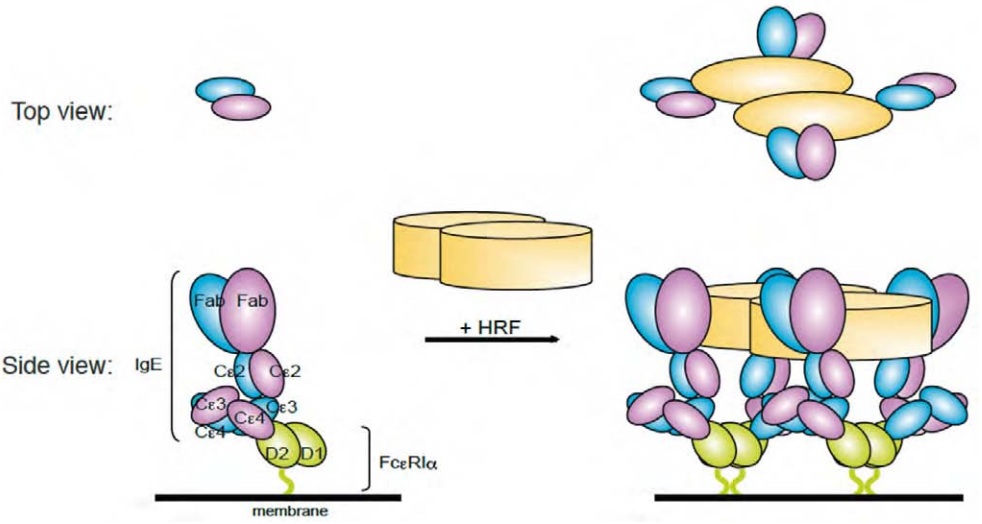

Figure 2: Working model of HRF-mediated FcεRI crosslinking. IgE binds the FcεRIα chain via the interaction between Cε3 and D2 domains. HRF can exist as a dimer and one HRF molecule can bind to two molecules of IgE via interactions with the N19 and H3 regions of HRF. The top view (*Top*) of IgE at the level of Fab and the side view (*Bottom*) of IgE-bound FcεRIα chain are shown on the left. After binding of an HRF dimer, four FcεRIα chain-nucleated complexes will be formed (*Right*). The cytoplasmic portion of the FcεRIα as well as β and γ chains of the FcεRI are omitted for clarity.

tolerance induction clinical protocols. Such a study, together with global gene expression profiling and other experiments, will help clarify the role of HRF as a biomarker and/or pathogenic factor in food allergy and/or tolerance induction or maintenance.

4. Kashiwakura, J., Okayama, Y., Furue, M., Kabashima, K., Shimada, S., Ra, C., Siraganian, R.P., Kawakami, Y., and Kawakami, T. Most highly cytokinergic IgEs have polyreactivity to autoantigens. ***Allergy Asthma Immunol Res.*** 4, 332-340 (2012)

5. Xiao, W., Kawakami, Y., and Kawakami, T. Immune regulation by phospholipase C-β isoforms. ***Immunol Res.*** 56, 9-19 (2013)

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Two major activities of invariant natural killer T (*i*NKT) cells orchestrate the immune regulation between innate and acquired immunity. One is a host defense activity, including IFN-γ production by *i*NKT cells stimulated with CD11c$^+$ dendritic cells. The other is induction of immune tolerance generated by multiple systemic administration of α-galactosylceramide (α-GalCer), a representative exogenous ligand for *i*NKT cells. Although the tolerance induction activity *i*NKT cells could potentially be applied to a variety of therapies for immune disorders, understanding of the detailed mechanism of action and development of suitable methods for its selective enhancement among the multivalent activities of *i*NKT cells have remained undiscovered. We previously found that a liposomal form of α-GalCer, but not an aqueous one, could predominantly enhance the immunoregulatory functions of *i*NKT cells, such as IL-10 production. Based on these results, we attempted to suppress IgE antibody formation in a mouse allergy model by the administration of liposomal α-GalCer. In the case of primary IgE antibody formation, a systemic injection of liposomal α-GalCer prior to immunization with alum-absorbed antigen resulted in a remarkable suppression of both IgE and IgG antibody formation. By contrast, both the secondary IgE antibody and total IgE response of fully antigen-sensitized mice after antigen challenge were significantly suppressed, but the IgG antibody response was not, even after repeated systemic injection of liposomal α-GalCer. The first aim is to clarify the mechanism of IgE isotype-specific suppression induced by treatment with liposomal α-GalCer.

Despite being a versatile delivery system, liposomes have some disadvantages, including having a tendency to clump upon storage and lacking sufficient stability for oral delivery. Stability in the presence of gastrointestinal (GI) tract bile salts can be improved through the use of phospholipids with high transition temperatures, such as dipalmitoyl-phosphatidylcholine (DPPC). Two new liposomal α-GalCer formulations will be investigated in this project – silica and chitosan coated liposomes. Both these formulations are still simple to produce but the liposomes are stabilized by the coating process.

Mechanism for IgE isotype-specific suppression by liposomal α-GalCer

In the course of our studies, we found that rhodamine-labeled liposomal α-GalCer was incorporated into splenic B220$^+$CD1d^{high} cells but not into B220$^+$CD1d^{low} cells, and much more than into CD11b$^+$ macrophages or CD11c$^+$ dendritic cells. Immunohistochemical observation clearly showed that rhodamine-positive dots corresponding to B220$^+$CD1d^{high} cells were localized at the marginal zone area in the spleen and that co-localization of *i*NKT cells

Recent Publications

1. Shimizu K, Mizuno T, Shinga J, Asakura M, Kakimi K, Ishii Y, Masuda K, Maeda T, Sugahara H, Sato Y, Matsushita H, Nishida K, Hanada KI, Dörrie J, Schaft N, Bickham K, Koike H, Ando T, Nagai R and Fujii S, Vaccination with antigen-transfected, NKT cell ligand-loaded, human cells elicits robust in situ immune responses by dendritic cells. ***Cancer Res***. 73, 62-73. (2012)
2. Duramad O, Laysang A, Li J, Ishii Y and Namikawa R. Pharmacologic Expansion of Donor-Derived, Naturally Occurring CD4(+)Foxp3(+) Regulatory T Cells Reduces Acute Graft-versus-Host Disease Lethality Without Abrogating the Graft-versus-Leukemia Effect in Murine Models. ***Biol Blood Marrow Transplant.*** 17, 1154-1168 (2011)
3. Ishii Y, Motohashi S, Shimizu K, Nakayama T, Taniguchi M, and Fujii S. Application of NKT cells in immunotherapy, ***Curr Immunol Rev.*** 6, 109-115 (2010)

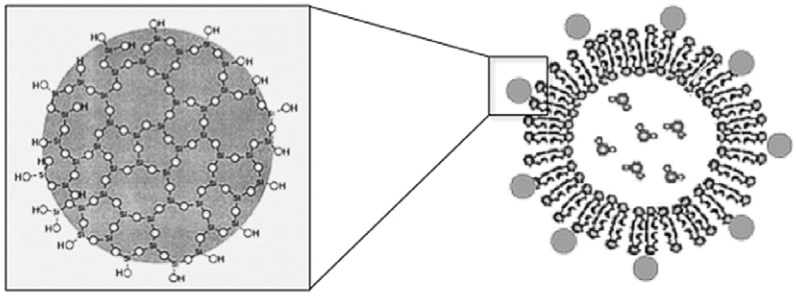

Figure 1 : Silica nanoparticle coated liposomes
Coating by colloidal silica (Ludox): Amorphous, nonporous, spherical particles with an average particle size of ~40 nm. The coating was formed by the electrostatic interaction between the negatively charged silica nanoparticle surface and the positively charged amine group of the phospholipid.

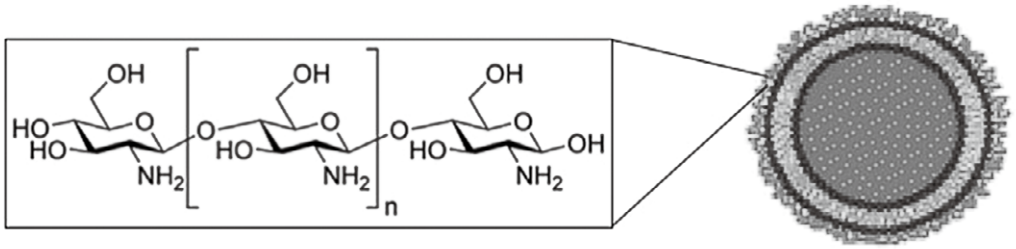

Figure 2 : Chitosan coated liposomes
Chitosan is biodegradable, mucoadhesive and can facilitate tight junction opening. Liposomes are coated by the electrostatic interaction between the negatively charged phosphate of dicetyl phosphate and the positively charged amine group of the chitosan.

after the rhodamine-liposome injection could be detected in the mice transferred with NKT-ES-*V* cells, in which *IRES-Venus* was inserted downstream of the *TCR Cα* stop codon in NKT-ES cells. This co-localization as well as IL-21 mRNA expression by *i*NKT cells were completely inhibited by pre-treatment of the recipient mice with anti-CXCL16 neutralizing mAb, suggesting that IL-21 production by *i*NKT cells was induced after their interaction with the liposomal α-GalCer-pulsed B220^{+}CD1d^{high} cells. Indeed, IL-21 mRNA expression by *i*NKT cells was detected after *in vitro* co-culture of *i*NKT cells isolated from normal mouse spleen with splenic B220^{+}CD1d^{high} cells derived from mice injected with liposomal α-GalCer. Since it has been reported that IL-21 is capable of inhibiting IgE class switching, we wondered if IL-21 production by *i*NKT cells might be involved in the IgE isotype-specific suppression generated by the liposomal α-GalCer. To explore this possibility, we analyzed the expression of IgE, IL-21 receptor and Bmf (Bcl-2 modifying factor), which would be activated by IL-21 in splenic B cells of IgE-producing mice, after the injection of the rhodamine-labeled liposomal α-GalCer. The expression level of IL-21 receptor mRNA in rhodamine-positive B cells was remarkably higher than that in rhodamine-negative B cells. Surprisingly, there was substantial expression of IgE and Bmf mRNA in rhodamine-positive B cells but not in rhodamine-negative B cells. These results suggest that liposomal α-GalCer was incorporated into the marginal zone B220-postive cell population including IgE-producing Bε cells, which would express Bmf and IL-21 receptor. The Bε cells would be induced to undergo apoptosis by IL-21 derived from *i*NKT cells after their interaction with α-GalCer-presenting Bε cells.

Manufacturing of liposomal α-GalCer formulations for oral administration

Oral tolerance has been expected to be utilized as one of the key immunotherapies for allergic diseases. If oral allergy vaccines were developed, allergy patients would be more likely to accept such a non-invasive treatment. However, oral immunotherapies still remain in the research stage of development. In order to apply oral allergy vaccines to clinical studies, vaccine design based on solid basic research is required. In an international research collaboration between RCAI and the University of Otago in New Zealand, we began to combine our α-GalCer liposome technology with our extensive experience in the formulation of vaccines using not only liposome but also silica, chitosan and emulsion based formulations for oral administration (Fig. 1 and 2). Now we are screening to find the best formulation of liposomal α-GalCer to induce oral tolerance. Then we will perform studies to understand the detailed mechanism of action, such as identification of target antigen-presenting cells and putative regulatory cells in the GI-tract.

Laboratory for

Human Disease Models

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Shinsuke Takagi (JRA)
Yuho Najima (JRA)

To bridge mouse research and human research, we have been creating humanized mouse models. Humanized mice developed by injecting human cord blood HSCs into newborn NOD/SCID/Il2rγKO (NSG) have enabled us to analyze differentiation and function of the human immune system *in vivo*. In this fiscal year, we aim to investigate the development of human innate immunity in NSG recipients. However, in the current humanized mouse models, human immune subsets differentiate from injected human hematopoietic stem cells within a mouse microenvironment. To better reconstitute human immunity in mice, we have to develop immune-compromised mice that also have a humanized microenvironment.

To this end, we are currently creating humanized thymic and bone marrow environments in NSG mice. To date, we found that human CTL responses against virus-infected cells can be identified in an HLA-expressing humanized mice. As another example, NSG with membrane-bound human stem cell factor supported the development of significantly higher levels of human myeloid subsets. In collaboration with Dr. Leonard Shultz at the Jackson Laboratory and Drs. Koseki and Ohara at RIKEN RCAI, we continue to investigate the significance of humanized mice with a humanized microenvironment and try to apply these systems into understanding of human diseases.

Development of human myeloid subsets in humanized NSG mice

While physiological development of human lymphoid subsets has become fairly well understood in humanized mice, *in vivo* development of human myeloid subsets has remained unevaluated. We have investigated *in vivo* differentiation and function of human myeloid subsets in NSG mouse recipients transplanted with purified human cord blood hematopoietic stem cells. At four to six months post-transplantation, we observed the development of human neutrophils, basophils, mast cells, monocytes, as well as conventional and plasmacytoid dendritic cells in the recipient bone marrow. Frequencies of monocytes and dendritic cells in the humanized mouse bone marrow (BM) were similar to those seen in the primary human BM, while the frequency of HLA-DR(-) granulocytes was lower in humanized mouse BM compared with human BM. Consistent with the expression of cytokine receptors and TLR by the engrafted human myeloid cells, *in vivo* administration of human G-CSF or LPS resulted in the recruitment of human myeloid cells into the recipient circulation or elevation of human inflammatory cytokines in the humanized mouse sera. Human bone marrow monocytes and alveolar macrophages in the recipients displayed intact phagocytic function. In particular, human BM-derived monocytes/macrophages were further confirmed to be capable of

Recent Publications

1. Takagi S, Saito Y, Hijikata A, Tanaka S, Watanabe T, Hasegawa T, Mochizuki S, Kunisawa J, Kiyono H, Koseki H, Ohara O, Saito T, Taniguchi S, Shultz LD, Ishikawa F. Membrane-bound human SCF/KL promotes *in vivo* human hematopoietic engraftment and myeloid differentiation. ***Blood*** 119, 2768-2777. (2012)
2. Tanaka S, Saito Y, Kunisawa J, Kurashima, Y., Wake, T., Suzuki, N., Shultz, LD., Kiyono, H., Ishikawa, F. Development of mature and functional human myeloid subsets in hematopoietic stem cell-engrafted NOD/SCID/IL2rγKO mice. ***J Immunol.*** 188, 6145-6155. (2012)
3. Shultz LD, Saito Y, Najima Y, Tanaka S, Ochi T, Tomizawa M, Doi T, Sone A, Suzuki N, Fujiwara H, Yasukawa M, Ishikawa F. Generation of functional human T-cell subsets with HLA-restricted immune responses in HLA class I expressing NOD/SCID/IL2rγnull humanized mice. ***Proc Natl Acad Sci USA.*** 107, 13022-13027. (2010)

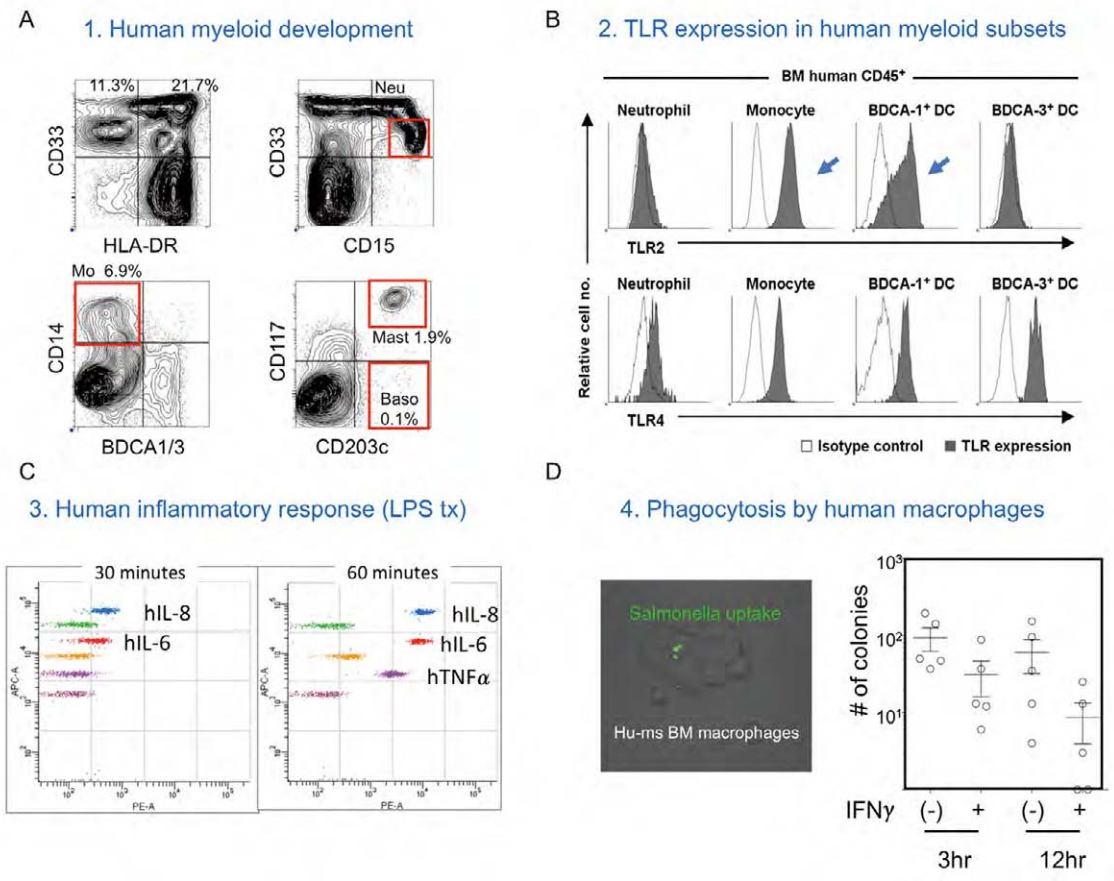

Figure : **Development of human myeloid subsets in the BM of an NSG recipient**
(A)CD15$^+$ neutrophils, HLA-DR$^+$CD14$^+$ monocytes, CD117$^+$ mast cells, and CD203c$^+$CD117$^-$ basohpils were present in the BM of an NSG recipient transplanted with cord blood HSCs. (B) Engrafted human myeloid subsets express TLR2 and TLR4 (*white*: isotype control). (C) *In vivo* administration of 15μg LPS into humanized NSG mice resulted in rapid elevation of human inflammatory cytokines in the sera. (D) Humanized mouse BM-derived macrophages phagocytized GFP-tagged *Salmonella* and inhibited *Salmonella* colony formation upon IFNγ stimulation.

phagocytosis and killing of *Salmonella Typhimurium* upon IFN-γ stimulation. *In vivo* human myelopoiesis established in the NSG humanized mouse system may facilitate the investigation of human myeloid cell biology, including *in vivo* analyses of infectious diseases and therapeutic interventions.

Human leukemia models

We are currently investigating two types of acute leukemia, acute lymphoblastic leukemia with Mixed lineage leukemia (MLL) gene rearrangement and adult-onset acute myeloid leukemia. For acute myeloid leukemia, we reported that transplantation of CD34$^+$CD38$^-$ leukemia cells into newborn NSG resulted in robust recapitulation of the patients' leukemic status in mice. In the recipient bone marrow, CD34$^+$CD38$^-$ cells generated non-stem AML cells such as CD34$^+$CD38$^+$ cells or CD34$^-$ cells. By contrast, MLL-rearranged acute lymphoblastic leukemia (ALL) shows a different hierarchy of leukemogenesis; i.e., leukemia developed in NSG recipients consisted of CD34$^-$CD19$^+$ ALL cells as well as CD34$^+$CD38$^+$CD19$^+$ cells. Furthermore, transplantation of CD34$^-$CD19$^+$ ALL cells resulted in the engraftment of CD34$^+$CD38$^+$CD19$^+$ cells and CD34$^-$CD19$^+$ cells in the recipient bone marrow.

Based on the distinct stem cell hierarchy in these two types of leukemia, we are attempting to identify therapeutic targets in each one, through comparison of gene expression profiles of leukemia stem cells and normal hematopoietic stem cells.

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 LD, Ishikawa F. Induction of cell cycle entry eliminates human leukemia stem cells in a mouse model of AML. ***Nat Biotech.*** 28, 275-280. (2010)

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

Research Unit for

Cellular Immunotherapy

Unit Leader : **Shin-ichiro Fujii**

Technical Staff : **Jun Shinga**

Student Trainee : **Yusuke Sato (JRA)**

Both innate and adaptive immunity are crucial for cancer immune surveillance, but precise therapeutic equations to restore such surveillance in cancer patients have yet to be developed. The forms of antigen, adjuvant and antigen-presenting cells may be crucial components of successful immunotherapy. Particularly, dendritic cells (DCs), as "nature's adjuvants", play a pivotal role in determining the character and magnitude of an immune response. The *ex vivo* loading of autologous patient DC with tumor specific antigens is one of the most promising current immunotherapeutic strategies. On the other hand, *ex vivo* DC therapy requires the generation of large numbers of DCs from individual patients and the quality of the DCs will likely depend on the patient's condition at the time of venipuncture to harvest DC precursors.

We have studied the role of DCs *in situ* for tumor immunity by focusing on the link between innate and adaptive immunity. We evaluated another *in vivo* DC targeting strategy that exploits the pro-inflammatory potential of dying cells together with the adjuvant activity of invariant natural killer T (*i*NKT) cells. We successfully developed a strategy in murine models for the induction of antigen-specific T cell responses using tumor-associated antigen expressing, α-GalCer-loaded allogeneic cells through *in situ* DC maturation. Based on these findings, we have attempted to launch preclinical studies of human artificial adjuvant vector cells (aAVCs), in collaboration with Dr. Shimizu (Therapeutic Model Research Unit).

We have been attempting to generate better strategies for immunotherapy, not only to establish vaccines and therapeutic models, but also to elucidate the basic nature of the immune response in these systems.

Vaccination with antigen-transfected, NKT cell ligand-loaded, human cells elicits robust *in situ* immune responses by dendritic cells.

We previously demonstrated the *in vivo* effectiveness of allogeneic fibroblast cells loaded with α-GalCer and

Recent Publications

1. Shimizu, K., Mizuno, T., Shinga, J., Asakura, M., Kakimi, K., Ishii, Y., Masuda, K., Maeda, T., Sugahara, H., Sato, Y., Matsushita, H., Nishida, K., Hanada, K., Dorrie, J., Schaft, N., Bickham, K., Koike, H., Ando, T., Nagai, R., Fujii, S. Vaccination with antigen-transfected, NKT cell ligand-loaded, human cells elicits robust in situ immune responses by dendritic cells. ***Cancer Res.*** 73, 62-73 (2013)
2. Fujii S., Shimizu K. DC-based immunotherapy targeting NKT cells. In Terabe M and Berzofsky JA (eds), ***Natural killer T cells: Balancing the regulation of tumor immunity.*** Springer New York Dordrecht Heidelberg London, 95-110 (2011)
3. Shimizu K., Asakura M., Fujii S. Prolonged antitumor NK cell reactivity elicited by CXCL-10-expressing dendritic cells licensed by CD40L^{+}CD4^{+} memory T cells. ***J Immunol.*** 186, 5927-37 (2011)

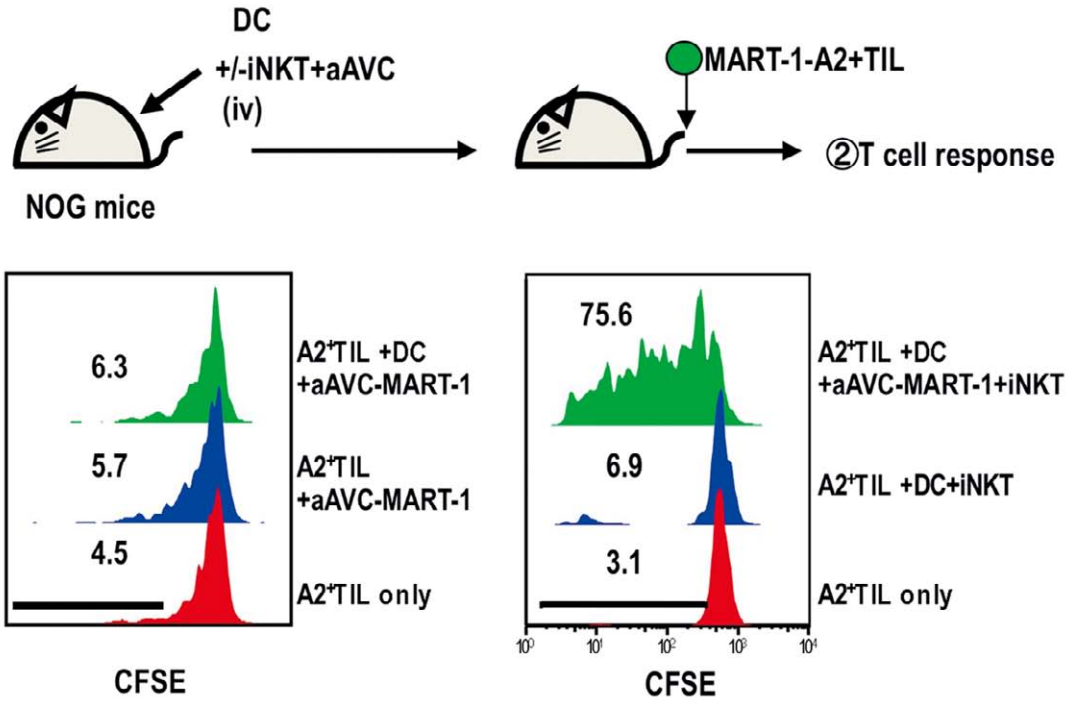

Figure : **The antigen presenting capacity of human DCs in NOG mice in response to aAVC**
Immature DCs generated *in vitro* from human monocytes were transferred into NOG mice and then *i*NKT cells and aAVC were transferred. Ten hours later, CFSE-labeled HLA-A2^{+}JKF6 cells (stable melanoma responding TIL cells) were adoptively transferred. Five days later, JKF6 T cell proliferation as a measure of human APC function was evaluated by dilution of CFSE. The numbers indicate the percentages of divided JKF6 T cells.

transfected with antigen-encoding mRNA, thus combining the adjuvant effects of *i*NKT cell activation with delivery of antigen to DCs (*Blood* 2009). In the current study, we established human artificial adjuvant vector cells (aAVC) consisting of human HEK293 embryonic kidney cells stably transfected with CD1d, loaded with α-GalCer and then transfected with antigen-encoding mRNA. When administered to mice or dogs, these aAVCs activated *i*NKT cells and elicited antigen-specific T cell responses with no adverse events. In parallel experiments, using the human dendritic cell (hDC)-transferred *NOD/SCID/IL-2rγc^{null}* immunodeficient (hDC-NOG) mouse model, we also showed that the human melanoma antigen MART-1 expressed by mRNA transfected aAVCs could be cross-presented to antigen-specific T cells by human DCs. Antigen-specific T cell responses elicited and expanded by aAVC were verified as functional in tumor immunity. Our results support the clinical development of aAVC to harness innate and adaptive immunity for effective cancer immunotherapy.

A strategy using *in vivo* DC maturation by NKT cells to generate adaptive immunity with T cell memory

We have been examining 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. With this strategy of using NKT cell ligand-loaded syngeneic tumor antigen expressing cells, the activation of adaptive immunity occurred through *in vivo* maturation of DCs and was able to optimize tumor antigen delivery systems to DCs. Moreover, we have found that DCs can play a role in cross presentation of tumor antigen and glycolipid to T cells and NKT cells, respectively. We also found that T cells responding to the specific antigen can be maintained as memory T cells, thus resulting in long-term protection. We have recently been elucidating the mechanism for this memory T cell response.

4. Asano K., Nabeyama A., Miyake Y., Qiu CH., Kurita A., Tomura M., Kanagawa O., Fujii S., Tanaka M. CD169-positive macrophages dominate antitumor immunity by crosspresenting dead cell-associated antigens. ***Immunity*** 34, 85-95 (2011)
5. Watarai H, Fujii S, Yamada D, Rybouchkin A, Sakata S, Nagata Y, Iida-Kobayashi M, Sekine-Kondo E, Shimizu K, Shozaki Y, Sharif J, Matsuda M, Mochiduki S, Hasegawa T, Kitahara G, Endo TA, Toyoda T, Ohara O, Harigaya K, Koseki H, Taniguchi M. Murine induced pluripotent stem cells can be derived from and differentiate into natural killer T cells. ***J Clin Invest.*** 120, 2610-2618 (2010)
6. Fujii S, Goto A, Shimizu K. Antigen mRNA-transfected, allogeneic fibroblasts loaded with NKT-cell ligand confer antitumor immunity. ***Blood*** 113, 4262-4272 (2009)

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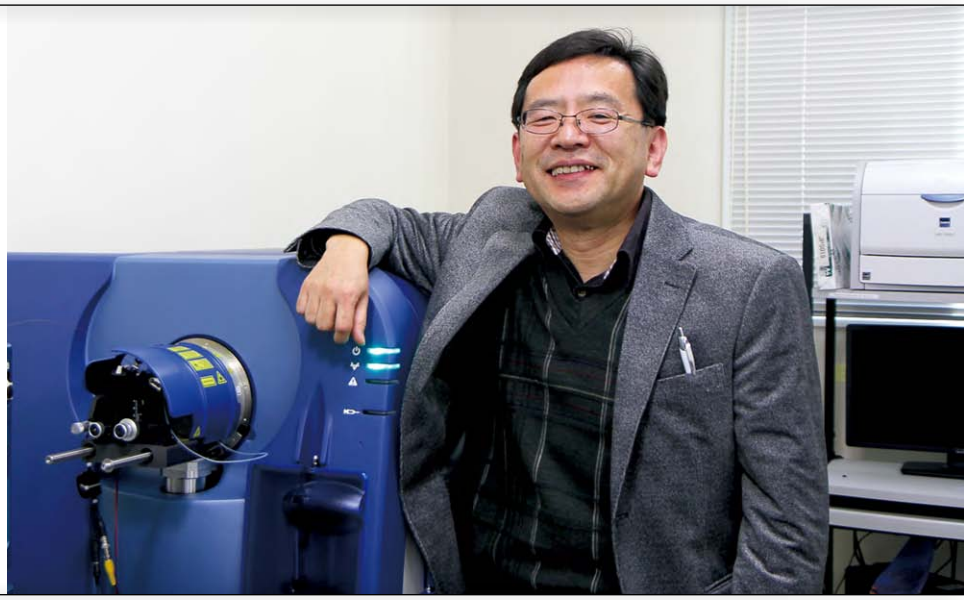
Student Trainee:
Nanako Shimura (JRA), Asahi Nakahara



The very basic mission of the immunogenomics group has been to function as a "Gateway" to genomics for immunologists. To achieve this mission at the center, we have divided our research activities into three components as follows: (1) central support activities; (2) strategic and collaborative research activities; and (3) exploratory research activities aimed at new technology development. The emergence of massively parallel DNA sequencing (MPS) technology has considerably changed the genomics research style and more or less affects all research activities. We have currently used MPS for various purposes and recently set up a high-throughput DNA sequencer (HiSeq1000, Illumina) as a part of the central support activities. The advantage of MPS is not its high capacity for data production: Because most conventional MPS methods are based on clonal amplification of a single DNA template, the generated data enable us to characterize a pool of heterogeneous DNA molecules at the single-nucleotide resolution. In other words, MPS could directly analyze genetic heterogeneity of a cellular ensemble of immune cells expressing different immunoglobulins or T-cell receptors in a quantitative manner. In this way, the MPS technology is particularly useful for analysis of diversity and dynamics of the immune system at single-cell resolution, which makes single-cell analysis more accessible to immunologists than before. Because we have also developed various single-cell analysis methods in the past, we consider that the combination of analyses in a population context and in real-time will surely clarify unexplored aspects of the dynamics of the immune system. As examples of such approaches, 1) construction of the pipeline of MPS data processing and 2) development of an inflammatory cytokine secretion assay from single cells are described below.

Construction of the data analysis pipeline for MPS

The currently available high-throughput MPS machine has a sequencing capacity of 300 x 10^9 bases (300 Gbase)/run in a 100-base pair-end sequencing mode. Thus, it is obvious that we must establish an in house pipeline for routinely managing such huge amounts of DNA sequencing data. However, the use of MPS varies widely, from DNA sequence determination (whole exon sequencing, conventionally termed "Exome sequencing") to measurement of the occurrence frequencies of particular DNA fragments mapped on the genome (RNA sequencing and ChIP-sequencing). In a realistic situation, multiple samples labeled with short identification tag sequences are simultaneously run on the same machine and eventually separately processed according to their analysis purposes. Taking these situations into consideration, we first established a sample

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3. Izawa, K., Hijikata, A., Tanaka, N., Kawai, T., Saito, MK., Goldbach-Mansky, R., Aksentijevich, I., Yasumi, T., Nakahata, T., Heike, T., Nishikomori, R., Ohara, O. Detection of Base Substitution-Type Somatic Mosaicism of the NLRP3 Gene with >99.9% Statistical Confidence by Massively Parallel Sequencing. ***DNA Res.*** 19, 143-152 (2012)

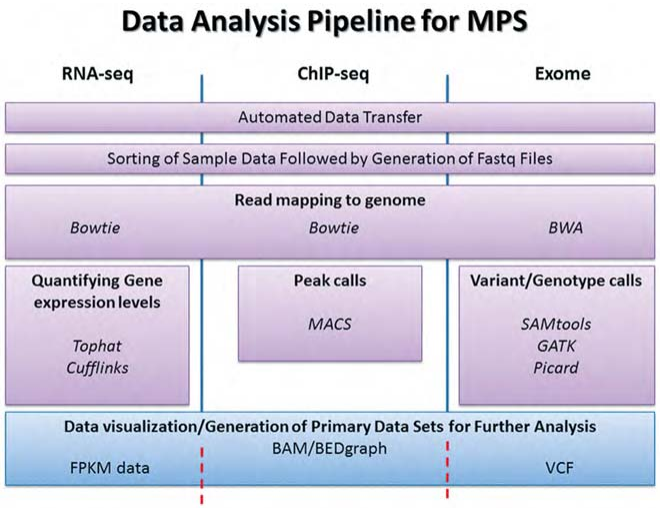

Figure 1: Diagram of the automated data processing pipeline for MPS
Abbreviations shown in italics are names of publicly available software for MPS data analysis. The pipeline is composed of these public software and the final data (FPKM data for RNA sequencing; BAM/BED graph for ChIP-sequencing; VCF for exome sequencing) are automatically generated without human intervention.

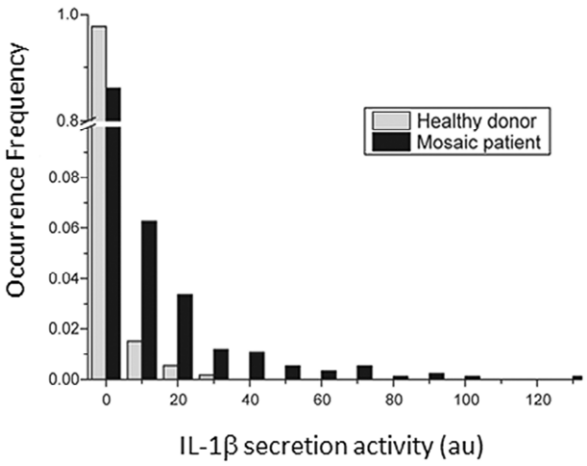

Figure 2: Distribution of IL-1β secretion activities of human monocytes at the single-cell level.
IL-1β secretion was measured by the Microengraving method at the single-cell level. As can be clearly seen, a considerable number of human monocytes in the CINCA patient secrete more IL-1β than those in the healthy donor without any inflammatory stimulation.

annotation system that clearly describes the sample attribute. After the sequencing run, the data are automatically transferred and processed according to their attributes as shown in Fig. 1. Taking advantage of the automated data processing pipeline thus constructed, it becomes possible to fully utilize the power of MPS at the center.

Monitoring of IL-1β secretion from single human monocytes for diagnosis of CINCA syndrome

Chronic infantile neurological cutaneous and articular syndrome (CINCA), also known as neonatal-onset multisystem inflammatory disease (NOMID), is a dominantly-inherited systemic autoinflammatory disease caused by a heterozygous germline gain-of-function mutation in the *NLRP3* gene. As a response to our recent finding of a high incidence of *NLRP3* somatic mosaicism in apparently mutation-negative CINCA/NOMID patients (Tanaka *et al*., *Arthritis Rheum*. 2011), we developed a rapid diagnosis method for somatic *NLRP3* mosaicism by using MPS (Izawa *et al*., *DNA Res*. 2012). However, the etiology of CINCA syndrome has still remained unclear. Although several investigators have suggested the possibility of mutation-induced aberrant IL-1β secretion from monocytes to spark and to expand the inflammation, little is known about the involvement of IL-1β secretion as a pathogenic mechanism. Thus, we have tried to measure IL-1β secretion by human monocytes from healthy donors and CINCA patients at the single-cell level by the Microengraving method (Love *et al*., *Nat. Biotechnol*. 2006). Fig. 2 shows the distributions of amounts of IL-1β secretion from single monocytes purified from a healthy donor and a CINCA patient and cultured without any stimuli. Interestingly, human monocytes from the CINCA patient contain a larger fraction of actively IL-1β secreting cells than those from the healthy donor even under these non-activating conditions, which suggests that the measurement of IL-1β from monocytes at the single-cell level might be used for rapid diagnosis of the CINCA syndrome. While more data must be obtained and statistical power calculated before using this assay for clinical CINCA diagnosis, these data offer an interesting example where the combination of MPS data and single-cell protein measurements allows us to explore the etiology of a complex inflammatory disease.

4. Takagi, S., Saito, Y., Hijikata, A., Tanaka, S., Watanabe, T., Hasegawa, T., Mochizuki, S., Kunisawa, J., Kiyono, H., Koseki, H., Ohara, O., Saito, T., Taniguchi, S., Shultz, LD., Ishikawa, F. Membrane-bound human SCF/KL promotes *in vivo* human hematopoietic engraftment and myeloid differentiation. ***Blood*** 119, 2768-2777 (2012)

5. Takagi, H., Fukaya, T., Eizumi, K., Sato, Y., Sato, K., Shibazaki, A., Otsuka, H., Hijikata, A., Watanabe, T., Ohara, O., Kaisho, T., Malissen, B., Sato, K. Plasmacytoid dendritic cells are crucial for the initiation of inflammation and T cell immunity *in vivo*. ***Immunity*** 35, 958-971 (2011)

Research Unit for

Immunoinformatics

Unit Leader:
S. Sujatha Mohan

Research Associate:
Subazini Thankaswamy Kosalai
(until December, 2012)

The main mission and focus of the RCAI Immunoinformatics research unit is to develop and maintain an open access Bioinformatics platform and data resource in order to gain insights into primary immunodeficiency diseases (PID). Towards this end, we have developed and established a molecular disease database named RAPID—Resource of Asian Primary Immunodeficiency Diseases (PID) (http://rapid.rcai.riken.jp), a web-based informatics platform that enables PID experts to easily mine collected genomic, transcriptomic, and proteomic data about PID-causing genes. 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.

At present, RAPID includes a total of 242 genes that are confirmed to cause over 260 PIDs. There are over 5000 unique mutations annotated from over 1800 citations as of January 2013.

Resource of Asian Primary Immunodeficiency Diseases (RAPID) phenotype ontology framework - PhenomeR: Towards establishment of an e-clinical support system for phenotype-based genetic analysis of PID using semantic web technology

The main challenge for *in silico* genotype-phenotype correlation for any genetic disease is to standardize phenotype ontology terms and the genotype. We have introduced a newly developed PID ontology browser for systematic integration and analysis of PID phenotype with the genotype data from RAPID. Towards this end, we have developed a user-friendly interface named, "PID PhenomeR" (http://rapid.rcai.riken.jp/ontology/v1.0/phenomer.php), which serves as a standardized phenotype ontology resource to present ontology class structures and entities of all observed phenotypic terms in PID patients from RAPID in standardized file formats - Web Ontology Language (OWL) and Resource Description Framework (RDF) using semantic web technology. PID PhenomeR consists of 1466 standardized PID terms that are classified under 24 and 29 semantic types and categories respectively as of December, 2012 (The manuscript describing this project "PID PhenomeR- An integrated platform for developing phenotype ontology structures for primary immunodeficiency diseases" has been submitted to *Database*, an Oxford University Press Journal). The standardization of PID phenotype terms for addition of new terms is in progress, using a unique semi-automated process including a logic based assessment method. The home page and overall work flow of PID Phenomer is shown in Fig. 1. In essence, PID PhenomeR serves as an active integrated platform for PID phenotype data, wherein the generated semantic framework is implemented in the integrated knowledge-base query interface i.e. SPARQL Protocol and RDF

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1. Telikicherla, D., Marimuthu, A., Kashyap, MK., Ramachandra, YL., Mohan, S., Roa, JC., Maharudraiah, J., Pandey, A. Overexpression of ribosome binding protein 1 (RRBP1) in breast cancer. ***Clin Proteomics.*** 9 (1): 7 (2012)
2. Telikicherla, D., Ambekar, A., Palapetta, SM., Dwivedi, SB., Raju, R., Sharma, J., Prasad, TsK., Ramachandra, Y., Mohan, S., Maharudraiah, J., Mukherjee, S., Pandey, A. A comprehensive curated resource for follicle stimulating hormone signaling. ***BMC Res Notes.*** 4; 408 (2011)
3. Raju, R., Nanjappa, V., Balakrishnan, L., Radhakrishnan, A., Thomas, JK., Sharma, J., Tian, M., Palapetta, SM., Subbannayya, T., Sekhar, NR., Muthusamy, B., Goel, R., Subbannayya, Y., Telikicherla, D., Bhattacharjee, M., Pinto, SM., Syed, N., Srikanth, MS., Sathe, GJ., Ahmad, S., *et al.* NetSlim: high-confidence curated signaling maps. ***Database (Oxford)*** bar032 (2011)

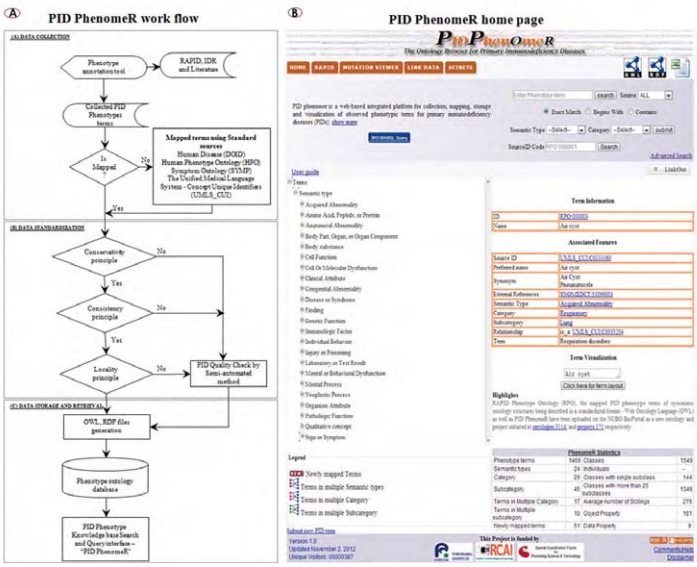

Figure 1 : Snapshot of PID Phenomer

A. Overview of PID PhenomeR work flow highlights various stages of processes such as (A) Data collection, (B) Data standardization and (C) Data storage and retrieval

B. Home page of PID PhenomeR presents term information along with associated features, query options (simple and advanced), term visualization, statistics and so on.

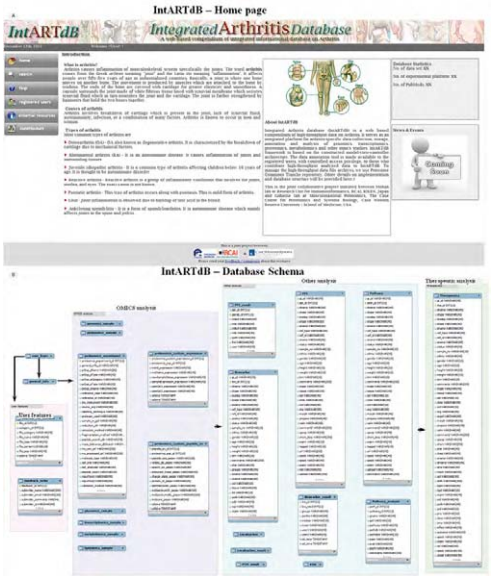

Figure 2 : Integrated Arthritis Database (IntARTdB)

A. Top page of IntARTdB (Formative stage of development)

B. IntARTdB schema shows four major compartments i.e. User features - upload and feedback; OMICs; Other studies - Protein-protein interaction, Pathway, Localization, Post-translational modification and Biomarker; therapeutics data annotation and analyses, all shaded in different colors. This schema is generated using the MySQL workbench

Query Language (SPARQL) endpoint for establishing a well-informed PID e-clinical decision support system. This kind of analysis should help bridge the gap between genotype and phenotype correlation, thereby improving phenotype-based genetic analysis of PID genes. Moreover, it should aid clinicians in confirming early PID diagnosis and also be helpful in implementing appropriate therapeutic interventions.

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

Integrated Arthritis database (IntARTdB) is a web based compendium of high-throughput data on arthritis. It serves as an integrated platform for arthritis-specific data collection, storage, annotation and analysis of genomics, transcriptomics, proteomics, metabolomics and other omics studies. The IntARTdB framework is based on the constructed model-view-controller architecture. The data annotation tool is made available to those who contribute high-throughput analyzed data to IntARTdB, with controlled access privilege through a streamlined-registration. The data entry and display of IntARTdB are performed using HTML front end using PHP interface as well as MySQL database at the backend. Each Omic (Genomics, Proteomics, Transcriptomics, Metabolomics, Lipidiomics and Glycomics) input information consists of general, sample and experimental annotation forms. Moreover, the database also hosts other studies such as PTMs, PPIs, Pathways, Biomarkers, Sub-cellular localization, *etc*. Once a registered user inputs general information, the annotation page will be subsequently accessed for loading, editing, viewing and updating Omics data according to individual access privilege. The customized interface is also developed to facilitate uploading of bulk data in text or CSV format files for direct storage in the database. Further, we use Proteome Commons Tranche repository to manage all high-throughput data file archives. Screenshots of this database top page and schema are shown in Fig. 2 (A & B).

Collaboration and funding

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

The IntARTdB project has been commenced in collaboration with the Gobezie lab at Musculoskeletal Proteomics, The Case Center for Proteomics and Systems Biology, Case Western Reserve University - School of Medicine, USA.

4. Ramadoss, SK. and Mohan, S. In Silico Identification of Prioritized Interacting Domains in Primary Immunodeficiency Disease Causing Genes. ***Intl J Biosci Biochem Bioinforma.*** 1, 84-88 (2011)

5. Hijikata, A., Raju, R., Keerthikumar, S., Ramabadran, S., Balakrishnan, L., Ramadoss, SK., Pandey, A., Mohan, S., Ohara, O. Mutation@A Glance: an integrative web application for analyzing mutations from human genetic diseases. ***DNA Res.*** 17, 197-208 (2010)

Laboratory for

Cellular System Modeling

Team Leader: **Mariko Okada (Hatakeyama)**

Senior Research Scientist: **Shinji Kondo (until May)**
Research Scientist: **Masaki Nomura**
Hisaaki Shinohara
Koichi Saeki (JSPS)

Technical Staff: **Noriko Yumoto, Kaoru Takahashi, Ki Sewon**
Student Trainee: **Norihiko Inoue (JRA)**

Dysregulation of intracellular signaling activity is strongly associated with a variety of diseases such as cancer, diabetes, inflammation and immune diseases. Our research interest is to identify important signaling dynamics related to cellular phenotypes, and to integrate signal transduction and transcriptional data using mathematical models to understand mechanistic regulation of the signal-transcription network. This approach aims to answer the question of how small quantitative changes in the initial signaling dynamics can induce qualitative changes in transcriptional profiles or expression of marker genes, changes that are often associated with diverse cellular phenotypes and disease states.

Spatiotemporal dynamics of NF-κB signaling in B cell differentiation

Activity of the transcription factor nuclear factor-κB (NF-κB) is spatiotemporally controlled and displays particular dynamic behaviors in the process of cellular determination. The current goal of our research is to identify the relationship between NF-κB dynamics and cell differentiation. Our quantitative experimental and mathematical analyses indicated that a TAK1-IKK positive feedback loop enforced by CARMA1 Ser-578 phosphorylation functions to produce a switch-like response to set a threshold in NF-κB activity and B cell proliferation. We are also trying to dissect interconnected signal-dependent networks in B cells by using ligand dose-response analysis. Such an analysis allows us to identify each signal-transcription relationship, how multiple signaling pathways coordinately determine transcriptional output of a B cell, and the positive and negative

Recent Publications

1. Hiroshima M, Saeki Y, Okada-Hatakeyama M, and Sako Y. Dynamically varying interactions between heregulin and ErbB proteins detected by single-molecule analysis in living cells. ***Proc Natl Acad Sci USA.*** 109, 13984-13989 (2012)
2. Saeki Y, Nagashima T, Kimura S, and Okada-Hatakeyama M. An ErbB receptor-mediated AP-1 regulatory network is modulated by STAT3 and c-MYC during calcium-dependent keratinocyte differentiation. ***Exp Dermatol.*** 21, 293-298 (2012)
3. Oyama M, Nagashima T, Suzuki T, Kozuka-Hata H, Yumoto N, Shiraishi Y, Ikeda K, Kuroki Y, Gotoh N, Ishida T, Inoue S, Kitano H, Okada-Hatakeyama M. Integrated quantitative analysis of the phosphoproteome and transcriptome in tamoxifen-resistant breast cancer. ***J Biol Chem.*** 286, 818-829 (2011)

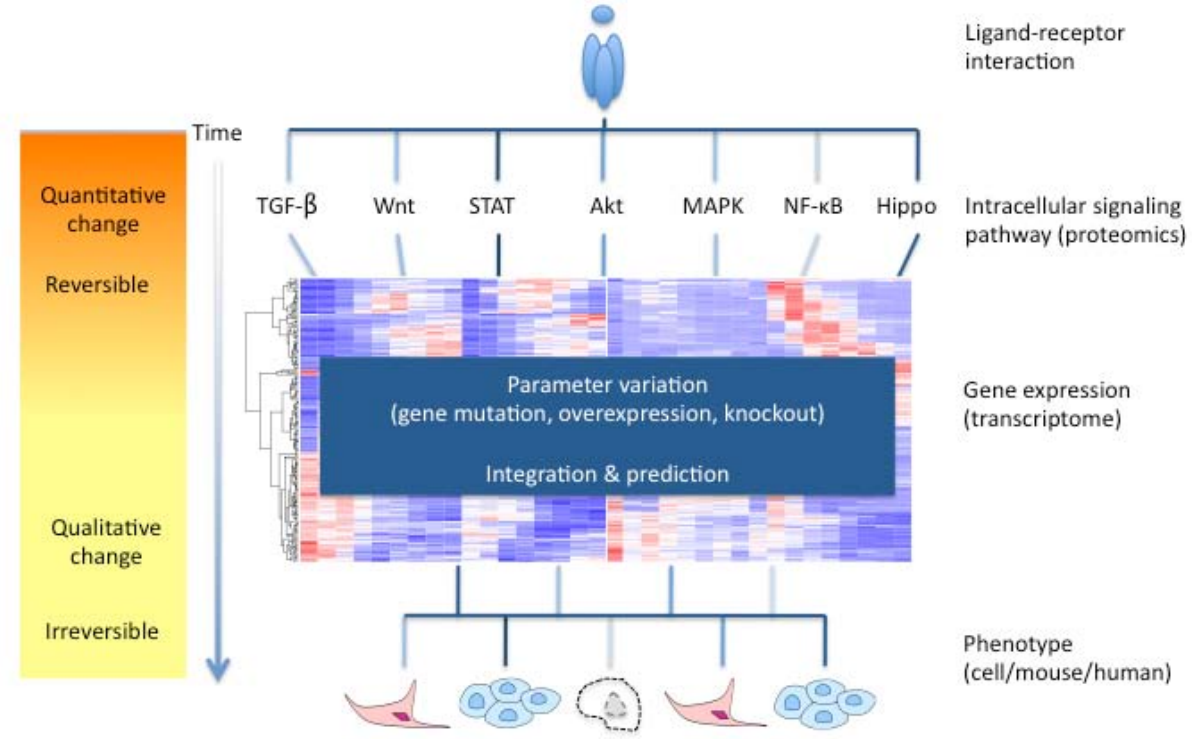

Figure : **Time-resolved integration of a signal-transduction network and its relationship with cellular and mouse phenotypes**

roles of signaling pathways for B cell functions.

Integration of data

We have been attempting to connect signal transduction pathways (proteomics), transcriptional signatures (transcriptome) and phenotypes using cellular and mouse models. Using this strategy, we identified a signal-early responsive gene network that potentially generates a tipping point for cellular differentiation (*Cell*, 2010), and predicted and then validated with clinical samples a marker gene, GSK3-β, for tamoxifen-resistant breast cancer (*JBC*, 2011). Currently our several joint research studies focus on transcriptome analysis of single gene knockout mice and its effect on intracellular signaling and metabolic pathways in mouse models. This strategy has successfully been filling the gap between gene signatures and cellular and mouse phenotypes.

Activation of membrane receptor-signaling pathways is first triggered by extracellular ligand-receptor interactions, and differences in the dynamics of these interactions often evoke induction of distinct cellular phenotypes. Inspired by the relationship between ligand-receptor interaction dynamics and cell fate decisions, we analyzed single molecule binding kinetics of a growth factor-ErbB membrane receptor interaction in living cells (in collaboration with Dr. Yasushi Sako, RIKEN ASI). This and our earlier analyses indicated that binding parameters of the two receptor-growth factor combinations are in a similar range, whereas the resultant cellular phenotypes stimulated by the growth factors are different. This analysis indicated that cytosolic reaction events rather than extracellular ligand-receptor kinetics seemed to play an important role in cell fate decisions mediated by the ErbB receptor (*Proc Natl Acad Sci USA*, 2012).

5. Nakakuki T, Birtwistle MR, Saeki Y, Yumoto N, Ide K, Nagashima T, Brusch L, Ogunnaike BA, Okada-Hatakeyama M*, Kholodenko BN.* Ligand-specific c-Fos expression emerges from the spatiotemporal control of ErbB network dynamics. ***Cell*** 141, 884-896 (2010) * corresponding authors.

Research Unit for

Thymic Environment

Unit Leader:
Willem van Ewijk

Preferential generation of K5⁺K8⁺ cTECs in the thymus following thymic injury caused by loss of thymocytes.

The stroma of the thymus consists of distinct sets of cortical and medullary thymic epithelial cells (TECs). Although early steps in the development of the thymic environments have recently been documented (*Immunity* 36, 298–309, 2012), the mechanism by which thymic environments are maintained postnatally is still unclear. We have identified a novel population of mature cortical (c) TECs that emerge postnatally in the depth of cortex. Microscopically defined by co-expression of cytokeratin (K) 5 and K8, these cTECs eventually populate the entire thymic cortex in aged mice.

We determined whether these K5⁺K8⁺ cTECs emerge as a consequence of K5 up-regulation in K5⁻K8⁺ cTECs as a mark of senescence, or newly arise as a result of a tissue repair process. For this purpose, a controlled perturbation of the thymic microenvironment was used to initiate tissue repair mechanisms. We thus investigated the emergence of K5⁺K8⁺ cTECs in organ cultures of 15 dpc fetal thymic (FT) lobes exposed to deoxyguanosine (dGuo). dGuo treatment depletes thymocytes and, consequently, ablates cross-talk signals required for normal TEC development. Subsequent transplantation of dGuo treated lobes under the kidney capsule of wild type mice leads to reconstitution of thymocytes. Two months later, the thymic tissue of both untreated and dGuo-treated grafts dramatically increased in size, the latter being slightly smaller. However, there was a clear difference with regard to the cTEC phenotype; a majority of cTECs in untreated FT grafts was K5⁻K8⁺, but almost all cTECs in the dGuo-treated FT grafts displayed a K5⁺K8⁺ phenotype (data not shown).

The thymic cortex is replenished by K5⁺K8⁺ cTECs generated as a consequence of clonal expansion during restoration of the injured thymic environment.

The above finding does not completely preclude the possibility of an age-related phenotypic change, because a certain stress, such as loss of thymic cross-talk signals, might accelerate senescence of cTECs. To determine whether K5⁺K8⁺ cTECs are newly generated by expansion of TEC progenitors, we employed a genetic model that allows clonal analysis of proliferating TECs. To this end, FT lobes were isolated from mice carrying both a Rosa26-creER allele and a loxP-stop-loxP EGFP transgene, and cultured for 6 days in the presence of dGuo (Fig. A). Subsequently, 4-hydroxy-tamoxifen (4-OHT) was added to the cultures for 6 hours at a low concentration. In a limited number of TECs, this treatment results in the nuclear translocation of the Cre-ER fusion protein, which initiates expression of GFP following Cre-mediated recombination. 4-OHT treated thymic lobes were then grafted under the kidney capsule of wild-type mice and reconstitution of thymopoiesis was followed over time. Under these experimental conditions, clonally expanded TECs, if present, could be visualized as

Recent Publications

1. Masuda, K, Germeraad WTV , Satoh R, Itoi M, Katsura Y, van Ewijk W, Kawamoto H. Activation of Notch signaling in thymic epithelial cells induces development of thymic microenvironments. ***Mol Immunol***. 46, 1756-1767 (2009)
2. Vroegindeweij, E, Itoi M, Satoh R, Zuklys S, Crobach S, Germeraad WTV. Cornelissen JJ, Cupedo T, Holländer G, Kawamoto H, van Ewijk W. Thymic cysts originate from Foxn1 positive thymic medullary epithelium. ***Mol Immunol.*** 47, 1106-1113 (2010)

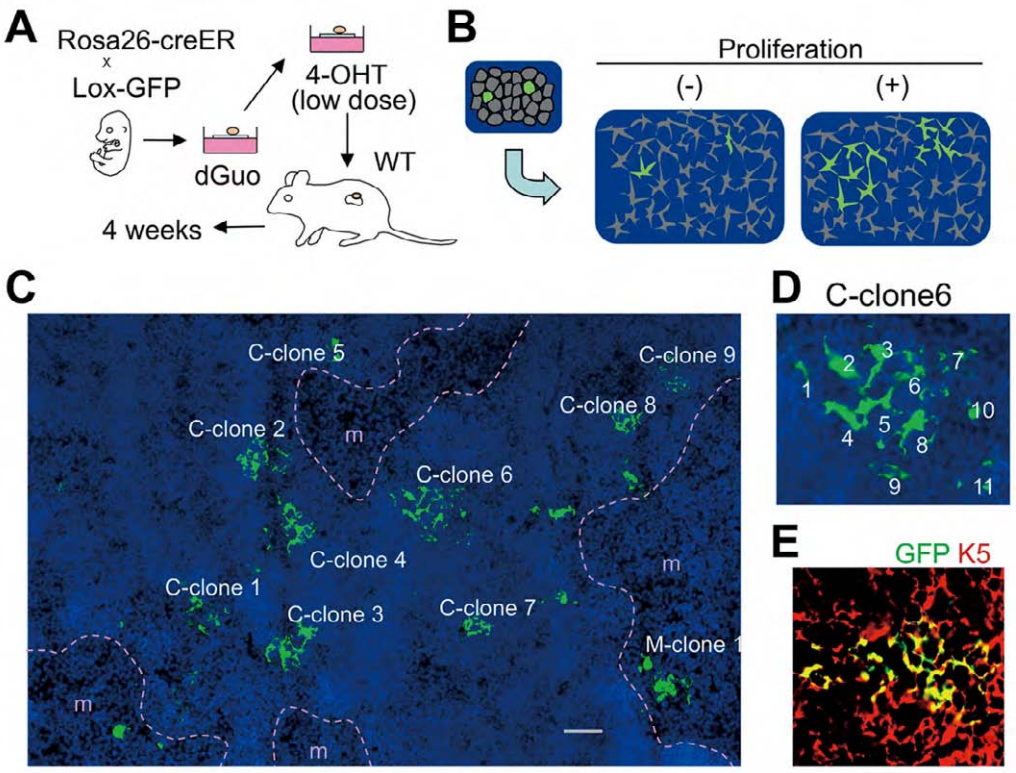

Figure : **Thymic cortex injured by loss of thymocyte-derived cross-talk signaling is replenished by K5⁺K8⁺ cTECs, generated by clonal expansion.**

A. A FT lobe from a 15 dpc fetus carrying both a Rosa26-CreER allele and a Lox-GFP transgene was incubated with dGuo for 6 days and subsequently with a low dose of 4-OHT, for 6 hours. The lobe was then transplanted under the kidney capsule of a 10 wk old wild type recipient mouse, and the graft was harvested 4 weeks later.

B. Rationale of the experimental system. By treatment of a low dose of 4-OHT, cre-mediated recombination is expected to occur randomly at a floxed site at very low frequency (lower than 1/100 cells in this case), resulting in the activation of GFP expression in cells separated from each other. If cTECs are formed without any proliferation in the restored thymic lobes, GFP⁺ cTECs will appear as individual cells, whereas if cTECs are formed by proliferation of precursor cells, GFP⁺ cTECs will be observed as independent colonies consisting of multiple cells.

C. A cryostat section of the graft was stained with anti-GFP (*green*), and counterstained with DAPI (*blue*). "C-clone" indicates a colony of clonally formed cTECs , whereas "M-clone" indicates clonally formed mTECs. Bar; 100 μm.

D. The enlarged image of C-clone 6 in Fig. 1C. About 10 cTECs were estimated to be present in the plane of this section.

E. A cryostat section of the graft of the same experiment as in Fig.1 C was stained with anti-GFP (*green*) and anti-K5 (*red*) antibodies, demonstrating the K5⁺K8⁺ TEC phenotype (*yellow*).

clusters of GFP-positive cells (Fig. B). Clusters of ten or more GFP⁺ cTECs were detected throughout the cortex of the rejuvenated thymic lobes, and the large majority of GFP⁺ cTECs was organized in such clusters (Fig. C). Each cluster was formed by several tenths of GFP⁺ cTECs, covering spherical areas larger than ϕ100 μm and thought to contain 500-1000 thymocytes (Fig. D). It is quite unlikely that these GFP⁺ cTECs represent a single cTEC, because the size of one cluster is much larger than a so called "thymic nurse cell", that usually contain fewer than 50 thymocytes. All these GFP⁺ cTECs were found to co-express K8 and K5 (Fig. E). Hence, the restoration of the cortical epithelial compartment following dGuo-treatment occurs predominantly via K5⁺K8⁺ cTECs generated as a consequence of clonal expansion, and not by an age-related phenotypic change of pre-existing K5⁻K8⁺ cTECs. The relation of these K5⁺K8⁺ cTECs with thymic epithelial stem cells remains to be elucidated.

Research Unit for

Immunoepigenetics

Unit Leader:
Miguel Vidal

Research Scientist: **Yang Li**

Polycomb group (PcG) complexes function as transcriptional repressors of genes determining cell identity states and the transitions between them. In stem cells, PcG complexes repress gene expression in ways that allow coordinate responses to inducing signals that promote cell state conversions taking place during differentiation. PcG complexes act both at the level of higher order chromatin structure and also locally. Their function depends, at least in part, on their activities as histone modifiers (mostly trimethylation of histone H3 and monoubiquitylation of histone H2A). PRC1 complexes, the subset of PcG assemblies with ubiquitin ligase activity (conferred by Ring1 protein ligases) are a heterogeneous set of entities. Mutually exclusive binding of chromodomain proteins or RYBP/Yaf2 proteins to Ring1 subunits specify two groups of PRC1 complexes. Functional analysis of these complexes is complicated by the presence of one or more paralogs and the potential for redundant activities. We have initiated work to approach this issue for the RYBP, Yaf2 homolog subunits.

Genetic interactions between RYBP and Yaf2 paralogs.

Our previous work showed prevalent association of RYBP with canonical Polycomb targets in ES cells; however, RYBP inactivation did not alter expression of these genes. By contrast, RYBP was also bound to germ line genes and retrotransposon elements and these were effectively repressed. Because of the existence of a closely related homolog, Yaf2 (Fig. 1, top), it was possible that the functional outcome of RYBP inactivation in ES cells was being modulated by Yaf2 compensatory activity. Thus, we set out to test this hypothesis by generating a compound *RYBP*, *Yaf2*-mutant ES cell. Fig. 1 (bottom) depicts a floxed allele designed (*Yaf2*^{f}, Jun Shinga, Developmental Genetics) for conditional deletion. Mutant mice were mated to introduce a tamoxifen-activated recombinase Cre and were also mated to *RYBP* mutant mice, so that *Yaf2*^{f} and *Yaf2*^{f}, *RYBP*^{f} expressing an inducible Cre gene were generated.

Preliminary gene expression analysis of these cells showed little Yaf2 activity (although the gene is expressed in ES cells) both alone and in combination with RYBP deficiency. Genome wide expression studies using RNA-Seq are presently ongoing in order to determine a comprehensive picture that would confirm or refute the current notion that whereas Yaf2 may control targets of its own, it does not compensate for RYBP on Polycomb targets. The unexpected anti-synergism seen at RYBP targets (Fig. 2) will need to be confirmed by biochemical studies that will be facilitated by the expression of a tagged-Yaf2 variant in our ES cell lines

Recent Publications

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

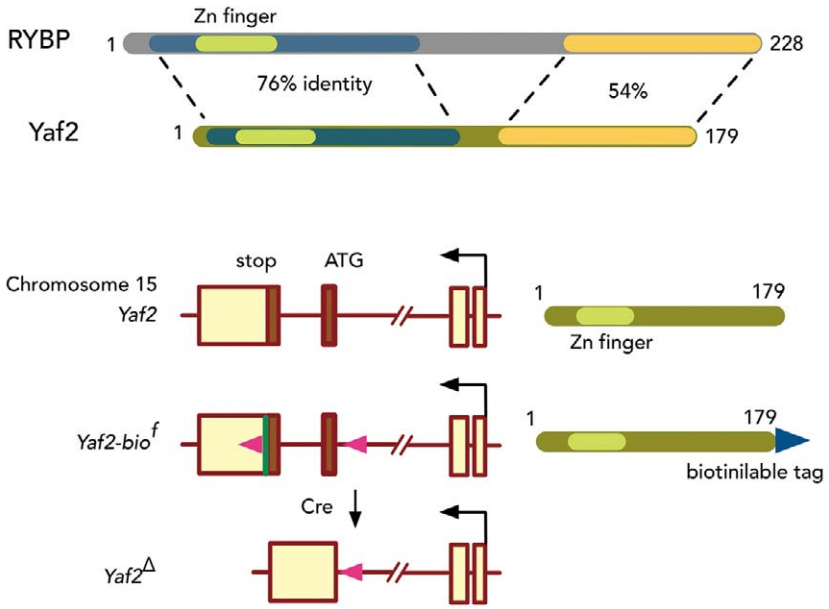

Figure 1: Murine RYBP, Yaf2 paralogs and Yaf2 allele for conditional inactivation.
Top: cartoons of RYBP and Yaf2 proteins depicting amino acid content, conserved regions and the Zinc finger domain; *bottom*: wild type *Yaf2* locus and the modified allele (*Yaf2-bio*^{f}) and their protein products, respectively. Deletion of sequences flanked by loxP sites (pink arrows) results in a null *Yaf2* allele (*Yaf2*$^{\Delta}$).

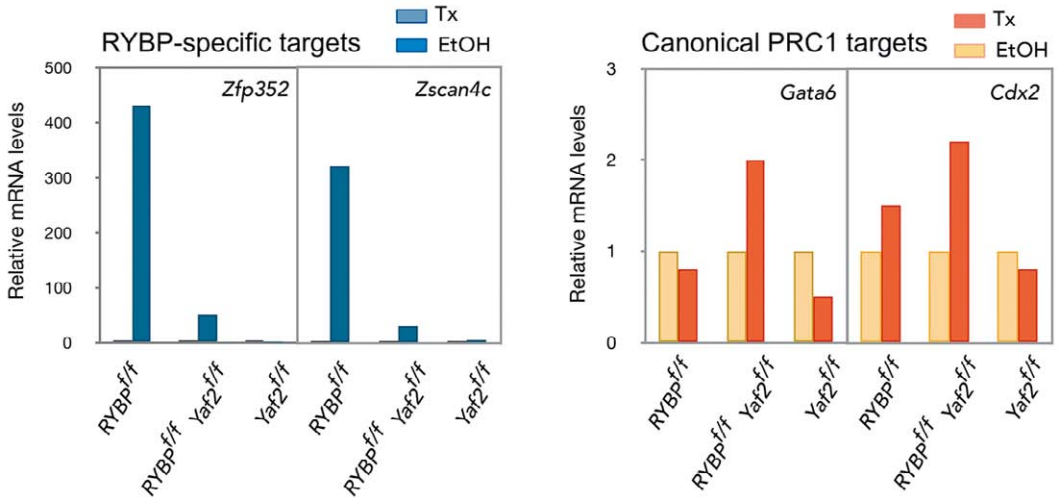

Figure 2: Gene expression in single *RYBP, Yaf2* and compound mutant ES cells.
Relative expression in wild type (ethanol-treated) and mutPant (Tamoxifen, Tx, treated) cells, measured by RT-qPCR, of representative genes among those repressed by RYBP (*left panel*) or silenced by canonical Polycomb-repression (*right panel*).

Research Unit for

Immune Crosstalk

Unit Leader:
Hilde Cheroutre

Defending the mucosal barrier against "the enemy" invading from the outside and the inside.

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

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

We have made milestone discoveries in both areas and showed that, for example, retinoic acid (RA) is a key factor in controlling mucosal immune responses and under tolerogenic conditions. RA promotes the induction of TGF-β-dependent Foxp3 iTreg cells whereas it suppresses the differentiation of inflammatory TGF-β-driven Th17 cells (Mucida *et al.*, *Science* 2007, Mucida *et al.*, *Immunity* 2009), .

We also showed that a novel affinity-based selective process operates at the mucosal interface of the intestine and preserves the optimal effector cells to become long-lived effector memory T cells (T_{EM}) that establish pre-existing and heightened protective immunity (Huang *et al.*, *Nature Immunology* 2011). Unlike central memory T cells (T_{CM}) that reside in lymphoid tissues, T_{EM} gain the capacity to reside long-term in non-lymphoid tissues such as the intestine. T_{CM} cells, which respond to antigen with robust clonal expansion, are effective at protecting against infections by pathogens that replicate systemically, but they are inadequate to prevent transmission of viruses, including the human immunodeficiency virus (HIV), or intracellular bacteria, which penetrate across mucosal epithelia. Effective resistance against transmission of such pathogens requires the presence of local, highly efficient antigen-specific T_{EM} prior to re-challenge. Nevertheless, because most of the current knowledge of immune memory has been gained from model systems that use systemic immunization routes for the generation of lymphoid T_{CM}, the generation of T_{EM} was very poorly understood.

We showed previously that the TCR repressor, CD 8αα, induced on activated CD8αβ T cells, marks those primary effector cells that preferentially differentiate into memory cells (Madakamutil *et al.*, *Science* 2004). The expression level of CD8αα is controlled by signal strength and it is most highly induced on those effector cells with the highest antigen-affinity. We found that mucosal T_{EM} are highly enriched for CD8αα-expressing memory cells, indicating that a selective mechanism might drive the preferential accumulation of high affinity effector cells at

Recent Publications

1. Mucida D, Husain MM, Muroi S, van Wijk F, Shinnakasu R, Naoe Y, Reis BS, Huang Y, Lambolez F, Docherty M, Attinger A, Shui JW, Kim G, Lena CJ, Sakaguchi S, Miyamoto C, Wang P, Atarashi K, Park Y, Nakayama T, Honda K, Ellmeier W, Kronenberg M, Taniuchi I, Cheroutre H. Transcriptional reprogramming of mature CD4⁺ helper T cells generates distinct MHC class II-restricted cytotoxic T lymphocytes. ***Nat Immunol***. 14, 281-289 (2013)

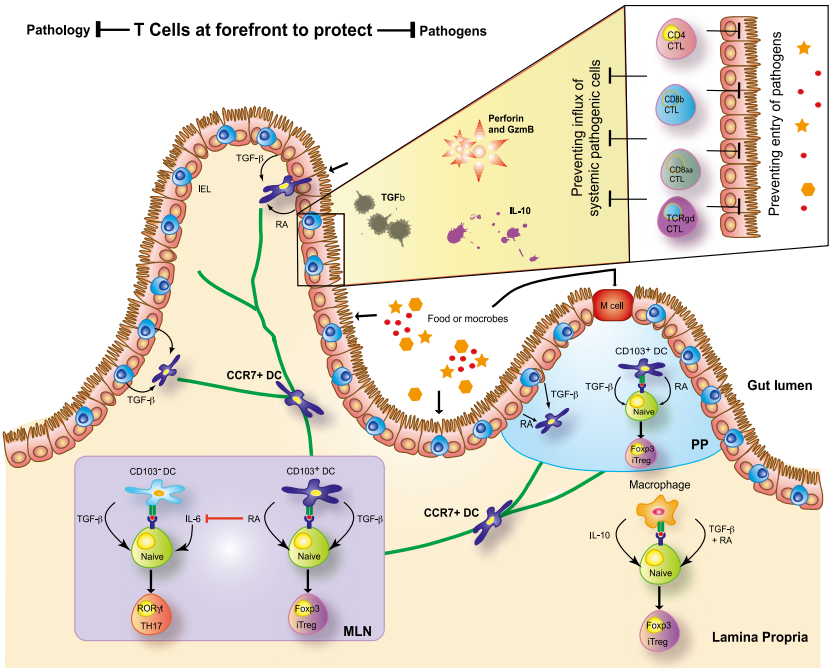

Figure : **Intraepithelial lymphocytes protect the mucosal barrier of the intestine from pathogen- and inflammation-induced pathology.**

T cells that reside within the epithelium of the intestine are phenotypically heterogeneous but they are all specialized to protect the mucosal barrier against pathogen- and immune cell-induced pathology. In contrast to the T cells in the periphery, epithelial T cells are antigen-experienced T cells that encountered their antigen initially during selection in the thymus (agonist selected CD8αα TCRαβ and TCRγδ precursor cells) or as mature cells in the periphery (CD8αβ CTL and CD4 CTL). Although the various epithelial T cells subsets display different antigen specificity and MHC restriction and although they follow different paths of effector differentiation, they all acquire cytolytic and regulatory capacity. The functional specialization of epithelial T cells adapts them toprovide optimal protection in the face of preserving the integrity of the delicate mucosal barrier.

the mucosal interface of the intestine.

In an earlier study we had shown that the high affinity ligand for CD8αα, the non-classical MHC class I molecule thymus leukemia antigen (TL), is constitutively expressed on mucosal epithelial cells. Using oral infection with the food borne pathogen *Listeria monocytogenes (Lm)*, we demonstrated the existence of an affinity-based selection mechanism controlled by TL expressed on APCs, which leads to the selective survival of high affinity, CD8αα$^+$C-D8αβ memory precursor cells. Furthermore, TL on the intestinal epithelial cells (IEC) continues to impose selection pressure and drives the affinity maturation of the resident mucosal CD8αβ T_{EM}, guaranteeing the optimal protective capacity of these memory cells.

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

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

Our research continues to elucidate mechanisms of mucosal immune protection and regulation and, in a recent study, we uncovered an unexpected degree of plasticity for CD4 T helper (Th) cells which, upon antigenic stimulation, are able to terminate the expression of the Th transcription factor, ThPOK, and differentiate into cytotoxic T lymphocytes (CTL). At steady state, these CD4 CTL remain quiescent and express a self-regulated phenotype. However under challenging conditions, these cells have the potential to transform into potent inflammatory killer effector cells (Mucida *et al.*, *Nat Immunol.* 2013).

Overall, based on the insights we are gaining from our research, a clear picture has begun to emerge showing that the immune defense of the intestine adapts to the local environment and specializes to provide the most efficient and immediate protection in the face of preserving the integrity of the most critical mucosal barrier of the body.

Central Facilities

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

FACS Lab

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

The FACS Lab provides a range of support for flow cytometry and cell sorting, procedures that are essential for nearly all immunological experiments. In 2012, the FACS Lab added three new machines, two Aria III and one Canto II. One of the newly installed Aria III is equipped with 5 lasers (blue, red, yellow-green, violet and near UV), which enables detection of 14 colors.

Table 1: Instruments in the FACS Lab

	FACS	Number of machines
FACS cell analyzer	Calibur	4
	Canto II	2
FACS cell sorter	Aria I	2
	Aria II	1
	Aria III	2
	Diva (digital vantage)	1
Imaging flow cytometer	Image Stream X	1
Mass cytometer	CyTOF	1

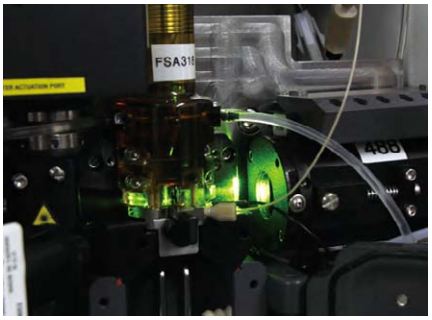

Figure 1: Yellow-green laser in Aria III

FACS Lab provided various services for the FACS users as follows.

1. Technical support and training
   In 2012, the facility offered 15 technical courses (6 for cell sorting, 7 for cell analysis (2 for Calibur and 5 for Canto II) and 2 for CytoF). A total of 64 RCAI researchers took the courses in 2012.

2. Cell sorting operation service
   The FACS Lab provides a cell sorting operation service, in which researchers can ask an experienced operator to conduct the sorting experiment. In 2012, there were 426 such requests.

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

Ikuo Ishige, Engineer from BM Equipment Co. Ltd., provided technical support for Image Stream X, including training courses, consultations for experiments and operation and analysis services.

Monoclonal Antibody Lab

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

The Monoclonal Antibody (mAb) Lab aims to produce mAbs that meet the needs of RCAI researchers, and also focuses on more targeted development of mAb with a strategic purpose, such as those for allergy-related molecules. This activity is now being done in cooperation with the program for Drug Discovery and Medical Technology Platforms for development of monoclonal antibodies as diagnostics and drugs. Two technical staff members, Tomomi Aoyama and Kazuyo Uchida, are engaged in producing, purifying and analyzing mAbs. In 2012, the Lab produced mAbs against 13 different antigens, which were requested by 8 laboratories. The lab also prepared human chimeric antibodies, such as an anti-Cry j 1 human IgE antibody.

Confocal Lab

Visiting Scientist: Yasutaka Wakabayashi (Leica Microsystems Co. Ltd.)

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

1. Inverted system with visual and a multi-photon (MP) laser suitable for time-lapse imaging of living cells and organs.

2. Inverted system with a 405 nm laser suitable for a time-lapse imaging of living cells in a controlled environment (CO_2, temperature, and humidity).

3. Leica SP5 confocal system, the successor of the SP2. Brighter and more striking images can be produced because of the improved optical system.

4. Upright system with visual and UV lasers suitable for standard fixed specimen observation.

5. Intravital upright system with single visual laser, double MP lasers and high speed scanner that can be used for *in vivo* imaging and for some other applications such as light stimulation.

6. To increase the convenience of animal experiments, an intravital system with visual laser, MP and OPO laser was relocated to the new animal area on the first floor.

During 2012, Confocal Lab provided training for 13 researchers. The total running time of the microscopes was over 2011 hours.

Animal Facility

Senior Research Scientist: **Takanori Hasegawa**

Technical Scientist: **Shinobu Mochizuki**
Masashi Matsuda

Technical Staff: **Tomoyuki Ishikura**
Yusuke Iizuka
Hiromi Tatsumi
Yurie Kawamoto
Chie Yoshida
Takahisa Shimazu

Animal caretakers (out sourced): 21 people

Figure 2: New animal facility room on the first floor.

Over 9,000 cages of SPF mice are maintained in the Animal Facility. The facility provides the following services for the users in the RIKEN Yokohama Institute.

1. Maintenance, generation and cryostocks of genetic resources
   The Animal Facility has been maintaining over 45,000 mice and 4 rats in the SPF area, 1,700 mice in an isolated area and several germfree mice. They have newly introduced 623 mouse lines into the SPF area by a combination of *in vitro* fertilization (IVF) and embryo transfer and generated cryostocks of genetic resources for 512 lines. The facility also maintains relatively large colonies for several commonly used strains, such as NOD/SCID/CγKO mice, Rag1KO and cre deleters, and provides them to users on demand. They have also provided technical assistance to generate chimeras (136 lines) and transgenic mice (10 lines) and to establish and maintain ES cells. They have also generated an internally available database for genetic resources.

2. Introduction of human BAC clones into NOD/SCID/CγKO mice
   This work was undertaken in collaboration with Dr. Osamu Ohara (RCAI Immunogenomics Group) and Dr. Fumihiko Ishikawa (RCAI, Human Disease Model Unit).
   The Animal Facility launched a new activity to improve the efficacy of transplantation of human hematopoietic stem cells into NOD/SCID/CγKO mice by "humanizing" the host strain. For this purpose, they have introduced large genomic fragments containing human genes encoding MHC, cytokines, adhesion molecules, virus receptors and others into NOD/SCID/CγKO mice. Up to now, they have established 15 BAC transgenics and confirmed the expression of human genes on a C57BL/6 background and started back-crossing them onto the NOD/SCID/CγKO mice using the speed-congenic method.

3. Creation of germ-free mice, maintenance and management
   This work is undertaken in collaboration with Dr. Hiroshi Ohno (RCAI Epithelial Immunobiology Group).
   The Animal Facility attempted sanitization of knockout mice and built a system that creates germ-free mice at the pace of 1 strain per month.

4. Construction of two new rooms for Animal Maintenance
   Two new rooms, fully equipped with autoclaves and cage washing systems, were added to the animal facility. These rooms were constructed on the first floor of the building, separately from the central animal facility on the 7th floor, and can be operated independently. In total, the facility has added 900 new cages where 4,500 animals can be kept. They have set up a multi-photon microscopy in one room that can be used for imaging analysis. In the other room, we have constructed vinyl isolator and bio-bubble facilities to breed and maintain (immunologically) humanized mice (Fig. 2).

Genomics Lab.

Research Scientist: **Takashi Watanabe**
Yoko Kuroki

Research Associate: **Mai Yamagishi**
Atsushi Hijikata

Technical Staff: **Tomoko Hasegawa**
Nobutake Suzuki
Akio Kobayashi
Fumie Yokoyama
Emi Abe

Figure 3: TripleTOF5600 System

The Genomics Lab provides various services to the members of the Center: proteomics analysis, multiplex suspension array, DNA microarray, DNA sequencing, cDNA/Genomic clone distribution, and primer/labeled probe distribution for qRT-PCR analysis of immune cells (Table 2).

Table 2: Services provided by the Genomics Lab in 2012

Proteomics	# of samples	# of teams
Two-dimensional electrophoresis	4	2
Mass Spectrometry Analysis	58	1
Multiplex suspension array	# of samples	# of teams
	3,740	12
Affymetrix Genechip (Exon array, Gene array, miRNA array)	# of samples	# of teams
Human	103	5
Mouse	514	18
Total	617	21
Sanger DNA sequencing	# of samples	# of teams
36cm capillary	13,926	16
50cm capillary	13,474	17
Total	27,400	33
cDNA clone delivery	# of clones	# of teams
	37	5
Primer/labeled probe delivery	# of sets	# of teams
	54	1

Because the speed of progress in genome technology is very fast, the Genomics Lab is keen to provide the Center with the most up-dated technology on demand. We have already introduced two next generation sequencers, a GS Junior [Roche] and a HiSeq 1000 [Illumina], which enable us to obtain vast amounts of massively parallel DNA sequencing data for genome DNA, transcripts, and so on. Both sequencers are already available in 2012. Additionally, we have recently introduced a mass spectrometer, a TripleTOF 5600 System [AB SCIEX] (Fig. 3). The system will make it possible to take quantitative proteomic approaches in various immunological studies. The members of the Center will be able to access to the TripleTOF 5600 System in early 2013.

Administrative Coordination Office

The Administrative Coordination Office (AdmCO) was established in April 2010 to support the research activities of RCAI. It consists of assistants, the IT team and facilities support staff.

The assistants support the Center and its laboratories by providing secretarial assistance and serve as Secretariats of the Center's annual events, such as the International Symposium co-organized with JSI, the International Summer Program, Harvard Summer School Program, Retreat, several joint workshops, the Graduate Students Tutorial Meeting, 21 seminars in the 2012 Seminar Series, etc. The IT team designs and maintains RCAI's unique server and databases, solves PC troubles and supports scientific meetings and conferences both inside and outside RCAI. The facilities staff advises on RCAI facilities and arranges construction required in remodeling laboratories and installing large equipment, etc.

In the summer of 2012, RCAI resumed international programs after one year hiatus caused by the Great East Japan Earthquake. In April, 2013, a new center will be launched and RCAI started preparing for it. From December, 2012, remodeling and relocation of laboratories took place sequentially. Mr. Ogata of the facilities staff advised each moving laboratory about laboratory layout, and planned the moving schedule. The IT team was busy virtualizing servers, which enabled RCAI members to acquire a more trustworthy IT environment and greatly contributed to secure the precious research data. Assistants helped laboratories move out and in, taking charge of machine recycling, mouse transfer, and a large number of MTAs, etc.

Using the centralized approach, the AdmCO is now able to provide smooth and efficient administrative support under the same standards to all RCAI laboratories. For example, even on the occasion of an assistant's sudden leave or illness, another AdmCO member can replace her without any interruption. The AdmCO, in cooperation with the Yokohama Research Promotion Division (YRPD), has been successfully coordinating and resolving various issues for the laboratories.

The strong spirit and constant efforts by the Administrative Coordination Office are now supporting the fundamental base of the RCAI activities and they will continue at the new center.

Table 3: Members of Administrative Coordination Office 2012

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

2012
Part 7

Data and Statistics

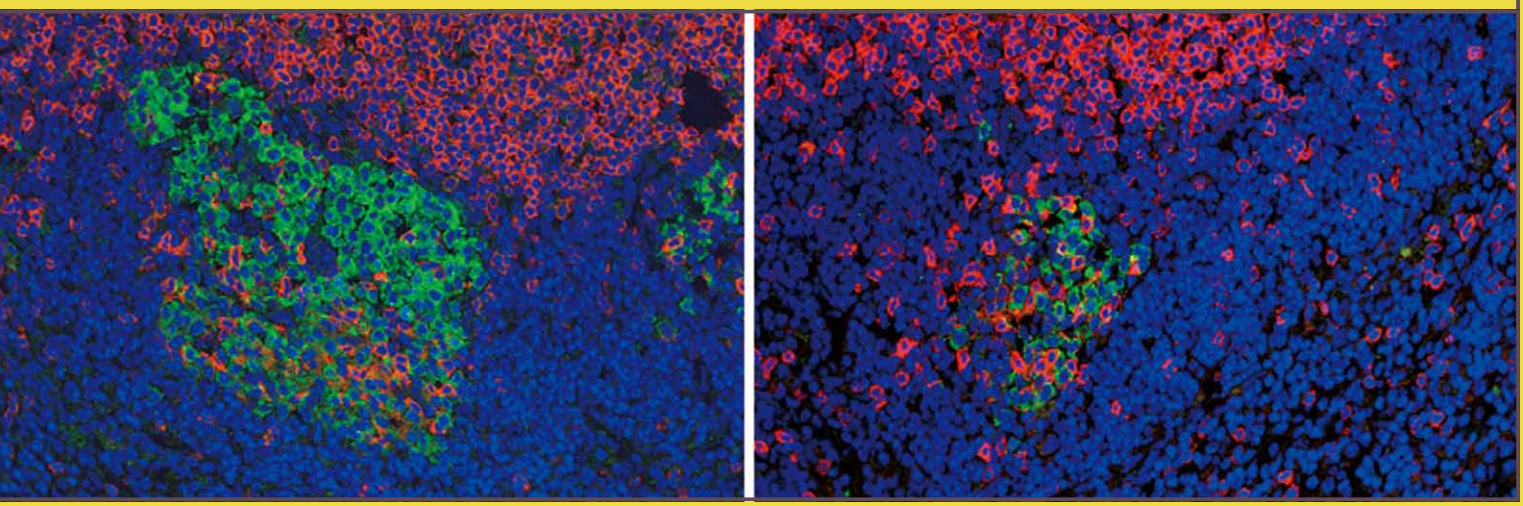

Table 1: RCAI publications FY2012

Journals	IF (2011)	FY2012
Annu Rev of Immunol.	52.8	1
Nat Rev Immunol.	33.3	1
Science	31.2	1
Nat Immunol.	26.0	6
Cell Stem Cell	25.4	3
Nat Med.	22.5	1
Immunity	21.6	4
Mol Cell.	14.2	1
J Exp Med.	13.9	4
Cell Host Microbe.	13.5	1
Immunol Rev.	11.1	1
J Allergy Clin Immun.	11.0	1
Blood	9.9	7
Proc Natl Acad Sci USA.	9.7	7
Curr Opin Immunol.	9.5	2
Circ Res.	9.5	1
EMBO J.	9.2	1
Genome Biol	9.0	1
PLoS Genet.	8.7	1
Cancer Res.	7.9	1
Development	6.6	1
Cell Mol Life.	6.6	1
Philos Trans R Soc Lond B Biol Sci.	6.4	1
Semin Immunol.	6.4	1
J Immunol.	5.8	12
Mol Cell Biol.	5.5	1
J Virol.	5.4	2
DNA Res.	5.2	1
Eur J Immunol.	5.1	1
Mol Biol Cell.	4.9	1
J Biol Chem.	4.8	3
PLoS One	4.1	1
Dev Biol.	4.1	2
Int Immunol.	3.4	4
Other Journals		41
Total		**119**

Table 2: Institutions ranking in immunology (Essential Science Indicators, The Thomson Coorporation, Sep., 2012)
When compared with institutions with more than 300 immunology papers, RIKEN's citations per paper was ranked 3rd in the world.

Institutes with more than 300 papers	Citations/ paper	Citations	Papers
ROCKEFELLER UNIV	**56.23**	24,572	422
OSAKA UNIV	**54.00**	55,021	1,019
RIKEN	**46.13**	**22,326**	**484**
SCRIPPS RES INST	**45.36**	31,029	684
KYOTO UNIV	**44.87**	27,731	618
SLOAN KETTERING CANC CTR	**44.79**	16,797	375
YALE UNIV	**44.16**	57,411	1,300
WALTER & ELIZA HALL INST	**43.25**	15,051	348
UNIV CALIF BERKELEY	**41.89**	16,126	385
LA JOLLA INST ALLERGY & IMMUNOL	**41.82**	22,208	531
WASHINGTON UNIV	**41.63**	43,004	1,033
BRIGHAM & WOMENS HOSP	**41.39**	32,614	788
NEW YORK UNIV	**38.62**	26,303	681
UNIV TEXAS HLTH SCI CTR HOUSTON	**37.99**	18,082	476
KYUSHU UNIV	**37.70**	16,775	445
UNIV CHICAGO	**37.48**	19,940	532
HARVARD UNIV	**37.41**	150,312	4,018

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Invited Presentations

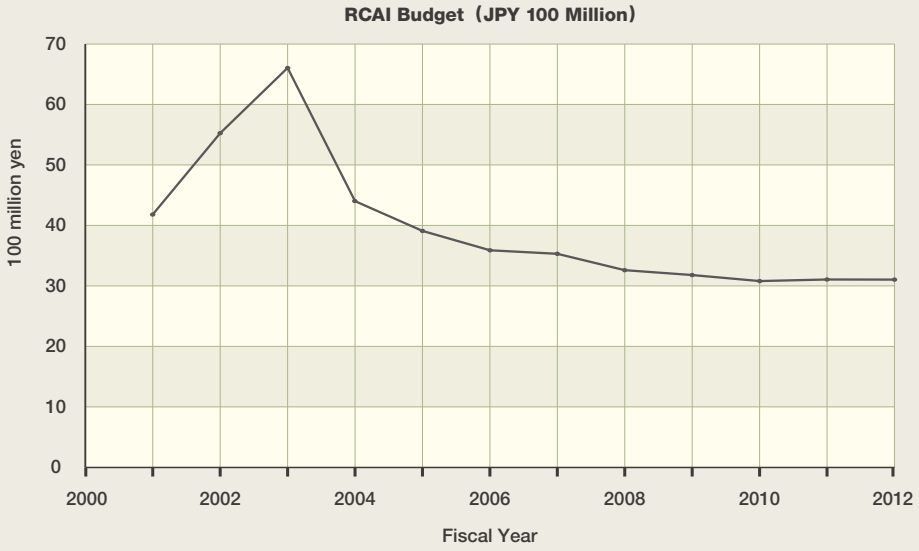
Month and year	Name of the speaker	Title of the presentation	Name of the meeting	Place of the meeting (City and country)
Apr-12	**Fagarasan, S.**	**Mucosal Immunology. Role of PD-1 in IgA synthesis in gut.**	Korean Immunology Conference	Seoul, Korea
Apr-12	**Taniguchi, M.**	**NKT cell system as a new therapeutic target**	The 112th Annual Congress of Japan Surgical Society	Chiba, Japan
Apr-12	**Wang, J-Y.**	**The IgM Fc receptor positively regulates B cell survival and antibody production**	Innovation Summit Tokyo 2012 - Chronic Inflammation and Autoimmune Diseases	Tokyo, Japan
Apr-12	**Sato, K.**	**Control of immune response by dendritic cells**	Seminar on Hiroshima Liver Conference	Hiroshima, Japan
Apr-12	**Ohno, H.**	**M cells, a unique subset of intestinal epithelial cells specialized for mucosal antigen-uptake**	The 2012 Spring Conference of the Korean Association of Immunologists	Seoul, Korea
Apr-12	**Ohno, H.**	**Gut microbiota and host protection - prevention of 0157-infectious death of mice by bifidobacteria**	The 22nd Tokyo Alcoholic Clinica Forum	Tokyo, Japan
Apr-12	**Ohno, H.**	**Role of Commensal Microbiota in Intestinal Immunity and Host Defense**	The 3rd International Forum, The 98th General Meeting of the Japanese Society of Gastroenterology	Tokyo, Japan
Apr-12	**Saito, T.**	**CRTAM-mediated cell adhesion regulates development of pathogenic effector T cells.**	ICAD Innovation Summit Tokyo 2012	Tokyo, Japan
Apr-12	**Cheroutre, H.**	**Mucosal Memory CD8 T Cells: Saving the Best for (to) Last**	Southwestern Medical Center at Dallas Excellence in Immunology Lecture Series	Dallas, USA
Apr-12	**Taniuchi, I.**	**Transcriptionaland epigentic control of thymocytes development**	Seminar at NIH	Bethesda, USA
Apr-12	**Cheroutre, H.**	**Mucosal Memory CD8 T Cells: Saving the Best for (to) Last**	Wake Forest University School of Medicine, Microbiology and Immunology Research Seminar	Winston-Salem, USA
May-12	**Koseki, H.**	**Polycomb repression in mammalian development**	Seminar, Dulbecco Telethon Institute	Rome, Italy
May-12	**Koseki, H.**	**Polycomb repression in mammalian development**	Seminar, European School of Oncology, European Institute of Oncology	Milan, Italy
May-12	**Koyasu, S.**	**Natural helper cells and Th2-type innate immunity**	99th Annual Meeting: The Americal Association of Immunologists	Boston, USA
May-12	**Ishikawa, F.**	**Creation of Humanized Mouse Model for Human Immunity and Diseases**	Workshop on Improving Animal Models for Regenerative Medicine, NIH	Bethesda, USA
May-12	**Fujii, S.**	**Licensing by invariant NKT cells harness the power of the dendritic cells (DCs)**	A Scientific Symposium in Memory of Ralph M. Steinman	New York, USA
May-12	**Koyasu, S.**	**Masters Lecture: Type 2 innate immune responses mediated by natural helper cells**	5th FIMSA Congress	New Delhi, India
May-12	**Fukada, T.**	**Zinc signal in growth and bone homeostasis**	The 66th Annual Meeting of Japan Society of Nutrition and Food Science	Sendai, Japan
May-12	**Kurosaki, T.**	**Activation Mechanisms of B Lymphocytes**	Freiburg Institute for Advanced Studies Seminar Series	Freiburg, Germany
May-12	**Koyasu, S.**	**Natural helper cell, a novel innate lymphocyte producing Th2 cytokines**	Xth Latin-American Congress of Immunology-ALAI2012	Lima, Peru
May-12	**Moro, K.**	**IL-33-dependent eosinophilia in the lung mediated by natural helper cells.**	20th International Symposium on Molecular Cell Biology of Macrophages 2012	Tokyo, Japan
May-12	**Moro, K.**	**The role of Natural helper cells in helminth infection and allergy**	77th Japan Congress of Interferon and cytokine	Kobe, Japan
May-12	**Moro, K.**	**The role of Natural helper cells during allergy**	Rochester conference	Tokyo, Japan
May-12	**Kurosaki, T.**	**Activation Mechanisms of B Lymphocytes**	Seminar, Max Planck Institute of Immunology and Epigenetics	Freiburg, Germany
Jun-12	**Takemori, T.**	**Memory B cells require memory T cells for their terminal differentiation.**	Immunological memory in Health and Diseases joint HKS and IMPAM meeting	Berlin, Germany
Jun-12	**Taniuchi, I.**	**Transcriptionaland epigentic control of thymocytes development**	Seminar at Medical University of Viena	Viena, Austria
Jun-12	**Saito, T.**	**Spatiotemporal regulation of T cell activation and co-stimulation.**	Gordon Research Conferences—Immunochemistry & Immunobiology	Les Diablerets, Switzerland
Jun-12	**Ohno, H.**	**Integrated 'omics' approach reveals that bifidobacteria protect from enterohaemorrhagic Escherichia coli infectious death through production of acetate.**	GI Research Academy	Tokyo, Japan
Jun-12	**Ohno, H.**	**Antigen delivery to the gut immune system through epithelium: M cells vs FAE?**	Gordon Research Conference, Lysosomes & Endocytosis	Andover, NH, USA
Jun-12	**Okada, T.**	**Imaging of lymphocyte dynamics during the germinal center formation**	RIKEN RCAI International Summer Program 2012	Yokohama, Japan
Jun-12	**Cheroutre, H.**	**Mucosal T cells: Shaped by the challenge**	RIKEN RCAI International Summer Program 2012	Yokohama, Japan,
Jun-12	**Taniguchi, M.**	**NKT cell-mediated adjuvant activity on antitumor responses.**	FOCIS2012	Vancouver, Canada
Jun-12	**Fukada, T.**	**"Zinc Signal": A new signal mechanistic regulating systemic growth and bone homeostasis**	12th Scientific Meeting of the Japanese Society of Anti-Aging Medicine	Yokohama, Japan
Jun-12	**Fujii, S.**	**NKT cell-mediated licensing of dendritic cells (DCs) in vivo**	RCAI-JSI International Symposium on Immunology	Yokohama, Japan
Jun-12	**Koyasu, S.**	**Critical role of GATA3 in natural helper cell differentiation and function**	RCAI-JSI International Symposium on Immunology 2012	Yokohama, Japan
Jun-12	**Ohno, H.**	**Function and differentiation of M cells, a unique subset of intestinal epithelial cells specialized for mucosal antigen-uptake**	RCAI-JSI Interntional Symposium on Immunology 2012	Yokohama, Japan
Jun-12	**Nishida, K.**	**Critical role of zinc transporter ZnT5/Slc30a5 in cytokine-mediated delayed-type allergic reaction**	The 23rd Annual Scientific Meeting of the Japanese Society for Biomedical Research on Trace Elements	Tokyo, Japan
Jul-12	**Fukada, T.**	**Biochemical characterization of human ZIP13 protein: A homo-dimerized zinc transporter involved in the Spondylocheiro dysplastic Ehlers-Danlos syndrome**	The 23rd Annual Scientific Meeting of the Japanese Society for Biomedical Research on Trace Elements	Tokyo, Japan
Jul-12	**Fukada, T.**	**Zinc signal study and Metallomics**	The 23th Annual Scientific Meeting of the Japanese Society for Biomedical Research on Trace Elements	Tokyo, Japan
Jul-12	**Ohno, H.**	**Differentiation and function of M cells, a unique subset of intestinal epithelial cells important for the induction of intestinal immunity**	The 2nd Dietary Sicence Forum	Tokyo, Japan
Jul-12	**Kurosaki, T.**	**Molecular Basis for Humoral Memory Response**	JSI Immunolgy Summer School 2012	Nasu, Japan
Jul-12	**Kurosaki, T.**	**Development and activation mechanisms of B lymphocytes**	JSI Immunolgy Summer School 2012	Nasu, Japan
Jul-12	**Saito, T.**	**Dynamicregulation of T cell activation**	JSI Immunolgy Summer School 2012	Nasu, Japan

Jul-12	**Kawamoto, H.**	**Lineage restriction process from hermatopoietic stem cells to T cell progenitors**	JSI Summer School Memorial Lecture for JSI Award	Nasu, Japan
Jul-12	**Fujii, S.**	**Development of artificial adjuvant vector cells targeting in vivo DCs**	The 6th Annual Meeting of Japanese Association of Cancer Immunology	Sapporo, Japan
Jul-12	**Kawamoto, H.**	**A novel model of hematopoiesis**	Kitano Hospital Core Lecture	Osaka, Japan
Aug-12	**Fujii, S.**	**Cross-talk between innate lymphocytes and dendnritc cells**	8th Lecture on Transplantation Immunology	Nagoya, Japan
Aug-12	**Fujii, S.**	**Discovery of dendritic cells by Prof. Steinman**	Men-eki Fushigi Mirai 2012	Tokyo, Japan
Aug-12	**Taniuchi, I.**	**Transcriptional and Epigenetic Views of CD4/CD8 Lineage Commitment in Thymus**	Seminar at Cancer Science Institute	Singapore, Singapore
Aug-12	**Taniuchi, I.**	**Transcriptional Control of CD4/CD8 Lineage Decision in Thymus**	Semiar at Walter and Eliza Hall Institute	Melbourne, Australia
Aug-12	**Okada, T.**	**Imaging of cellular dynamics during the adaptive immune response**	14th International Congress of Histochemistry and Cytochemistry, Kyoto, Japan	Kyoto, Japan
Aug-12	**Sakata-Sogawa, K. & Tokunaga, M.**	**Quantitative analysis of single molecule imaging of physicochemical field for genetic activities**	The 50th Annual Meeting of the Biophysical Society of Japan, Symposium	Nagoya, Japan
Sep-12	**Koyasu, S., Furusawa, J.-I., Moro, K.**	**Critical role of GATA3 in natural helper cell differentiation and function**	Cold Spring Harbor Asia Conferences "Frontiers of Immunology in Health and Diseases"	Suzuhou, China
Sep-12	**Honda, K.**	**Treg-inducing commensal Clostridium species derived from the human microbiome**	Cold Spring Harbor Asia Conferences "Frontiers of Immunology in Health and Diseases"	Suzuhou, China
Sep-12	**Ishikawa, F.**	**Humanized mouse research**	INSERM Necker Hospital	Paris, France
Sep-12	**Taniuchi, I.**	**Transcriptional Control of CD4/CD8 Lineage Decision in Thymus**	Seminar at Weatherall Institute of Molecular Medicine	Oxford, UK
Sep-12	**Ohno, H.**	**M cells, a unique subset of intestinal epithelial cells specialized for mucosal antigen-uptake**	The 11th Awaji International Forum on Infection and Immunity Program	Awaji, Japan
Sep-12	**Hase, K.**	**Intestinal microbiota shapes intestinal T cell balance via epigenetic modifications**	The 71st Annual Meeting of the Japanese Cancer Association	Sapporo, Japan
Sep-12	**Koseki, H.**	**Polycomb repression in mammalian development**	University of Luxembourg, Luxembourg Center for Systems Biomedicine team reatreat	Nennig, Germany
Sep-12	**Ohara, O.**	**Integra1ve Medical Sciences at RIKEN RCAI: to understand the process of disease development**	Australia-Japan workshop on biomedical breakthroughs and systems biology	Tokyo,Japan
Sep-12	**Moro, K.**	**Critical roles for natural helper cells in IL-33 dependent lung eosinophilia upon helminth infection**	The 12th Annual International and Innate Immunity Society Meeting	Tokyo, Japan
Oct-12	**Fujii, S.**	**Artificial cellular vaccine as front-line immune therapy against cancer**	RIKEN cutting edge symposium	Tokyo, Japan
Oct-12	**Okada, T.**	**Imaging of cellular dynamics underlying the immune tissue organization**	The 25th Annual Mouse Molecular Genetics Conference	Pacific Grove, USA
Oct-12	**Koseki, H.**	**Polycomb repression in mammalian development**	Seminar, Life Sciences Institute, University of British Columbia	Vancouver, Canada
Oct-12	**Okada, T.**	**Imaging of cross-presenting dendiritic cells in the lymph node**	University of California, San Francisco, Immunology Seminar Series	San Francisco, USA
Oct-12	**Cheroutre, H.**	**A transcriptional switch reprograms mature CD4 T helper cells to cytotoxic T lymphocytes.**	International Symposium on Immune Tolerance and Mucosal Immunology. Sichuan University	Chengdu, China
Oct-12	**Ishikawa, F.**	**Targeting chemotherapy-resistant AML stem cells**	74th Japan Society of Hematology Meeting, Asian Joint Symposium	Kyoto, Japan
Oct-12	**Ohno, H.**	**The role of M cells in intestinal immunity**	International Endotoxin & Innate Immunity Society Meeting 2012, Homeostatic Inflammation Symposium, Japan Endotoxin and Innate Immunity Society 2012	Tokyo, Japan
Oct-12	**Ohno, H.**	**Function and differentiation of M cells, a unique subset of intestinal epithelial cells specialized for mucosal antigen-uptake**	The 12th International Symposium on Dendritic Cells	Daegu, Korea
Oct-12	**Ohno, H.**	**Multi-omics approach reveals that bifidobacteria protect from enterohemorrhagic Escherichia coli infection through production of acetate**	The 34th Naito Conference, Infection, Immunityand their Control for Health	Sapporo, Japan
Oct-12	**Hori, S.**	**Resolving the controversy over regulatory T cell plasticity**	The 3rd International Conference on Regulatory T Cells and Th Subsets and Clinical Application in Human Diseases	Shanghai, China
Oct-12	**Shiroguchi, K.**	**System-wide digital counting of nucleic acid molecules using next generation sequencer with optimized single-molecule barcodes**	International meeting of "Spying Minority in Biological Phenomena", Grant-in-Aid for Scientific Research on Innovative Areas, MEXT, Japan	Taipei, Republic of China
Oct-12	**Taniguchi, M.**	**NKT cells. Target for the treatment of intractable diseases based on a new concept.**	Joint Symposium on Tsukuba University/Tokyo University of Science, Research Institute for Biomedical Sciences.	Tokyo, Japan.
Oct-12	**Hase, K.**	**Epithelial immune functions at the interface between self and non-self**	IEIIS2012 Homeostatic Inflammation Satellite Symposium - Infection, Inflammation and Immunity	Tokyo, Japan
Oct-12	**Tanaka, T.**	**Negative regulation of inflammatory responses by LIM proteins.**	The 12th Biennial International Endotoxin & Innate Immunity Society Meeting, Symposium L3, Chronic inflammation	Tokyo, Japan
Oct-12	**Fujii, S.**	**Development of artificial adjuvant vector cells as new type of cancer vaccine**	Tokyo University of Pharmacy and Life Sciences' Seminar	Hachioji, Japan
Oct-12	**Ohara, O.**	**Towards an understanding of immune cell sociology based on single-cell analysis**	International Symposium on Bio-Interactomics	Sevilla, Spain
Oct-12	**Moro, K.**	**Development of an innate Th2 producer 'natural helper cell**	International Symposium on genetic and epigenetic control of cell fate	Kyoto, Japan
Nov-12	**Koseki, H.**	**Mechanisms underlying Polycomb repression**	Grants-in-Aid for Scientific Research on Innovative Areas: "Genetic and Epigenetic Control of cell Fate"	Kyoto, Japan
Nov-12	**Takemori, T.**	**Immunological Memory consists of multiple layers.**	International Symposium: from Cell sorting to Immunlogical Memory	Berlin, Germany
Nov-12	**Kawamoto, H.**	**Molecular mechanisms for the production and maintenance of the T cell lineage**	The International Symposium on Genetic and Epigenetic Control of Cell Fate	Kyoto, Japan
Nov-12	**Fujii, S.**	**Development of vaccine linking innate and adaptive immunity**	Hoshima University Seminar Series	Tokyo, Japan
Nov-12	**Kurosaki, T.**	**B Cell Intrinsic and Extrinsic Mechanisms for Rapid Responsiveness of IgG1 Type Memory B Cells**	Keystone Symposia: B Cell Development and Function	Keystone Resort, USA
Nov-12	**Hori, S.**	**Control of autoimmunity by Foxp3+ regulatory T cells**	International Symposium on Glyco-minded Biology of Diseases as a Basis of Pharmaceutical Sciences	Tokyo, Japan
Nov-12	**Sato, K.**	**Control of immune response by dendritic cells**	The 70th Special Seminar on Clinical Allergy Society	Tokyo, Japan

Nov-12	**Kawamoto, H.**	**Lymphocyte Development**	Hong Kong University Pasteur Institute Immunology Course Lecture	Hong Kong, China
Nov-12	**Kurosaki, T.**	**Challenging for Mysteries of Immune Memory**	The second WPI joint outreach symposium "Let's enjoy the world's advanced science!"	Tsukuba, Japan
Nov-12	**Kurosaki, T.**	**Activation Mechanisms of Memory B cells**	Graduate School Seminar, University of Occupational and Environmental Health	Kitakyushu, Japan
Dec-12	**Fagarasan, S.**	**IgA synthesis: a form of adaptation extending beyond gut.**	42nd Annual Scientific Meeting Australasian Society for Immunology.	Melbourne, Australia
Dec-12	**Kawamoto, H.**	**Myeloid-based model of hematopiesis: revison of the classical myeloid-lymphoid dichotomy concept**	Osaka University Medial Tissue Engeneering Forum	Osaka, Japan
Dec-12	**Kawamoto, H.**	**Epigenetic mechanisms maintaining the T cell and thymic epithelial cell lineage**	2012 Annual Meeting of the Japanese Society for Immunology	Kobe, Japan
Dec-12	**Ohara, O.**	**Systems immunology: Tackling the complexity of the immune system**	2012 Annual Meeting of the Japanese Society for Immunology	Kobe, Japan
Dec-12	**Watarai, H.**	**Invariant natural killer T cells mediate airway inflammation through IL-25 and IL-18.**	2012 Annual Meeting of the Japanese Society for Immunology	Kobe, Japan
Dec-12	**Hase, K.**	**Epithelial Notch signaling secures lymphoid organogenesis and immune homeostasis in the gut**	2012 Annual Meeting of the Japanese Society for Immunology	Kobe, Japan
Dec-12	**Hori, S.**	**Genetic and environmental control of regulatory T cell fitness in tissues**	2012 Annual Meeting of the Japanese Society for Immunology	Kobe, Japan
Dec-12	**Taniguchi, M.**	**NKT cells as an ideal target for anti-tumor immunotherapy.**	2012 Annual Meeting of the Japanese Society for Immunology	Kobe, Japan
Dec-12	**Wang Ji-Yang**	**The Fc receptor for IgM (FcμR) positively regulates B cell survival and activation**	The 42nd Annual Meeting of The Molecular Biology Society of Japan	Kobe, Japan
Dec-12	**Okada, T.**	**Two-photon imaging of cellular dynamics during adaptive immune responses**	The 42nd Annual Meeting of The Molecular Biology Society of Japan	Fukuoka, Japan
Dec-12	**Hase, K.**	**Roles of intestinal M cells in mucosal infection and immunity**	The 85th Annual Meeting of the Japanese Biochemical Society	Fukuoka, Japan
Dec-12	**Fukada, T.**	**Zinc signal: A new regulatory system in bone and immunity**	The 85th Annual Meeting of the Japanese Biochemical Society	Fukuoka, Japan
Dec-12	**Ohno, H.**	**Understanding the host-gut microbiota interaction with multi-omics approach**	The 85th Annual Meeting of the Japanese Biochemical Society	Fukuoka, Japan
Jan-13	**Saito, T.**	**Dynamic and cytoskeletal regulation of T cell activation**	RCAI-Michigan Joint Workshop	Ann Arbor, USA
Jan-13	**Koseki, H.**	**Polycomb potentiates Meis2 activation in midbrain by mediating precursory interaction of promoter with tissue-specific enhancer**	RCAI-Michigan Joint Workshop	Ann Arbor, USA
Jan-13	**Ishido, S.**	**Novel immune regulation by MHC II ubiquitination**	The 2nd Innovative Area Symposiumy	Kobe, Japan
Jan-13	**Koyasu, S., Moro, K.**	**Natural helper cell, a Th2-type innate lymphocyte**	IFReC-SIgN Winter School 2012 on Advanced Immunology	Osaka, Japan
Jan-13	**Fagarasan, S.**	**Regulation of IgA synthesis and bacteria in the gut by PD-1**	International Symposium on Immunological Self	Kyoto, Japan
Jan-13	**Okada, M.**	**A switch in NF-kappaB immune signaling**	First Annual Winter q-bio Meeting	Honolulu, USA
Feb-13	**Kawamoto, H.**	**A revised model of hematopoiesis**	Five hub-Institute Meeting	Kobe, Japan
Feb-13	**Kawamoto, H.**	**Thymic epithelial cells and thymic cyst**	Special Lecture at the Annual meeting of Japanese Association for Research on the Thymus	Sapporo, Japan
Feb-13	**Fagarasan, S.**	**New roles of PD-1 in immune homeostasis**	New Horizons in the Immune System	Tokushima, Japan
Feb-13	**Fagarasan, S.**	**IgA synthesis: A form of functional immune adaptation extending beyond gut**	Keystone symposia. X1 2013	Colorado, USA.
Feb-13	**Saito, T.**	**Dynamic imaging analysis of T cell activation regulation**	2nd New Zealand-RIKEN-Chiba Joint Workshop	Auckland, New Zealand
Feb-13	**Fujii, S.**	**iNKT cell-triggered in vivo DC targeting immunotherapy**	2nd New Zealand-RIKEN-Chiba Joint Workshop	Auckland, New Zealand
Feb-13	**Fukada, T.**	**Zinc biology as medical science: why zinc is essential for us?**	The 4 th Kinki Zinc-Nutritional Research Meeting	Osaka, Japan
Feb-13	**Cheroutre, H.**	**Selective Differentiation of Mucosal Effector Memory T cells: Saving the Best for (to) Last**	Department of Immunology, Mayo Clinic College of Medicine.	Rochester, USA
Feb-13	**Cheroutre, H.**	**Autoimmunity: New Players, Old Faces**	Benaroya Research Institute at Virginia Mason, Wilske Lecture Series, Science & Medicine	Seattle, USA
Mar-13	**Honda, K.**	**Microbiota's Influence on Immunity**	3rd Schloss Elmau Meeting on Resistance and Disease promoting Principles of Innate Immunity	Elmau, Germany
Mar-13	**Hori, S.**	**Stability and adaptability of Foxp3+ regulatory T cells**	The 7th International Symposium on the Clinical Use of Cellular Products	Erlangen, Germany
Mar-13	**Ohno, H.**	**Multi-omics approach reveals that Bifidobacteria protect from enterohaemorrhagic Escherichia coli infection through production of acetate: Importance of sugar transporter**	3rd TNO Beneficial Microbes Conference: international conference on the health impact and future potential of beneficial microbes	Noordwijkerhout, The Netherlands
Mar-13	**Ohno, H.**	**Gut microbiota and healtch care focusing on the immunological analysis with lactic acid bacteria and bifidobacteria**	Metropolitan Bio Network Japan Seminar	Tokyo, Japan
Mar-13	**Fukuda, S.**	**Integrated omics approach identified acetate produced by probiotic bifidobacteria to protect host from enteropathogenic infection**	The 3th Stage Surface Barrier Immunology Study Group	Osaka, Japan
Mar-13	**Honda, K., Atarashi, K., Tanoue, T., Morita, H., Hattori, M.**	**Induction of intestinal regulatory T cells**	World Immune Regulation Meeting-VIII	Davos, Switzerland
Mar-13	**Mohan, S.**	**A peek overview of emerging informatic tools for mutation analyses in general and PID perspectives**	1st National conference on Primary Immunodeficiency Diseases, The Postgraduate Institute of Medical Education and Research (PGIMER)	Chandigarh, India
Mar-13	**Mohan, S.**	**The current status and emerging trends in Asian primary immunodeficiency network**	1st National conference on Primary Immunodeficiency Diseases, The Postgraduate Institute of Medical Education and Research (PGIMER)	Chandigarh, India
Mar-13	**Honda, K.**	**Intestinal Commensal Bacteria-Mediated Treg Induction**	Keystone Symposia, The Microbiome	Keystone, USA
Mar-13	**Fukada, T.**	**Zinc signal: the action of Zn ion controlling cellular and physiological events**	The 86th Annual Meeting of the Japanese Pharmacological Society	Fukuoka, Japan
Mar-13	**Tanaka, T.**	**Negative regulation of T-helper cell differentiation by LIM proteins.**	The 132th Annual Meeting of the Pharmatheutical Sciety of Japan, Special Symposium OS06, Frontiers in Researches of Immune Regulators	Sapporo, Japan

Date	Speaker	Title	Affiliation	Country	
Apr 16th	**Dr. Seishi Ogawa**	**Novel pathway mutations in myeloid dysplasia**	Project associated professor, Cancer Genomics,Project, University of Tokyo	Japan	
Apr 25th	**Dr. Stephen Schoenberger**	**Cellular and Molecular Determinants of CD8$^+$ T Cell Memory**	La Jolla Institute for Allergy & Immunology	USA	
May 17th	**Dr. Toshiaki Kawakami**	**Atopic Dermatitis due to the impairment of phospholipase C-β3**	La Jolla Institute for Allergy & Immunology	USA	
May 21st	**Dr. Yoichi Gondo**	**The new utilization system of the RIKEN ENU mutant mouse library (in Japanese)**	RIKEN BioResource Center	Japan	The fourth RCAI-CGM Joint Seminar
May 24th	**Dr. Hodaka Fujii**	**Locus-specific biochemical epigenetics by insertional chromatin immunoprecipitation (iChIP)**	Research Institute for Microbial Diseases, Osaka University	Japan	
May 28th	**Dr. Yoshio Kodera**	**Comprehensive approach to develop diagnostic biomarker peptides in serum. -Establishment of discovery and validation methods targeting low-abundance peptides-**	Director, Center for Disease Proteomics Associate Professor, Department of Physics Kitasato University School of Science	Japan	RIKEN RCAI Seminar Series 2012
May 29th	**Dr. Motonari Kondo**	**Roles of bone marrow microenvironment in lymphoid and myeloid lineage choice by lymphoid-specified multipotent progenitors**	Associate Professor, Department of Molecular Immunology Toho University School of Medicine	Japan	
Jun 5th	**Dr. Akihiro Fujimoto**	**Whole genome sequence analysis of a human genome and cancer genome**	Researcher, Riken Center for Genomic Medicine	Japan	RIKEN RCAI Seminar Series 2012
Jun 11th	**Dr. Hans Clevers**	**Lgr5 stem cells in self-renewal and cancer**	Professor and Director, the Hubrecht Institute in Utrecht,University Medical Centre Utrech	Netherlands	
Jun 11th	**Dr. Yin Chun Lin**	**Global analysis of long-range genomic interactions in developing B cells**	Assistant Project Scientist, Department of Biological Sciences, University of California, San Diego	USA	
Jun 12th	**Dr. Ryo Yoshida**	**Bayesian statistical modeling and computational technologies in systems biology and chemoinformatics**	Associate Professor, The Institute of Statistical Mathematics	Japan	RIKEN RCAI Seminar Series 2012
Jun 14th	**Dr. Makoto Nakanishi**	**Regulation of maintenance DNA methylation through novel histone modification**	Professor, Nagoya City University	Japan	
Jun 20th	**Dr. Martin Frith**	**Reliable large-scale sequence comparison**	Senior Researcher,Computational Biology Research Center, National Institute for Advanced Industrial Science and Technology (AIST)	Japan	RIKEN RCAI Seminar Series 2012
June 25th	**Dr. Hisataka Kobayashi**	**Molecular cancer imaging; New optical diagnostic technologies and beyond**	National Cancer Institute, National Institutes of Health	USA	RIKEN RCAI Seminar Series 2012
Jul 2nd	**Dr. Atsushi Iwama**	**Role of the polycomb group proteins in the maintenance of hematopoietic stem cells and restriction of tumor development**	Professor, Graduate School of Medicine, Chiba University	Japan	
Jul 10th	**Dr. Todd Taylor**	**Metagenomics: an introduction and applications to immunology**	Team Leader, RIKEN Quantitative Biology Center	Japan	RIKEN RCAI Seminar Series 2012
Jul 11th	**Dr. Shunsuke Chikuma**	**Regulation of T cell function by epigenetic TRIM28**	Assistant Professor, Graduate School of Medicine, Kyoto University	Japan	
Jul 12th	**Dr. Masakatsu Yamashita**	**Menin-Bach2 axis controls senescence-associated secretory phenotype (SASP) in CD4 T cells.**	Head of the Laboratory, Lab. of Med. Genomics, Kazusa DNA Research Institute	Japan	
Jul 17th	**Dr. Jun Sese**	**Revisiting Multiple Testing – Statistics for Genome-Wide Experiments and Discovery of Combinatorial Regulations of Transcription Factors**	Associate Professor,Dept. of Computer Science, Tokyo Institute of Technology	Japan	RIKEN RCAI Seminar Series 2012
Jul 24th	**Dr. Hideya Kawaji**	**Computational approaches in data-driven transcriptome**	Unit Leader, RIKEN Omics Science Center	Japan	RIKEN RCAI Seminar Series 2012
Jul 26th	**Dr. Takehiko Kamijo**	**Role of Polycomb in Tumorigenesis**	Head of Division of Biochemistry and Molecular Carcinogenesis, Chiba Cancer Center Research Institute	Japan	
Jul 31st	**Dr. Keisuke Nagao**	**Langerhans cells and the mammalian hair in skin immunology**	Assistant Professor, Keio University School of Medicine	Japan	RIKEN RCAI Seminar Series 2012
Aug 9th	**Dr. Mari Sato**	**Born/Bone this way (in Japanese)**	Division of Hematology, Department of Internal Medicine, Kobe University Graduate School of Medicine	Japan	
Aug 10th	**Dr Takuji Yamada**	**Enterotypes of the human gut microbiome**	Lecturer, Tokyo Institute of Technology	Japan	
Sep 18th	**Dr. Satoru Miyano**	**Uncovering Molecular Networks in Cancer by Supercomputer**	Professor, Human Genome Center, The Institute for Medical Science, The University of Tokyo	Japan	RIKEN RCAI Seminar Series 2012
Sep 25th	**Dr. Hiroyuki Kurata**	**Integration of omics data into metabolic and gene regulatory networks**	Professor, Kyushu Institute of Technology	Japan	RIKEN RCAI Seminar Series 2012
Sep 25th	**Dr. Min-Tze Liong**	**Probiotics &Hypercholesterolemia: Trials, Challenges & Personalized Nutrition**	Associate Professor,Bioprocess Technology Division School of Industrial Technology, Universiti Sains Malaysia	Malaysia	

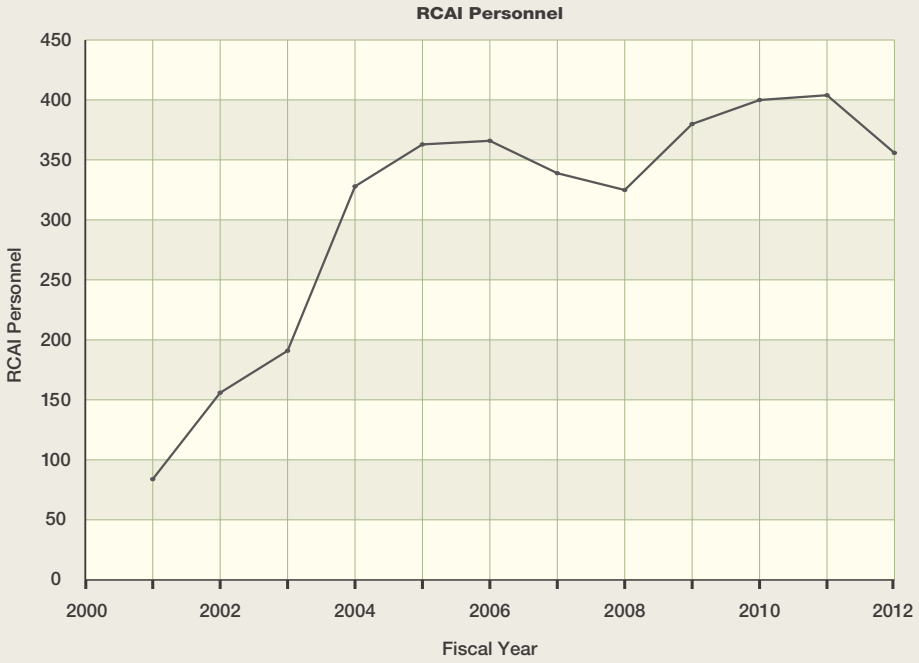
Sep 27th	**Dr. Hitomi Mimuro**	**Crosstalk between Helicobacter pylori and Host Cells**	Associate Professor, Department of Infectious Diseases Control,International Research Center for Infectious Diseases,The Institute of Medical Science, The University of Tokyo	Japan	
Oct 9th	**Dr. Hiroyuki Aburatani**	**Epigenome dynamics**	Professor, Research Center for Advanced Science and Technology , The University of Tokyo	Japan	RIKEN RCAI Seminar Series 2012
Oct 18th	**Dr. Matteo Barberis**	**Timing in systems biology: the cell cycle**	Senior Post-doctoral Research Associate, Theoretical Biophysics, Institute for Biology, Humboldt University Berlin Post-doctoral Research Associate, Max Planck Institute for Molecular Genetics	Germany	
Oct 16th	**Dr. Tatsuo Shibata**	**Basal dynamics of signal transduction system in chemotaxis cells**	Unit Leader, RIKEN Center for Developmental Biology	Japan	RIKEN RCAI Seminar Series 2012
Oct 23rd	**Dr. David Schlessinger**	**Genetic variants associated with age-related traits and diseases in the Sardinian population**	Chief, Laboratory of Genetics, National Institute on Aging, National Institutes of Health	USA	RIKEN RCAI Seminar Series 2012
Oct 23rd	**Dr. Françesco Cucca**	**Finding genetic factors in the Sardinian population for both levels of immune cells and molecules, and correlated autoimmune disease pathogenesis**	Director, Institute of Genetics and Biomedical Research, National Research Council	USA	RIKEN RCAI Seminar Series 2012
Oct 30th	**Dr. Yuichi Wakamoto**	**Dynamic persistence of bacteria against antibiotic drugs**	Research Center for Complex Systems Biology, The University of Tokyo	Japan	RIKEN RCAI Seminar Series 2012
Nov 2nd	**Dr. Cornelis Murre**	**Global analysis of long-range genomic interactions and the establishment of lymphocyte cell fate**	Professor, Division of Biological Sciences, University of California, San Diego	USA	
Nov 5th	**Dr. Shuji Ishihara**	**Phase map analysis of complex cell morpholigy**	Assistant Professor, Graduate School of Arts and Sciences, The University of Tokyo	Japan	RIKEN RCAI Seminar Series 2012
Nov 8th	**Dr. Kristian Helin**	**Roles of TET and Polycomb Group Proteins in Stem Cells and Cancer**	Director and Professor, Biotech Research & Innovation Centre, University of Copenhagen	Denmark	
Nov 9th	**Dr. François Karch**	**Long-distance regulatory interactions in the Drosophila bithorax complex: from boundaries, PREs and ncRNAs to the female post-mating response**	Associate Professor, Department of Genetics and Evolution, University of Geneva	Switzerland	
Nov 13th	**Dr. Ichiro Hiratani**	**Developmental regulation of nuclear genome organization and DNA replication timing**	Assistant Professor, National Institute of Genetics	Japan	
Nov 20th	**Dr. Toshiro Sato**	**Intestinal stem cells in homeostatic and regenerative state**	Project Assistant Professor, Department of Gastroenterology, Keio University School of Medicine	Japan	RIKEN RCAI Seminar Series 2012
Nov 22nd	**Dr. Frederic Geissmann**	**Development and functions of myeloid cells**	Professor, Center for Molecular and Cellular Biology of Inflammation King's College London	UK	
Nov 27th	**Dr. Neil Brockdorff**	**Targeting epigenetic systems in a changing landscape**	Group Leader, Department of Biochemistry, University of Oxford	UK	
Dec 4th	**Dr. Yui-Hsi Wang**	**Novel IL-9-producing innate helper cells promote oral antigen-induced anaphylaxis.**	Division of Allergy and Immunology, Cincinnati Children's Hospital Medical Center	USA	
Dec - 10th	**Dr. Harry W. Schroeder, Jr.**	**Immune Deficiency, Autoimmunity and Control of the Antibody Repertoire**	University of Alabama at Birmingham Division of Clinical Immunology and Rheumatology	USA	
Dec - 11th	**Dr. Paul Wilmes**	**Eco-Systems Biology of the human microbiome highlights the potential importance of small RNAs in human-microbe interactions.**	ATTRACT Research Fellow, Luxembourg Centre for Systems Biomedicine, University of Luxembourg	Germany	
Dec - 18th	**Dr. Masahiro Morita**	**RNA biology through the 5' cap structure and 3' poly(A) tail**	Postdoctoral Fellow, Dr. Nahum Sonenberg's lab, Department of Biochemistry and Goodman Cancer Research Centre, McGill University	Canada	
Dec - 18th	**Dr. Daron M. Standley**	**A structural view of immune function**	Immunology Frontier Research Center (IFReC), Osaka University	Japan	RIKEN RCAI Seminar Series 2012
Jan - 23rd	**Dr. Shinichi Nishikawa**	**Development of Hematopoietic Stem Cell: A final scenario**	Group Director, Center for Developmental Biology, RIKEN	Japan	
Jan - 28th	**Dr. Becca Asquith**	**Efficiency of the CD8+ T cell response to persistent viral infection**	Senior Lecturer in Within-Host Dynamics, Imperial College London	UK	
Feb - 15th	**Dr. Wilfred T.V. Germeraad**	**Development of new Immunotherapies for cancer**	Associate Professor, Department of Internal Medicine, Division of Haematology, GROW School for Oncology and Developmental Biology Maastricht University	Netherlands	
Mar - 7th	**Dr. Denis Duboule**	**Long range regulation of HoxD genes during mammalian development**	Professor, Department of Genetics and Evolution, University of Geneva	Italy	
Mar - 7th	**Dr. Yusuke Miyanari**	**Reprogramming and nuclear dynamics**	Postdoctral fellow, Institute of Genetics and Molecular and Cellular Biology (IGBMC)	France	
Mar - 13th	**Dr. Eugene Oltz**	**Defining the Malignant Epigenome in Follicular Lymphoma**	Professor of Pathology & Immunology, Washington University School of Medicine	USA	



RCAI Budget FY2001-2012 (JPY 100 Million)

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

RCAI Budget (JPY 100 Million)	
2001	41.74
2002	54.23
2003	60.48
2004	40.10
2005	39.02
2006	35.90
2007	34.56
2008	32.61
2009	31.86
2010	30.83
2011	31.06
2012	31.04



RCAI Personnel	
2001	84
2002	156
2003	191
2004	328
2005	363
2006	366
2007	339
2008	326
2009	380
2010	400
2011	404
2012	354

RCAI staff composition (as of Mar. 2013)

Category	Number
Director	1
Senior Advisor	2
Group Director	6
Team Leader	14
Unit Leader	12
Coordinator	2
Senior Scientist	8
Research Scientist	50
Special Postdoctoral Researcher	5
Foreign Postdoctoral Researcher	1
Research Associate	7
Junior Research Associate	16
International Program Associate	7
Student Trainee	41
Research Fellow	2
Research Consultant	2
Visiting Scientist	49
Senior Technical Scientist	1
Technical Scientist	5
Technical Staff I	31
Technical Staff II	66
Assistant	22
Temporary Employment	4
Total	354

RCAI Patents (as of Mar. 2013)

There were 16 patents filed from April 2012-March 2013.

RIKEN Research Center for Allergy and Immunology
http://www.rcai.riken.jp/english/index.html

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RIKEN 2013-047