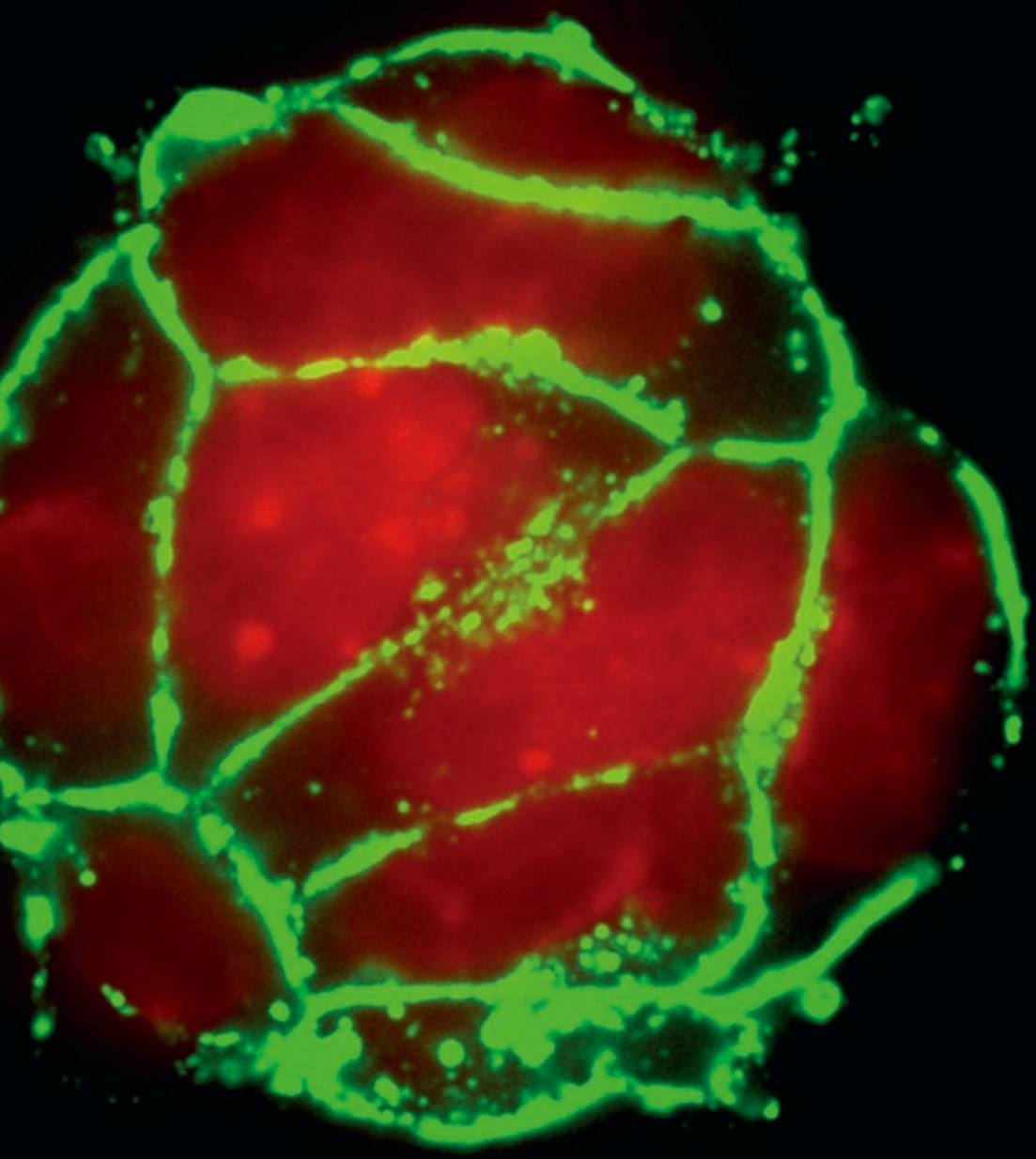
RIKEN RCAI Annual Report 2010

RIKEN Research Center for Allergy and Immunology



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Director's Report

Earthquake

FY2010 was a turbulent period. The disastrous magnitude 9.0 Tohoku-Pacific Ocean Earthquake hit the North Eastern region of Japan on March 11, 2011. It was the most powerful earthquake we have ever experienced in Japan and the fifth largest in the world. It shifted the earth's axis by 10 cm and moved the mainland of Japan ~2.5 m. The zone plate boundary between the Pacific and North America plates was lifted up along a 500 km long stretch, which caused a massive Tsunami more than 15 m high. Currently, there are 12,157 confirmed deaths and 15,496 have been reported missing, and the numbers are still growing. I feel great sympathy for those who lost their lives, their surviving family members and friends, including RCAI members who lost their families.

On Friday, Mar. 11, we felt two strong quakes at 2:46 p.m. and 3:15 p.m. at our Center. Soon after the first quake, the electric power went out. The trains were stopped and no traffic signals were working, which caused major traffic jams. Most RCAI staff had to stay overnight in the Center. The Group Directors discussed how to maintain emergency power as long as possible, especially for the SPF animal facility. The blackout continued for 10 hours, which was well beyond our expectation. It was very fortunate that all RCAI members were safe, and there was no major damage to research samples or mice.

My thoughts go out to the people now suffering. There are around 1.1 million people in the shelters. Many of them cannot return home because of the severe damage resulting from the earthquake, tsunami and/or the Fukushima nuclear accident. Hoping to be of assistance in rebuilding the educational and research environment in the region, RCAI, in collaboration with the Japanese Society for Immunology (JSI) has been requesting information about the damage and needs of the affected laboratories. RCAI together with immunology researchers in the Max Planck Institute Germany, La Jolla Institute for Allergy and Immunology and the NIH USA, and INSERM/Pasteur Institute France also offer support to immunology researchers there. Based on the responses, we are instituting targeted strategies to meet these needs.

Policies for innovation

According to the Science and Technology Policy for FY2011-2015 drafted by the Council for Science and Technology Policy, the government will shift their policies from "Science and Technology" to "Science, Technology and Innovation". Social contributions resulting from scientific research are now more expected in the society. According to Dr. Noyori,

RIKEN's president, RIKEN will also shift its focus to innovative science or solution research. RCAI has been making important social contributions since its establishment, e.g., development of an allergy vaccine, novel immune cell therapy for cancer, humanized mouse models and artificial lymph nodes, etc. However, I am very concerned that too rapid a shift to solution research will reduce the researchers' motivation and the attractiveness of the institute, which will result in a weaker scientific base and lower performance, not only in basic science but also in social contributions.

The Science and Technology Policy FY2011-2015 was scheduled to be approved by the cabinet by the end of March, 2011. However, the cabinet decided on a reconsideration of the policy because of the earthquake and the nuclear accident.

Budget

During FY2010, we faced a government plan for severe and steady cuts in the research budget. In June 2010, the Japanese cabinet decided to fix "general expenditures" of the government at 71 trillion yen/year for FY2010-FY2013. The general expenditure category includes social security, education, science, public works, national defense, etc. Because of the projected steady increase in social security spending of 1.3 trillion yen per year during this period, this policy required reductions in the budgets for other areas including science. Based on this cabinet decision, the Ministry for Education, Science and Technology (MEXT) decided to reduce RIKEN's budget by 3.5% per year, and RIKEN decided to reduce each Center's budget 9% per year. The only rescue plan suggested by the government was a so-called "Policy Contest" to revitalize Japan. The selected policies would be supported by an extra special budget (total 1 trillion yen). RCAI was fortunate that the allergy vaccine project was selected and funded with 300 million yen as a part of a health innovation policy. However, when we think of the current government situation, it is inevitable that our research budget will be cut in the very near future. We have to seek different funding mechanisms.

Research achievements

In FY2010, the creativity of RCAI scientists continued to yield important research achievements. I would like to mention especially the advances in gut immunity. Two achievements in this area were awarded the Outstanding Contribution of the Year.

It is widely believed that probiotics, i.e. “good” intestinal bacteria such as bifidobacteria and lactobacilli, benefit health, although scientific evidence in support of this notion is largely non-existent. Drs. Shinji Fukuda and Hiroshi Ohno took advantage of a simplified gnotobiotic mouse model, in which one or two well-studied bacterial species are used to colonize germfree mice, to tackle this issue. Combining metabolomic, transcriptomic and genomic analyses, they have identified for the first time the molecular mechanisms by which bifidobacteria protect mice from death caused by infection with enterohemorrhagic *E. coli* O157. Although this must be just the tip of iceberg for a wide range of probiotic functions, the multiple ‘omics’ approach they established here can be applied to understand other mechanisms involved in these functions, studies that will contribute to the improvement of public health. (Fukuda, et al., *Nature*, 469:543, 2011)

IgA is the major antibody of the humoral immune response in the gut. The mechanisms of gut IgA synthesis and the role of secreted IgA on mucosal surfaces are critical issues, whose understanding will aid in the design of oral vaccines and therapy of inflammatory bowel diseases. In this field, Drs. Keiichiro Suzuki and Sidonia Fagarasan have shown that intestinal IgA plays a critical role in the maintenance of a “healthy” gut microbiota. They have further contributed to our understanding of the induction and regulation of gut-IgA responses, and recently showed that $Foxp3^+$ T cells and follicular dendritic cells in Peyer’s patches have unique characteristics that promote IgA generation in the gut. Together these findings provide new insights into the mechanisms of intestinal IgA synthesis, and into the dynamic interactions between host immune cells and gut-environmental factors. (Suzuki K, et al., *Immunity*, 33:71, 2010)

The following publications received this year’s Excellent Paper Award:

Drs. Yuichi Aiba and Tomohiro Kurosaki showed that, contrary to textbook models, long-lived memory B cells are localized adjacent to contracted germinal centers in the spleen (*PNAS*, 107:12192, 2010). Drs. Kenichi Asano and Masato Tanaka

identified the APC responsible for crosspresentation of tumor antigens to $CD8^+$ T cells; $CD169^+$ macrophages phagocytose dead tumor cells transported via lymphatic flow (*Immunity*, 34:85, 2011). Mr. Tomohiro Fukaya, Mr. Hideaki Takagi and Dr. Katsuaki Sato showed a crucial role for the B7 family of costimulatory molecules in generating regulatory T cells to establish oral tolerance (*Blood*, 116:2266, 2010). Drs. Tomokatsu Ikawa and Hiroshi Kawamoto identified the earliest known checkpoint during T cell development in the thymus and showed that is dependent on the Bcl11b transcription factor (*Science*, 328:93, 2010). Drs. Noriko Yumoto, Takashi Nakakuki, Mariko Okada-Hatakeyama and collaborators were able to show how a spatially distributed, signaling-transcription cascade robustly discriminates between transient and sustained ERK activities at the c-Fos system level (*Cell*, 141:1, 2010). Drs. Shinya Tanaka, Yasutaka Motomura and Masato Kubo identified a critical enhancer element for regulating GATA-3-mediated *IL4* transcription in Th2 cells (*Nature Immunology*, 12:77, 2011). Drs. Hiroshi Watarai, Daisuke Yamada, Shin-ichiro Fujii, Haruhiko Koseki and Masaru Taniguchi demonstrated the feasibility of expanding functionally competent NKT cells via an induced pluripotent stem cell (iPSC) phase, an approach that may be adapted for NKT cell-targeted therapy in humans (*Journal of Clinical Investigation*, 120:2610, 2010). Drs. Tomoharu Yasuda, Kohei Kometani and Tomohiro Kurosaki showed that Erk kinases control plasma cell differentiation by regulating expression of a key transcriptional repressor Blimp-1 (*Science Signaling*, 4(169):ra25, 2011). Drs. Tadashi Yokosuka and Takashi Saito used high resolution imaging to demonstrate the spatiotemporal basis of the negative regulation of T cell activation by the CTLA-4 costimulatory molecule (*Immunity*, 33:326, 2010).

Nurturing research scientists

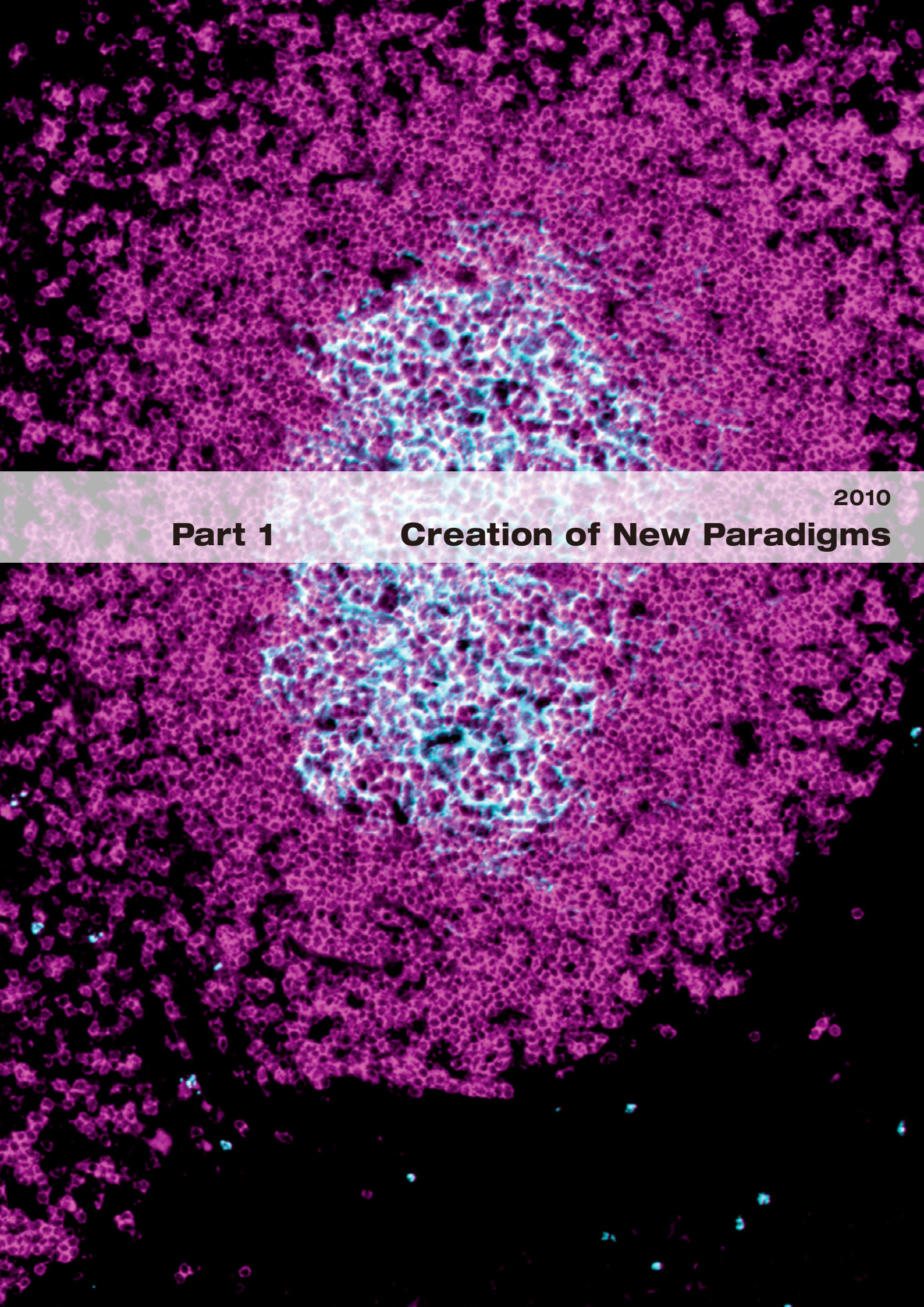
I believe that it is RIKEN's role and mission to create new research paradigms. For this purpose, I think it very important to nurture young investigators, who have the fresh power and mental flexibility to challenge new concepts. Unfortunately, in Japan the situation seems to be evolving in the wrong direction. The career path is becoming narrow. There are fewer tenured positions, and the safest shortcut to publication is to pursue the research already ongoing in the laboratory, rather than breaking new ground. In view of this problem, we established a new program 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 Investigators (age below 40) will head independent research laboratories but will be mentored by multiple specialists in related research fields, i.e. the leaders in various RIKEN Institutes. At the end of FY2010, we selected the first three investigators: Dr. Hayato Naka-Kaneda - aging of hematopoietic stem cells, Dr. Shinji Nakaoka - mathematical modeling of immune responses, and Dr. Koji Hase - epigenetic regulation of the host-environment interface. I hope that these individuals will grow under the mentorship from multiple fields and will become the pioneers in new research fields.

In the end of FY2010, we also appointed Dr. Hilde Cheroutre (LIAI) as the leader of RCAI's International Research Unit, "Research Unit for Immune Crosstalk". She will conduct collaborative research on $CD8\alpha\alpha$ with Dr. Ichiro Taniuchi in RCAI. I expect her research will impact not only the biology of $CD8\alpha\alpha$ but also the field of central tolerance and thymic selection and T cell development, and promote our understanding of protective immunity and the design of effective vaccination strategies.

The future

We are at the juncture for a real revitalization of Japan. Big changes must occur after this tragedy. New ideas and structures will be necessary in the social system, energy policies, economics, politics, technology and science. It will be a long way to go, but I believe that we Japanese, especially the young generations, have the strength of mind and the toughness of spirit that will be needed to overcome any difficulties that we may encounter.

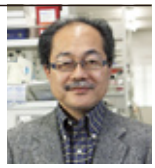
Mar. 31, 2011
Masaru Taniguchi



2010

Part 1 Creation of New Paradigms

Bacterial Guests Double as Bodyguards

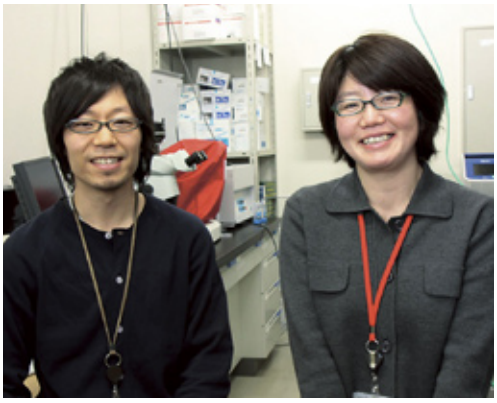


Hiroshi Ohno

‘Good citizens’ in the human gut bacterial community produce protective compounds that help prevent the onset of food poisoning

Infection with the O157:H7 strain of *E. coli* can cause various symptoms that range from diarrhea to blood cell loss, encephelopathy and kidney failure. The causative factor is Shiga toxin (Stx), a bacterially secreted compound that makes its way from the intestine into the bloodstream, and ultimately binds to target receptors on cells in the kidney and brain.

According to Hiroshi Ohno of RIKEN RCAI, the acquisition of protection against O157:H7 appears to be also related to diet. In addition to nutrition, mother’s milk from breast-feeding helps to establish the community of gut bacteria. These include the bifidobacteria, which have subsequently been associated with conferring O157:H7 resistance. A recent study from a team led by Ohno and Masahira Hattori at The University of Tokyo has revealed the defining characteristics of these defenders of human health.



Shinji Fukuda *(left)* **and Yumi Chiba** *(right)*

To characterize the protective properties of several different strains of bifidobacteria, the researchers used mice that lacked gut bacteria of their own. Completely ‘germ-free’ mice typically died within a week of exposure to O157:H7, but all animals pre-colonized with a protective strain of *Bifidobacterium longum* (Fig.), termed ‘BL’ survived. Mice carrying BL also showed considerably lower levels of Stx in their bloodstream, and the lining of their intestines appeared healthier.

Ohno, Hattori and their colleagues examined additional preventive and non-preventive bifidobacteria strains, and the researchers learned that the preventive strains tended to generate higher levels of acetate as a metabolic byproduct. Acetate, in turn, switches on the activity of a group of anti-inflammatory genes. Experiments with human colon epithelial cells showed that acetate treatment was protective against the negative effects of Stx, which otherwise induced the formation of ruptures in layers of cultured cells. This suggests that acetate-producing bacteria prevent poisoning by actively preserving the integrity of the intestinal wall and keeping Stx out of the bloodstream.

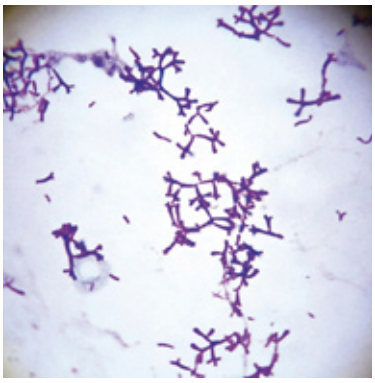


Figure :
Certain strains of *Bifidobacterium longum* and other bifidobacterial species residing within the gut can help fight off the ill effects of *E. coli* food poisoning.

A comparison of the complete genomic sequences of five different bifidobacterial strains revealed a subset of genes encoding proteins involved in uptake and metabolism of the sugar fructose that appear to be exclusively present in preventive strains. Accordingly, disabling these genes drastically undermined the protective qualities of such strains. They also determined that it was possible to cut out the bacterial ‘middleman’, and demonstrated that considerable protection against Stx lethality could be achieved by simply feeding mice a diet that was enriched in acetylated starch.

The team points out that all bifidobacteria strains produce acetate during the process of glucose metabolism, protecting the upper stretches of the colon. However, the selectively expressed fructose-metabolizing pathway is likely to provide important protection in the lower region of the colon, a portion of the digestive tract where glucose is likely to be largely depleted.

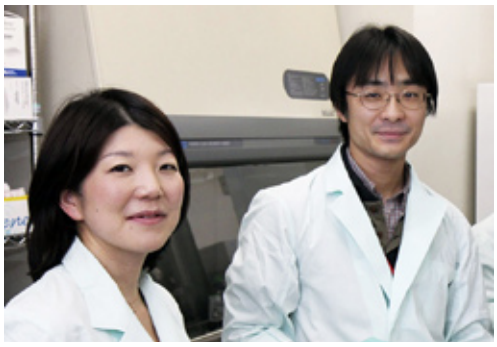
Ohno and his colleagues see their bacterial detective work as a powerful proof of concept for understanding the health implications of the close relationship between microbes and their hosts, and for advancing the development of microorganism-mediated ‘probiotic’ therapeutic strategies in the future. “The beneficial effects of probiotics are diverse, and acetate alone could not explain everything; we would therefore like to elucidate the mechanisms underlying other probiotic effects,” he explains, “and we would also like to apply the ‘multi-omics’ approach we took here for analyzing more complex gut ecosystems.”

ORIGINAL RESEARCH PAPER

Fukuda, S., Toh, H., Hase, K., Oshima, K., Nakanishi, Y., Yoshimura, K., Tobe, T., Clarke, J.M., Topping, D.L., Suzuki, T. *et al*. Bifidobacteria can protect from enteropathogenic infection through production of acetate. *Nature* **469**, 543-547 (2011).

Sensing of Environmental Stimuli Promotes IgA Generation in the Gut

Sidonia Fagarasan



Mikako Maruya *(left)* **and Keiichiro Suzuki** *(right)*

Immune responses occur in organized lymphoid structures such as Peyer's patches (PPs) in the gut and lymph nodes in the peripheral tissues (pLNs). Upon antigen stimulation, both PPs and LNs undergo extensive remodeling. T cell accumulation and extensive B cell proliferations result in the development of germinal centers (GC). However, several characteristics distinguish GCs in gut PPs from GCs in pLNs. First, GCs are always present in PPs, as they are induced through the constant stimulation by commensal bacteria. By contrast, GCs in pLNs develop only upon systemic infections or after deliberate immunization. PP GCs also differ from pLN GCs in regard to the type of antibodies produced. B cells in PP GCs produce IgA, whereas activated B cells in pLN GCs mainly generate IgG antibodies. IgA secreted into the gut lumen joins innate immune defenses to provide mucosal protection by regulating bacterial communities in the gut.

The molecular and cellular mechanisms underlying the continuous presence of GCs in PPs and the almost exclusive generation of IgA were incompletely understood. Keiichiro Suzuki and Sidonia Fagarasan, RIKEN RCAI, speculated that follicular dendritic cells (FDCs) are critical in B cell activation and class switching from IgM to IgA. To evaluate the hypothesis, they isolated PP and pLN FDCs from immunized mice and investigated their gene expression profiles. They found that the PP FDCs had features that were distinct from pLN FDCs. Genes highly expressed in PP FDCs encoded proteins important for lymphocyte recruitment and retention (CXCL13), B cell survival (BAFF), and activation of TGF-β, a cytokine important for IgA class switching.

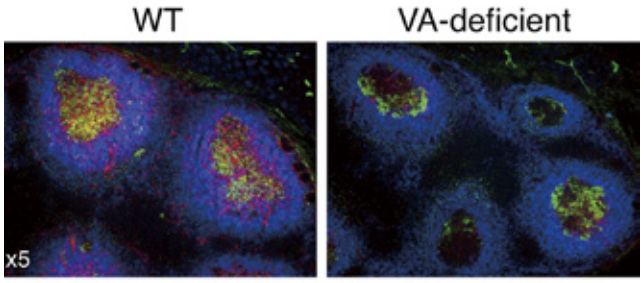


Figure:

Reduced expression of CXCL13 by PP FDCs in the absence of vitamin A.

Immunofluorescent microscopy analysis of PPs horizontal sections from the WT mice and VAD mice, stained for FDCs in green (CD21), CXCL13 in *red* and naïve B cells (IgD) in *blue*.

Suzuki and Fagarasan reasoned that these differences are due to FDCs recognition of specific gut environmental stimuli that modifies their functions in PP GCs, thus contributing to IgA production. In PP, bacterial antigens continuously activate innate immunity through Toll-like receptors (TLRs) and food components such as retinoic acid (RA) constantly activate RA receptors (RARs). To test this hypothesis, they stimulated pLN FDCs with TLR ligands and RA *in vitro*. Surprisingly this synergistic stimulation enhanced the transcription of PP signature genes such as *Cxcl13, Tnfsf13b*, which encodes BAFF, and TGF-β1 activators in pLN FDCs. Next, the effect of these environmental factors *in vivo* was also confirmed. In both Myd88$^{-/-}$ mice, which are defective in TLR downstream signaling, and in mice kept on a diet deficient in vitamin A, the RA precursor, the expression of CXCL13 and BAFF by PP FDC was severely reduced. Thus, FDCs are critical for production of CXCL13 and BAFF upon gut environmental stimulation. They further showed that TLRs and RARs regulate the conversion of latent TGF-β1 to its active form, which enhanced IgA class switching of B cells.

"The pathway supported by FDC is quite efficient. FDCs sense the bacterial components and food components in the gut lumen, and secrete CXCL13 to recruit B cells and T cells. They also secrete TGF-β1 that help B cell switch from IgM to IgA, and BAFF that enhances the survival of these switched cells and their differentiation into plasma cells," Suzuki said. "However, this is not the only way to generate IgA in the gut. Actually, there is another stromal cell type, and there are many other interesting regulatory factors, studied by the members of Sidonia's lab. This field is becoming more and more interesting."

ORIGINAL RESEARCH PAPER

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

Crossing the Line

Understanding of blood cell lineages advances with the discovery of a transcription factor crucial to T cell differentiation

Hiroshi Kawamoto

A master gene that underpins the development of specific blood cell lineages has been identified by a research team led by Hiroshi Kawamoto at RIKEN RCAI. The team has published its findings in the journal *Science*.

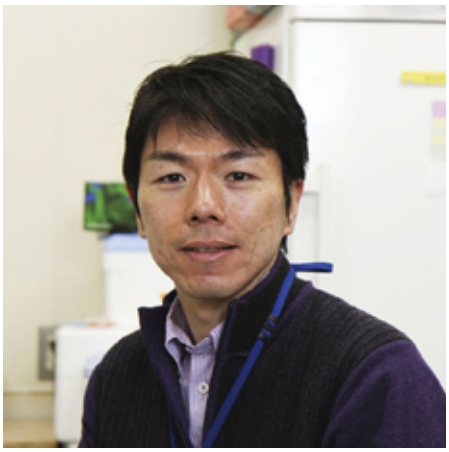
Precursor cells in the immune system, known as hematopoietic progenitor cells, can give rise to multiple immune cell types. Kawamoto and his team cultured multipotent progenitor cells from mice that could become T cells that shape the immune response, B cells that generate antibodies, or myeloid cells that can engulf pathogens. Their special culture system could stimulate the Notch signaling pathway, which is required for progenitor cell renewal, and included immune system regulators such as interleukin-7 (IL-7).

The researchers found they could induce the immune progenitor cells to lose their ability to become B cells under these conditions. However, this halted development of the cells past this stage, as the progenitors were unable to cease proliferating and mature into either T cells or myeloid cells.

Kawamoto and colleagues then observed that removing IL-7 from the cell culture medium was sufficient to drive the progenitors to mature into T cells. They found that withdrawing IL-7 induced the expression of the transcription factor Bcl11b, which is known to be expressed in T cells. Interestingly, even when IL-7 was present in the cell culture medium, they could push immune progenitor cells into becoming T cells by forcing Bcl11b to be expressed in the cells. This suggested to the researchers that this transcription factor drives this step in the commitment of these immune progenitor cells to the T cell lineage.

The team also showed that progenitor cells lacking the *Bcl11b* gene were unable to mature into T cells, and could continue to proliferate (Fig.). This is consistent with previous findings by other research teams that disruption of the function *of Bcl11b* is linked to leukemia and lymphoma, which may be caused by the inability of the progenitor cells to mature properly into T cells, and to instead continue to proliferate. Kawamoto and his colleagues think that Bcl11b may drive progenitor cells to take on the T cell fate by suppressing the genes that characterize the myeloid cell lineage.

"Our findings may facilitate the study of the molecular mechanisms of T cell lineage commitment by elucidating the exact timing for this commitment," explains Kawamoto, "and by identifying a master gene for the establishment of the T cell lineage."



Tomokatsu Ikawa

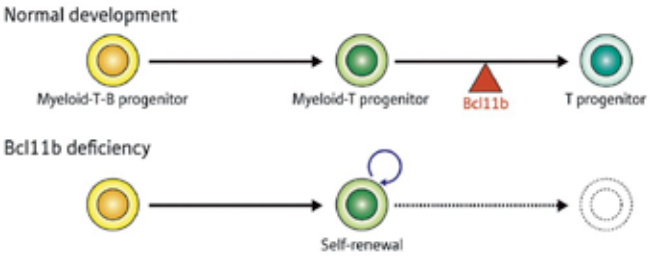


Figure :
The transcription factor Bcl11b regulates the formation of T cells from immune progenitor cells.

ORIGINAL RESEARCH PAPER

Ikawa, T., Hirose, S., 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).

Maintaining Independence

A set of neighboring immune-system genes each receive separate activation instructions despite being controlled by a common factor

Masato Kubo

As part of the immune response to foreign antigens, naïve T cells mature into different types of helper T cells. T_H1 cells and T_H17 cells, for example, secrete a subset of signaling factors known as cytokines that promote inflammatory responses to viral infections, while T_H2 cells secrete cytokines that promote antibody secretion by B cells and drive allergic reactions.

The GATA-3 protein is known as a 'master switch' for T_H2 differentiation, stimulating production of cytokines such as interleukin (IL)-4 and IL-13, but new findings from a team led by Masato Kubo at RIKEN RCAI have revealed an unexpected degree of complexity in this activation process.

Yasutaka Motomura

"The idea that genes encoding T_H2 cytokines are coordinately regulated … has been widely accepted," says Kubo. Many of these genes are situated in the same chromosomal neighborhood, and some scientists believe that the chromosome physically loops so that DNA-bound GATA can regulate multiple sites simultaneously. However, Kubo and colleagues found that GATA appears to independently bind multiple, distinct sites that each confer regulatory control over individual T_H2-associated genes.

One of these sites, HS2, specifically governs IL-4 expression, and GATA binding at this site induces chemical modification of the DNA segment containing the *Il4* gene, leading to increased cytokine production. Naïve T cells from mice lacking this chromosomal region give rise to T_H2 cells that generate normal levels of most cytokines, but fail to produce IL-4; these animals also show fundamental defects in their allergic response.

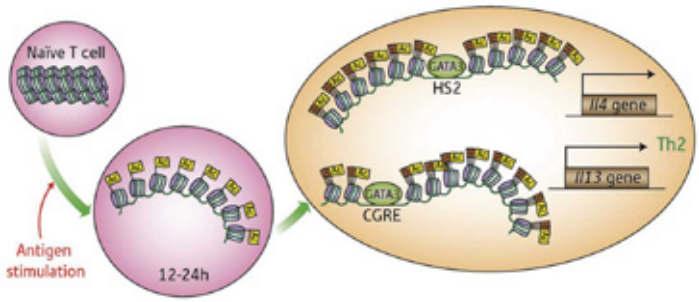


Figure:
Naïve T cells (*left*) begin to mature into T_H2 cells within 12–24 hours of being stimulated by the presence of a foreign antigen (*middle*). As a component of this process, a 'master switch' protein drives the production of T_H2 cytokines IL-4 and IL-13 by binding to and promoting chemical modification in the vicinity of the HS2 or CGRE sites, respectively (*right*).

In parallel, the researchers identified a second GATA-binding site, CGRE, which specifically regulates production of IL-13. Like HS2, GATA interaction with this site is associated with targeted chemical modification of a nearby stretch of DNA containing the *Il13* gene, and disruption of CGRE essentially eliminates production of this cytokine while leaving IL-4 production unaffected (Fig.). "These results came as a surprise," says Kubo. "They indicate that the independent recruitment of GATA-3 to locus-specific regulatory elements controls the expression status of individual genes encoding TH2 cytokines." These findings also parallel previous data suggesting that GATA coordinates expression of IL-5, another T_H2 cytokine, independently of IL-13.

Other types of immune cells also secrete T_H2 cytokines, and Kubo and colleagues now hope to determine whether their findings represent a broadly used mechanism for regulating production of these cytokines. "Our next priority will be exploring the relative contribution of these discrete elements to transcriptional regulation of IL-4 and IL-13 among these cell types," he says.

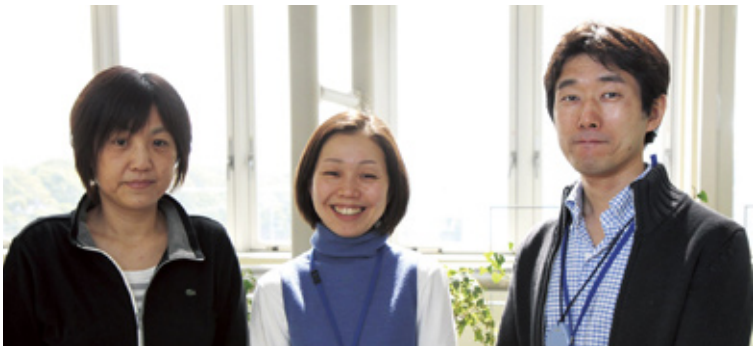
ORIGINAL RESEARCH PAPER

Tanaka, S., Motomura, Y., Suzuki, Y., Yagi, R., Inoue, H., Miyatake, S. & Kubo, M. The enhancer HS2 critically regulates GATA-3-mediated *Il4* transcription in T_H2 cells. ***Nature Immunology*** **12**, 77-85 (2011).

Fighting Their Way to the Middle

Immune cells get switched off by the accumulation of dense clusters of inhibitory proteins

Takashi Saito



Masako Takamatsu *(left)*, **Wakana Kobayashi** *(center)* **and Tadashi Yokosuka** *(right)*

Foreign entities within the body get chopped into pieces by antigen-presenting cells (APCs), which display the resulting chunks on their surface. These antigens can subsequently be recognized and bound by T cell receptors (TCRs), and the interaction between a T cell and an antigen-bearing APC eventually triggers the onset of an immune response against the antigen.

Inappropriate responses by this system, however, can give rise to disastrous medical consequences, and there is a keen interest in developing more sophisticated ways to modulate how T cells react to perceived threats. "Ultimately, we would like to regulate T cell function and activation in order to overcome autoimmune diseases, allergy, or infectious diseases," explains Takashi Saito, RIKEN RCAI in Yokohama.

New findings from a team led by Saito and RCAI colleague Tadashi Yokosuka could ultimately prove valuable for such efforts, by revealing insights into the mode of action of cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), an inhibitor of TCR signaling. When T cells associate with APCs, they form what is known as an 'immunological synapse', a juncture where numerous proteins assemble into elaborate complexes, such as the central supramolecular activation cluster (cSMAC). By establishing experimental conditions that simulate this cellular interaction, the researchers were able to monitor the dynamic rearrangements that take place at the cSMAC and its environs.

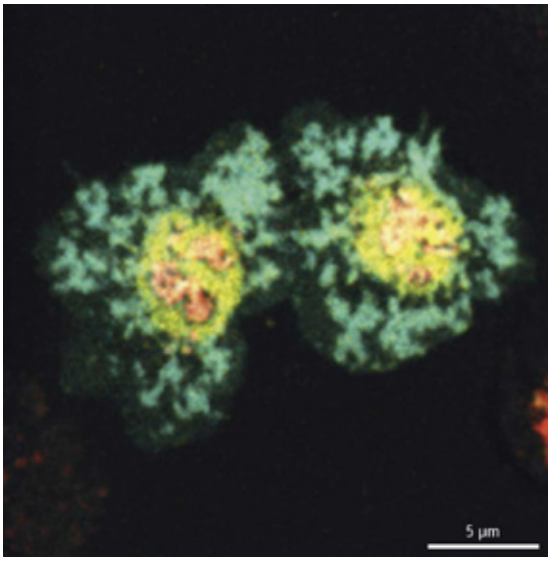


Figure :
Over time, clusters of CTLA-4 molecules (*yellow*) gather alongside TCRs (*red*) within the cSMAC; in doing so, they displace CD28 (*green*) and suppress TCR signaling.

CD28, a T cell protein that promotes TCR signaling, typically assembles at a narrowly defined region of the cSMAC, where it interacts with CD80, a protein expressed on the surface of APCs. Saito, Yokosuka and colleagues determined that CTLA-4 gradually gathers at the immunological synapse and subsequently forms into clusters within the exact same area of the cSMAC as CD28, where it directly competes to bind CD80 (Fig.). "Positive regulation by CD28 and negative regulation by CTLA-4 are induced at the same place in the cell," says Saito. "Without accumulating at the cSMAC, CTLA-4 cannot inhibit T cell activation."

In addition to illuminating a mechanism by which T cell responses get fine-tuned, these findings could ultimately yield benefits for patients suffering from a variety of conditions. "Anti-CTLA-4 antibody therapy has been utilized for cancer patients, enhancing tumor immunity by inhibiting regulatory T cells, and CTLA-4-based fusion proteins have been used to block autoimmune diseases such as arthritis," says Saito. "Our findings will enable us to explore new therapeutic concepts based on the inhibition of the dynamic movement of regulatory molecules such as CTLA-4."

ORIGINAL RESEARCH PAPER

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).

The Long and Short of Cell Signaling

By bolstering a sophisticated computational model with quantitative experimental data, researchers begin to decipher the workings of a complex signaling network

Mariko Okada-Hatakeyama

Like a telegraph transmission, the significance of a cellular signal can change greatly depending on whether it arrives as a brief 'dot' or a sustained 'dash' (Fig.). For example, transient activation of extracellular receptor kinase 1/2 (ERK) by epidermal growth factor (EGF) causes cells to divide, while prolonged ERK activation induced by heregulin (HRG) instructs these same cells to differentiate.

Cell biologists have struggled to untangle the relationship between this signaling network and cell fate, but a collaborative effort between Mariko Okada-Hatakeyama at RIKEN RCAI and Boris Kholodenko at University College Dublin in Ireland has achieved an important breakthrough by pairing quantitative experiments with computational modeling.

Figure:
Like dots and dashes from a Morse code transmission, brief and sustained cell signals can have different meanings for cell fate.

Okada-Hatakeyama's team previously examined the expression of *c-fos*, a so-called 'immediate early gene' whose expression is induced shortly following ERK activation, and obtained somewhat contradictory findings. "Early gene expression time-course profiles were the same regardless of whether the upstream ERK signal is transient or sustained," she says. "However, levels of c-Fos protein were 'all or none' for sustained and transient signals, respectively."

Based on an initial interpretation of their computational model of this pathway, Okada-Hatakeyama, Kholodenko and colleagues proposed that the effects of both HRG and EGF on *c-fos* expression were modulated purely by dual-specificity phosphatases (DUSPs), enzymes that inhibit ERK's capability to induce *c-fos*. However, experiments with a forced reduction of DUSP in cultured cells did not fully replicate these predictions. "There were long and serious discussions about whether our experiment was working properly or the model was wrong," says Okada-Hatakeyama.

This reassessment led to experiments that enabled the researchers to demonstrate the existence of a second, previously unknown mechanism for repression of c-fos expression that is triggered only in response to HRG. Together, these two ERK-activated 'negative feedback' systems appropriately control *c-fos* transcription in the process of cellular differentiation.

In parallel, their model also revealed how the combination of ERK-induced *c-fos* expression and sustained signaling activity by ERK outside the nucleus lead to steady production of c-Fos protein. This system architecture results in a highly stable signaling arrangement that filters out extraneous background noise and induces all-or-none output, according to Okada-Hatakeyama. "We learned from this study that cells possess very simple but robust system structures that can fight against unwanted perturbations," she says. "This cellular signaling network is still a 'black box' and what we can do with computational modeling is very much limited … but we hope to untangle the cell decision program someday."

ORIGINAL RESEARCH PAPER

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

RELATED ARTICLE

Nagashima, T., Shimodaira, H., Ide, K., Nakakuki, T., Tani, Y., Takahashi, K., Yumoto, N., & Hatakeyama, M. Quantitative transcriptional control of ErbB receptor signaling undergoes graded to biphasic response for cell differentiation. *The Journal of Biological Chemistry* **282**, 4045-4056 (2007).

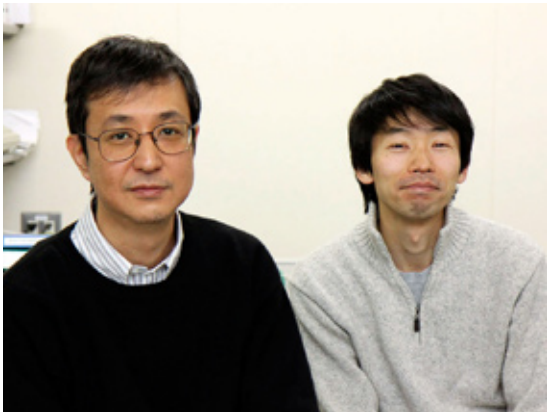
Ready and Waiting

A subset of immune cells remain ideally positioned to respond quickly to the reappearance of previously encountered pathogens

Tomohiro Kurosaki

Like a burglar tripping an alarm, infectious threats within the body set off a chain reaction of signaling events that enable the immune system to mount a proper defensive response. Once the crisis is averted, populations of target-specific 'memory' B cells ensure that any return visit by the same pathogen will be dealt with promptly and harshly.

Memory B cells initially arise within germinal centers in spleen and lymph nodes, but it has proven challenging to determine whether they continue to reside there or circulate throughout the body. By applying sophisticated cellular imaging techniques, a team led by Tomohiro Kurosaki of RIKEN RCAI has now resolved this question for at least one major subset of these cells.



Yuuichi Aiba *(left)* **and Kohei Kometani** *(right)*

B cells are primarily categorized based on the immunoglobulin protein chains they incorporate into their antibodies, and Kurosaki's team primarily focused their attention on immunoglobulin G-expressing (IgG^+) cells. Using a variety of fluorescent labeling strategies, they were able to determine that IgG^+ memory cells remain clustered close to the germinal centers long after the initial immune response in mice injected with the immunostimulatory molecule nitrophenol. By comparison, immunoglobulin M-expressing (IgM^+) memory cells are found scattered at discrete sites throughout the spleen.

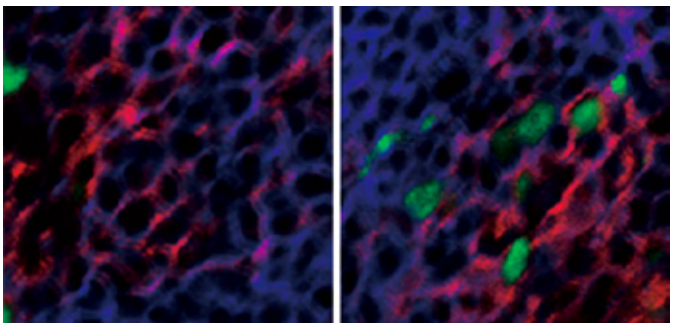


Figure :
A fluorescent indicator of cell division (*green*) reveals that IgG memory B cells (*purple*) are largely quiescent during an initial antigen challenge (*left*), but undergo activation and proliferation in response to a secondary challenge (*right*).

Subsequent experiments with a fluorescent indicator of cell division showed that IgG^+ cells replicate rapidly in response to a secondary challenge with nitrophenol (Fig.), and that this process is dependent on direct interaction with helper T cells, which are also located in close proximity to germinal centers. The communication between these two cell types appears to directly contribute to elevated production of antigen-specific antibodies. "Although preliminary, our data suggest that IgG^+ memory B cells are more prone to differentiate into antibody-producing plasma cells than IgM^+ memory B cells, which may contribute to regeneration of the memory pool after a secondary antigen challenge," says Kurosaki.

The memory cell-mediated immune response is generally faster and more robust with regard to target recognition than the 'first encounter' with a given pathogen, and Kurosaki believes that these findings represent an important step toward understanding the efficiency of the memory cell response. "Before our study, people believed that memory B cells leave the germinal centers and are recirculated all over the body by the lymphatic system and blood," he says. "However, our study clearly demonstrates that some IgG—but not IgM—memory B cells reside continuously near germinal centers and thus enable rapid activation after antigen re-challenging."

ORIGINAL RESEARCH PAPER

Aiba, Y., Kometani, K., Hamadate, M., Moriyama, S., Sakaue-Sawano, A., Tomura, M., Luche, H., Fehling, H.J., Casellas, R., Kanagawa, O. *et al*. Preferential localization of IgG memory B cells adjacent to contracted germinal centers. *Proceedings of the National Academy of Sciences USA* **107**, 12192-12197 (2010).

Awaiting Orders to Retaliate

Signaling proteins that help immune cells develop also enable those cells to mount an effective counterattack against infections

Tomohiro Kurosaki

When immune system B cells are alerted to the presence of a threat within the body, they form structures called germinal centers, which serve as *ad hoc* headquarters for marshaling a targeted immune response. These cells subsequently differentiate into plasma cells, which produce antibodies directed against foreign entities, or memory cells, which retain the capacity to become plasma cells if the same threat reappears in the future.

The extracellular signal-regulated kinase proteins (ERK1/2) are integrally involved in the early stages of this process, making it a challenge to assess their subsequent contributions. "If we delete both ERK genes entirely, differentiation of B cells is impaired and we cannot analyze the function of ERKs during the immune response," explains Kohei Kometani, a researcher in Tomohiro Kurosaki's group at RIKEN RCAI.

To address this challenge, Tomoharu Yasuda and Kometani developed transgenic mice in which ERK expression is lost only after the initial differentiation of B cells. Their initial results were striking; following vaccination with a highly immunogenic antigen, ERK-deficient mice showed a 10- to 40-fold reduction in antibody production (Fig.). This selective deletion of the two ERK genes led to a sharp decrease in the number of antigen-specific plasma cells but had little effect on memory cell counts. The researchers determined that these signaling factors appear to directly facilitate plasma cell maturation. "It was surprising that ERKs regulate differentiation but do not affect cell proliferation, because many people think of the ERKs as important molecules for cell growth," explains Kometani.

Several proteins known as transcription factors contribute to the maturation of plasma cells by turning key genes on or off. Blimp-1 is among the most important of these, as it also helps to inhibit transcription factors that maintain germinal center B cells. Yasuda and Kometani determined that the gene encoding Blimp-1 is a primary target of ERK signaling. They also identified another protein, Elk-1, which appears to be an important intermediary in this process.

As their findings also indicate that other signaling pathways are likely to intersect with ERK signaling in this developmental process, Yasuda and colleagues hope to explore this complexity in the future. "Harmful or excess antibody production are sometimes the cause of autoimmunity and allergy," he says, "and from this point of view, it may be interesting to check the involvement of not only the ERKs, but also the molecules upstream and downstream."

Kohei Komtani *(left)* **and Noriko Takahashi** *(right)*

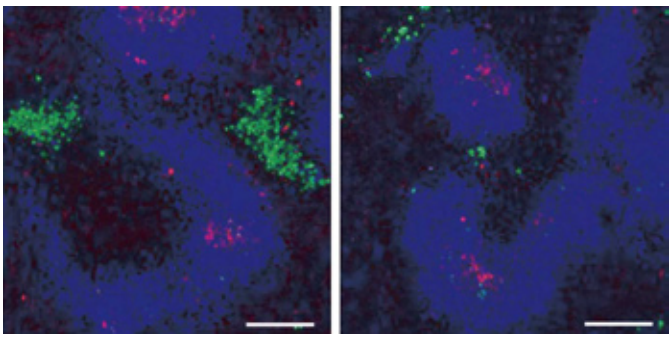


Figure:

Fluorescently labeled spleen tissue from wild-type (*left*) and ERK-deficient (*right*) mice reveals a dramatic difference in the production of antibodies (*green*) in response to an antigen challenge. *Blue* indicates expression of B220, a B cell specific marker, while *red* indicates labeling for a marker commonly found in germinal centers. Scale bars, 200 μm.

ORIGINAL RESEARCH PAPER

Yasuda, T., Kometani, K., Takahashi, N., Imai, Y., Aiba, Y. & Kurosaki, T. ERKs induce expression of the transcriptional repressor Blimp-1 and subsequent plasma cell differentiation. *Science Signaling* **4**, ra25 (2011).

Creating a Life-saving Killer

Cancer may be kept in check by a method for generating patient-specific immune cells with antitumor activity


Shin-ichiro Fujii

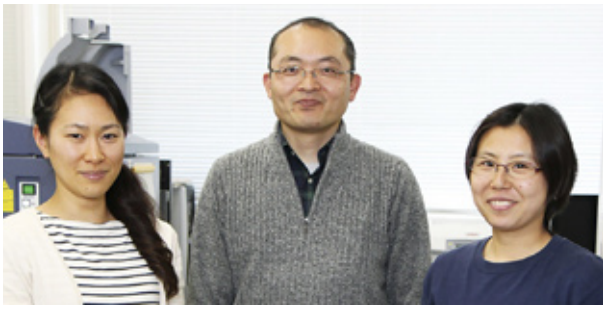

Haruhiko Koseki


Masaru Taniguchi

Upon receiving the appropriate activating signal, natural killer T (NKT) cells live up to their name, releasing a torrent of molecules that trigger the protective immune response necessary to eliminate pathogens or even thwart tumor growth.

These cells represent a promising clinical tool, as demonstrated in a recent clinical trial in which lung cancer patients received NKT-stimulating injections of dendritic cells that had been pretreated with α-galactosylceramide (α-GalCer). Some 60% of treated patients exhibited a striking seven-fold improvement in their median survival time relative to their untreated counterparts. "The effects are superior to other molecular-targeted cancer drugs," says Masaru Taniguchi of RIKEN RCAI, whose team participated in this study. "However, this therapy is not applicable to two-thirds of patients because of their limited number of NKT cells."


Daisuke Yamada


Yuko Nagata *(left)*, **Hiroshi Watarai** *(center)* **and Sakura Sakata** *(right)*

To solve this problem, Taniguchi teamed up with RCAI colleagues Haruhiko Koseki and Shin-ichiro Fujii to develop a method for generating transplantable NKTs. They derived these from induced pluripotent stem cells (iPSCs), embryonic-like cells that are typically generated via virus-mediated delivery of 'reprogramming genes' into skin cells. However, NKT maturation involves a complex genomic rearrangement event, making them difficult to derive from conventional iPSCs. As such, Taniguchi and Koseki devised a novel approach for generating mouse iPSCs from existing NKTs (Fig.), which have already undergone this rearrangement. They used these iPSCs to generate large numbers of new NKTs *in vitro*.

Their method efficiently produced mature NKTs, which rapidly established a stable population within the liver upon transplantation into mice. To the researchers' pleasant surprise, these new NKTs displayed typical activation behavior in response to α-GalCer-treated dendritic cells and proved capable of coordinating an effective immune response. "In general, cells generated from *in vitro* culture die quickly *in vivo* or are killed by host immune cells … however, this was not the case here," says Taniguchi. "We detected iPSC-derived NKT cells with adjuvant activity and tumor-eradicating effects two weeks after cell transfer."

Taniguchi, Koseki and colleagues are now keen to begin working with human cells. This transition will involve many new challenges, but the researchers see great clinical potential in their approach—particularly in the US, where the Food and Drug Administration has approved development of cell-based therapies. "NKT cell-targeted adjuvant cell therapy is applicable to any type of cancer patient, because it can overcome their immunodeficient status and enhance antitumor responses," says Taniguchi. "At present, the delivery of patient dendritic cells is the main limiting factor."

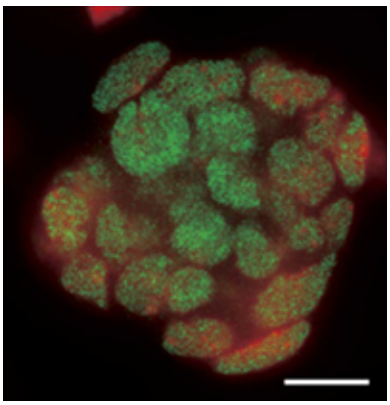


Figure :
A cluster of undifferentiated iPSCs derived from reprogrammed NKTs with high expression levels of the Oct3/4 pluripotency factors (*green fluorescence*) (DAPI (*red*), scale bar, 10 micrometers).

ORIGINAL RESEARCH PAPER

Watarai, H., Fujii, S., Yamada, D., Rybouchkin, A., Sakata, S., Nagata, Y., Iida-Kobayashi, M., Sekine-Kondo, E., Shimizu, K., Shozaki, Y. et al. Murine induced pluripotent stem cells can be derived from and differentiate into natural killer T cells. *The Journal of Clinical Investigation* **120**, 2610-2618 (2010).

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Giving Tumor Vaccines a Proper Introduction

Masato Tanaka

Therapies that target specialized cells residing within the lymph nodes may help to rally tumor-killing immune responses

Given how effectively the immune system can eliminate foreign threats such bacteria and viruses, hopes are high for the development of strategies that might turn these same defense mechanisms against cancerous targets. However, attempts to train the immune system to recognize malignancies via the intravenous injection of vaccines that present tumor-derived antigens have fallen short.

According to Kenichi Asano, a researcher with Masato Tanaka's group at RIKEN RCAI, this is the result of 'tolerance' mechanisms that protect against autoimmune disease. "Billions of cells die every day, and cell corpses must be removed swiftly from our body in order not to induce detrimental effects," he says. In this scenario, macrophage cells in the spleen clean house by devouring such debris in a process known as phagocytosis, thereby preventing dead cells from triggering an inflammatory response.

Tumor cells delivered into the lymphatic system via subcutaneous injection, however, can successfully elicit a strong immune response, and new research from Asano and colleagues explains why this is the case. In order to rouse an effective reaction, phagocytic cells must present recognizable chunks of those dead cells to tumor-killing cytotoxic T lymphocytes (CTLs). The researchers identified a very specific subset of macrophages within the lymph nodes that perform this task.

Intriguingly, these cells, which are distinguishable by their expression of the cell-surface protein CD169, are non-migratory and reside stably within the sinuses of the lymph node, awaiting their prey like spiders in a web. Dead cancer cells delivered to these sinuses via the lymphatic system are rapidly digested by the macrophages (Fig.), which in turn cross-present the resulting antigens to CTLs. By selectively killing off these macrophages with diphtheria toxin, the researchers were able to essentially disable the immune response. "Without CD169 macrophages, tumor-directed T cells were no longer activated—that means these cells dominate anti-tumor immunity after tumor cell death," says Asano.

These findings help explain why the dead cells that slough off of tumors into the lymphatic system during radiation or chemotherapy are sometimes sufficient to provoke an immune response, and could provide the foundation for far more effective cancer immunotherapy strategies. "I believe it is very promising to mount anti-tumor immunity in patients with solid tumors by delivering tumor antigens specifically to CD169 macrophages," says Asano. "It's my dream to invent artificial materials that possess the characteristics of dead cells and are safe for administration to patients."

Kenichi Asano

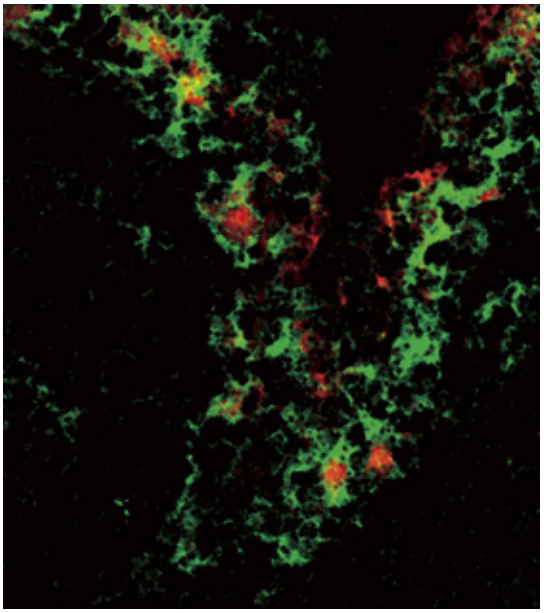

Figure:
CD169-positive macrophages (*green*) residing within the lymph sinuses rapidly consume the dead tumor cells (*red*) that make their way through the lymphatic system following subcutaneous injection.

ORIGINAL RESEARCH PAPER

Asano, K., Nabeyama, A., Miyake, Y., Qiu, C.-H., Kurita, A., Tomura, M., Kanagawa, O., Fujii, S.-I. & Tanaka, M. CD169-positive macrophages dominate antitumor immunity by crosspresenting dead cell-associated antigens. *Immunity* **34**, 85-95 (2011).

Holding Fire on the 'Good Guys'

Tolerating the foreign materials in food that mice and humans need hinges on the presence of B7 proteins

Katsuaki Sato

An international team of molecular biologists led by RIKEN researchers has unraveled key details of the molecular mechanism whereby the body's immune system determines what to attack among the organisms and food taken into the mouth, and what to leave alone or tolerate. The researchers have shown the pivotal role of two proteins found on the surface of cells that stimulate the immune system into action, the dendritic antigen-presenting cells (APCs). The work may lead to new therapies for immune disorders, and to ways of boosting the effectiveness of oral vaccines.

Hideaki Takagi *(left)* **Tomohiro Fukaya** *(center)* **and Yumiko Sato** *(right)*

Animals could not survive without the nutrients and beneficial micro-organisms they ingest when feeding. But the foreign material taken in through the mouth and passing through the gut also contains harmful substances and organisms. So the immune system must balance active protection against pathogens and toxins with a non-responsiveness to food and commensal bacteria known as oral tolerance. In the past, researchers have proposed two mechanisms for oral tolerance—reducing the numbers of effector T cells, the immune-system foot soldiers that move against foreign material and suppressing their action by means of specialized regulatory T cells.

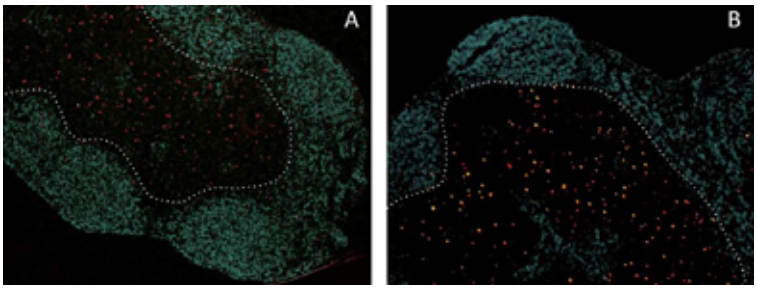

Figure :

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

The triggering of effector T cells depends on interaction with two distinct types of proteins on the surface of the APCs, antigens that are markers of foreign material and co-stimulatory proteins of the B7 family, which regulate the response. Both must be present, however, to initiate any action.

Using mice deficient in B7 co-stimulatory proteins Katsuaki Sato and colleagues from RIKEN RCAI, together with researchers from other laboratories in Japan, and in the US and France, found that oral tolerance demanded the presence of B7-H1 and B7-DC proteins. In fact, without these proteins the immune response was enhanced. Of the APCs, the dendritic cells of the mesenteric lymph nodes, in the membranes surrounding the digestive system, display higher levels of these proteins.

When the researchers investigated the role of the two B7 proteins, again using B7-deficient mice, they discovered the proteins induced the generation of regulatory T cells rather than normal effector cells (Fig.). These regulatory T cells then damp down the immune response promoting tolerance. During inflammation, however, their action is swamped.

The research group wants to continue analyzing the role of different groups of dendritic cells in live mice, Sato says. "In particular, we wish to identify the molecular basis of the regulation of the function of these cells."

ORIGINAL RESEARCH PAPER

Fukaya, T., Takagi, H., Sato, Y., Sato, Kaori, Eizumi, K., Taya, H., Shin, T., Chen, L., Dong, C., Azuma, M., Yagita, H., Malissen, B. & Sato, K. Crucial roles of B7-H1 and B7-DC expressed on mesenteric lymph node dendritic cells in the generation of antigen-specific CD4$^+$Foxp3$^+$ regulatory T cells in the establishment of oral tolerance. *Blood* **116**, 2266-2276 (2010).

Hirano Receives the Japan Prize 2011

Dr. Toshio Hirano received the Japan Prize 2011 in the field of "Bioscience and Medical Science", together with Dr. Tadamitsu Kishimoto. The award was given for their discovery of interleukin-6 (IL-6) and its application in treating diseases.

They originally purified IL-6 protein, which plays a vital role in the production of antibodies, and then succeeded in cloning the gene in 1986. In addition, they have identified a wide range of other IL-6 functions and their research has contributed to the progress of biomedical science and the development of therapeutic drugs for inflammatory diseases.

Discovery of interleukin-6

Hirano was born in 1947 in Osaka. He entered the Faculty of Medicine at Osaka University in 1966. In his 5th year of medical school, he heard a lecture by the late Yuichi Yamamura, a pioneer in immunological research in Japan. Yamamura always made the students think about the real causes of a disease. Yamamura's attitude, to consider the actual nature of a phenomenons intrigued Hirano, leading him to consider autoimmune disorders, where the immune system revolts and attacks our own bodies.

In 1973, Hirano graduated from the Faculty of Medicine at Osaka University and went on to study immunology at the National Institute of Health (NIH) in Baltimore, Maryland, U.S., where he became acquainted with Kishimoto. When he arrived at the airport, Kishimoto, who was already studying at Johns Hopkins University in Baltimore, came to pick him up. It was the start of their long-term relationship.

At that time, immunology was a field in which cutthroat competition was already underway. Researchers around the world were competing to understand the nature of interleukins. In 1975, Kishimoto began studies with Robert Good at the Memorial Sloan-Kettering Cancer Center in New York City, and showed the presence of a new candidate interleukin. Meanwhile, Hirano returned to Japan in 1976, and continued his own research at Habikino Hospital and the School of Medicine at Kumamoto University to identify a novel interleukin acting on B cells to induce immunoglobulin production, eventually culminating in the discovery of interleukin 6 together with Kishimoto at 1986.

However, this was only the beginning of research on the interleukin. One characteristic of these molecules, which came to be called cytokines, is that one type can have many functions, and there are many companion molecules that can have similar, overlapping functions. Because of this redundancy, even if the biological activities of the discovered substance were studied, it would be difficult to prove that it really was a new substance. The academic society at that time only acknowledged a substance as new if the encoding gene was successfully cloned.

In 1982, Kishimoto became a professor of the Institute for Molecular and Cellular Biology established

***Back row*: Chairman Yoshikawa and his wife, President Ito and his wife. *Front row*: Hirano and his wife, Kishimoto and his wife**

at Osaka University. In the following year, Kishimoto welcomed Hirano as his research partner. They adopted leading-edge genetic engineering techniques and continued in their gene hunt. Initially, they were unsuccessful and continued in their low-profile experiments for days and then for years, often struggling with frustration. Hirano recalls the period of the long dark night when only the people who believe could go forward.

However, in May 1986, they finally succeeded in capturing the long-sought gene. With so much joy, Hirano and colleagues jumped into a swimming pool with their clothes on during the lab's summer camp. In 1988, at an international conference, the name interleukin 6 (IL-6) was given to the new cytokine.

Opening the way from basic research

After the successful gene cloning, a highly pure IL-6 was obtained by means of genetic modification techniques, and progress was also seen in the clarification of the mechanism of IL-6 activity. The research group with both doctors as core members also discovered the structure of the IL-6 receptor. In addition, they identified the intracellular signals that transmit IL-6 information to the cell nucleus.

What proved fortunate was that as the research progressed, it became evident that IL-6 not only enhances antibody production, but also has a wide variety of other functions. For example, it can stimulate the production of a liver protein, CRP, when acute inflammation occurs in the body. It also increases production of platelets, blood components that coagulate blood in the event of an injury. The more research is done, the more functions are discovered, thus contributing to the progress of medicine. Particularly noteworthy was the discovery that IL-6 is involved in rheumatoid arthritis, which is a representative autoimmune disorder. The two doctors had already noted from an early stage of their research that IL-6 is deeply involved in inflammatory reactions within the body, but it was newly discovered that a large quantity of IL-6 exists in the joint fluid of rheumatoid arthritis patients. Based on this discovery, important insight was obtained about its pathogenic mechanism. Research originating from the discovery of IL-6 has broadened its horizons from leading-edge medicine to bioscience. Based on basic research, Kishimoto has jointly developed, with a pharmaceutical company, an antibody drug Tocilizumab that inhibits IL-6 action. After being approved in Japan in 2008, the drug has been approved in 70 countries worldwide including Europe and the U.S.

At a press-conference by the Japan Prize 2011, Hirano spoke.

"With a firm belief in the importance of attaining the true nature of things, I have been challenging and confronting difficulties one by one as if I was climbing up mountains and cliffs to reach the extreme summit. Through these fundamental research efforts, we were blessed with the discovery of IL-6, which opened up the way for the development of therapeutic medications for diseases like rheumatoid arthritis. It is something I could never have imagined when I graduated from the medical department of Osaka University 39 years ago. Today, I am determined as ever to keep contributing to the society through medical research and developing the next generation of researchers."

"I believe that Japan, for the sake of its future, must contribute to the peace and prosperity of mankind by acknowledging the importance of fundamental science, and engaging in scientific and technological development. I sincerely hope that this prize will reaffirm the importance of fundamental scientific research and set a vision and dream for the young people."

Hirano gave a lecture at RCAI on May 13, 2011, and RCAI members celebrated his receipt of the award.

Photos and the information courtesy of The Japan Prize Foundation

http://www.japanprize.jp

The Japan Prize is awarded to honor the achievements of people throughout the world who have contributed to the progress of science and technology and the advancement of world peace and prosperity. In principle, the Prize is given for work done in any field of science and technology, but each year two particular fields are designated based on trends within these areas and other considerations. Laureates receive "Japan Prize" certificates of merit, prize medals and a cash award of 50 million yen for each field.

Outstanding Contribution of the Year 2010

Keiichiro Suzuki (Photo 1) and Sidonia Fagarasan (Photo 2) in the Laboratory for Mucosal Immunity, and Shinji Fukuda (Photo 3) and Hiroshi Ohno (Photo 4) in the Laboratory for Epithelial Immunobiology received RIKEN RCAI's Outstanding Contribution of the Year Award 2010 at a ceremony held on April 22, 2011 for their recent contributions to advancing the field of mucosal immunology. This award was established in 2006 to recognize RCAI investigators who make outstanding contributions to the Center. The awardees, who were selected by the Director, received a plaque and a monetary prize.

Suzuki and Fagarasan received the award for their studies on gut-IgA synthesis and interactions between host and environmental factors in the gut. They recently showed that Foxp3$^+$T cells and follicular dendritic cells in Peyer's patches have unique characteristics that promote IgA generation (Tsuji et al. 2009; Suzuki et al. 2010). At the ceremony, Suzuki presented the part of their work in which he made major contributions.

The starting point of their studies was the analysis of activation-induced cytidine deaminase (AID) knockout mice (Fagarasan et al. 2002; Suzuki et al. 2004), which lack IgA because of a defect in isotype switching and are also unable to undergo somatic hypermutation of their Ig variable region genes. When the composition of gut commensal flora in these mice was analyzed, it was quite different from that in normal mice. Sticky anaerobic organisms called segmented filamentous bacteria were expanded and had penetrated into the gut epithelial cells, causing strong stimulation to the host immune system. As a result, there was hyper-proliferation of germinal center B cells and the mice had larger Peyer's patches and isolated lymphoid follicles (ILF). The expansion of germinal center B cells was seen not only in the gut but also in systemic immune organs, e.g., spleen, indicating that IgA is critical for the regulation of both gut and systemic immune systems.

To address where IgA is generated, these investigators used aly/aly mice, which have no lymph nodes, Peyer's patches, ILF, or follicular structures, and no IgA$^+$ cells in the gut because of functional defects in lymphocytes and stromal cells. Transfer of normal bone marrow did not normalize IgA production. However, to their surprise, bone marrow cells together with stromal cells, which were reconstituted by injection of normal lamina propria cells, partially restored IgA$^+$ cells. Thus, lamina propria stromal cell, were necessary for the generation of IgA in the absence of organized follicular structures like the Peyer's patches (Fig. 1) (Suzuki et al. 2005).

However, in the normal situation, most of the IgA$^+$ cells are derived from Peyer's patches, and without them, IgA levels declined significantly, indicating that Peyer's patches are important for the efficient generation of IgA. So, Suzuki and Fagarasan next studied how IgA$^+$ cells are generated in Peyer's patches. To address the importance of stromal cells for IgA generation in Peyer's patches, they decided to study follicular dendritic cells (FDC), a specialized stromal cell in the germinal center (Fig. 2). The isolation of these cells was quite difficult, however, and Suzuki had to struggle for one year just for the FDC purification.

Photo 1 : Keiichiro Suzuki

Photo 2 : Sidonia Fagarasan

Photo 3 : Shinji Fukuda

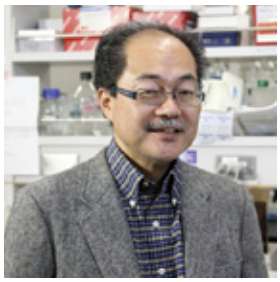

Photo 4 : Hiroshi Ohno

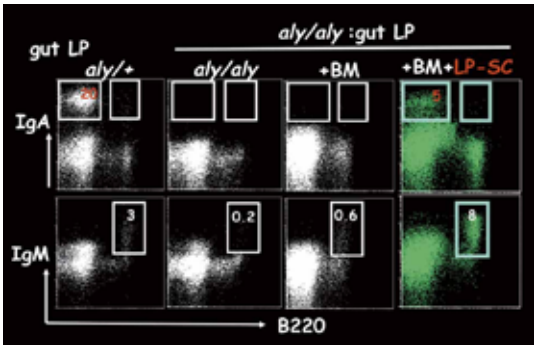

Figure 1: LP stromal cells support gut IgA generation

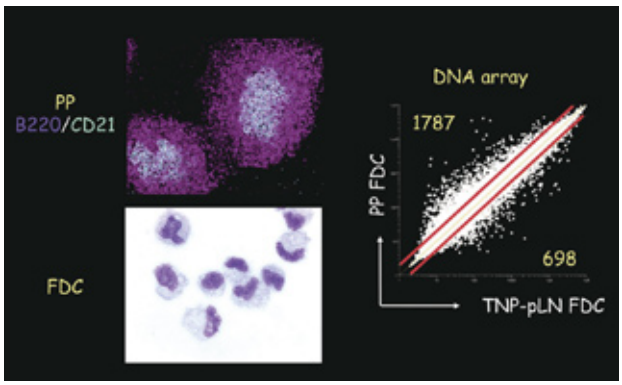

Figure 2: PP and pLN FDCs have distinctive gene expression profiles

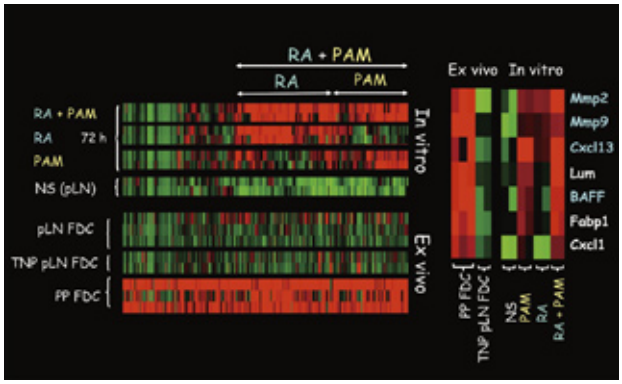

Figure 3: In vitro TLR and RA stimulations partially recapitulate the "PP FDC signature"

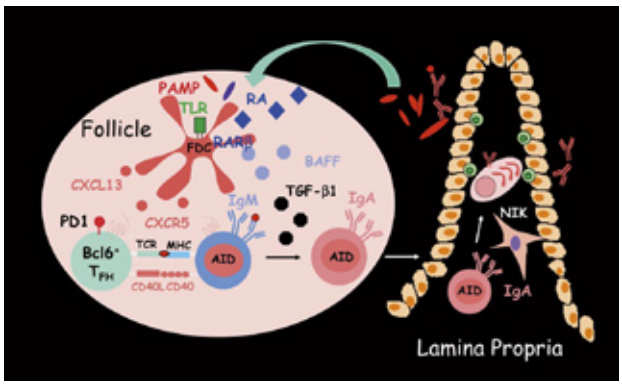

Figure 4: Gut IgA synthesis within and outside of Peyer's patches

A comparison of gene expression profiles revealed that FDCs of Peyer's patch and lymph node are quite different (Fig. 3). Suzuki speculated that FDCs in the Peyer's patch sense environmental cues, such as gut bacteria or food components. To test this idea, they stimulated lymph node (LN) FDC with bacterial components (TLR ligands) and/or food components (retinoic acid) *in vitro*. Many PP FDC signature genes were recapitulated in LN FDC when they were co-stimulated, indicating that FDCs induce IgA generation through these molecules, which include CXCL13 for lymphocyte recruitment, BAFF for lymphocyte survival, and TGFβ activators (Suzuki et al. 2010).

To summarize (Fig. 4), the FDCs in PPs sense bacterial and food components in the gut lumen, and secrete CXCL13 to recruit B cells and T cells. They also secrete TGF-β1 that induces IgM^+ B cells to switch to IgA, and BAFF that enhances the survival of the switched cells and their differentiation into plasma cells in the lamina propria.

Suzuki moved to Kyoto Univ. in April, 2011 to set up his own lab. It was almost 10 years ago that he joined Fagarasan's laboratory. "I was a Ph.D. student from a clinical department at that time, and didn't know anything about immunology or experiments. All experimental skills, knowledge and critical way of thinking were provided by Sidonia," he said. "Now, I have to work on my own feet. I am pretty sure that all of the experiments I performed in Sidonia's lab will help me a lot. I also would like to thank all of you for a lot of help, many discussions, chatting, and sometimes drinking. Everything was a very good experience. This is the real treasure, and in my future, I am pretty sure that this experience will help me a lot as well," Suzuki said at the ceremony.

Shinji Fukuda and Hiroshi Ohno received the award for their multi-omics-based approach to study host-gut microbe interactions (Fukuda et al. 2011).

It is widely believed that probiotics such as bifidobacteria and lactobacilli are good for health, although scientific evidence for their mechanisms of action is largely nonexistent. Enterohemorrhagic *Escherichia coli* (EHEC) causes illnesses ranging from mild diarrhea to severe diseases such as hemorrhagic colitis and hemolytic uremic syndrome. EHEC O157 produces the lethal Shiga toxin and it is responsible for public health problems worldwide.

To study the probiotic effects against EHEC O157 infection, Fukuda and Ohno took advantage of a simplified gnotobiotic mouse model, in which germ-free mice were colonized with individual strains of bifidobacteria. Initial experiments revealed striking differences between strains. They found that mice colonized by one bifidobacterium subspecies, *B. longum*, were able to survive when fed *E. coli* O157, while mice without the bifidobacteria died of infection within 7 days. However, another bifidobacterium strain, *B. adolescentis*, had no such protective effect (Fig. 5).

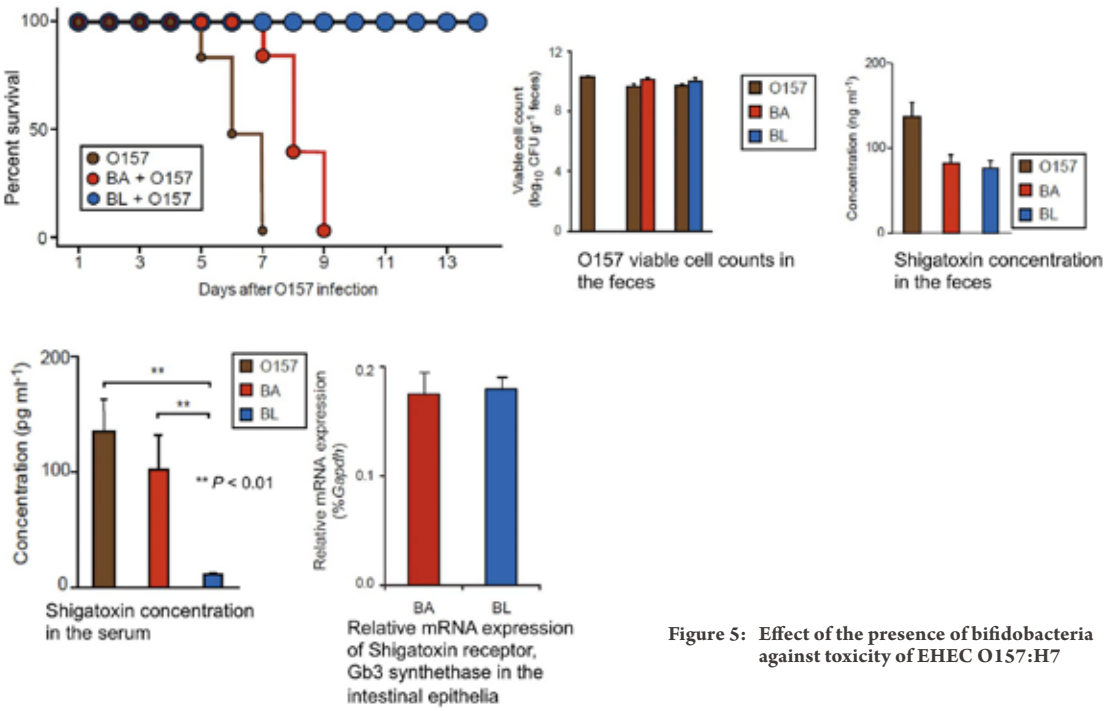

Figure 5: Effect of the presence of bifidobacteria against toxicity of EHEC O157:H7

Neither the concentration of Shiga toxin in the gut, nor the number of *E. coli* O157 was different between the mice colonized with the protective or non-protective bifidobacteria. However, the blood concentration of Shiga toxin was markedly lower in the surviving mice colonized by probiotic *B. longum*. Histological analysis showed a slight inflammation only in the distal colon of the dying mice, but not in the surviving mice, suggesting that probiotic bifidobacteria protect epithelial cells and prevent systemic infiltration of Shiga toxin.

By analysis of fecal metabolic profiles using a combination of techniques from genomics, transcriptomics and metabolomics, Fukuda and Ohno succeeded in pinpointing the source of this difference as the production of acetate, which enhanced intestinal epithelial defenses. The key player in this protection was a carbohydrate transporter present in protective strains of bifidobacteria such as *B. longum*, but absent from the non-protective strains. The carbohydrate transporter enabled these bacteria to utilize fructose to produce sufficient amounts of acetate in the distal colon to promote an anti-inflammatory effect. However, non-probiotic bifidobacteria could not produce sufficient amounts of acetate due to the lack of this transporter. After enteropathogenic infection, inflammation developed and the toxin infiltrated into the body.

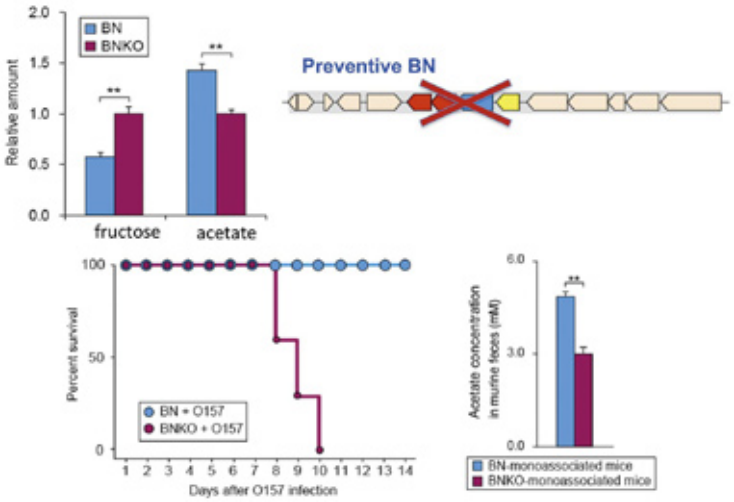

Figure 6: Non-preventing bifidobacteria overexpressing probiotic transporter prevents O157 lethal infection

To confirm this idea, the investigators knocked-out the carbohydrate transporter genes in *B. longum*, and colonized mice. The mice colonized with the mutant strain had a significantly reduced capacity to catabolize fructose and a reduction of acetate production. More importantly, survival after infection with *E. coli* O157 was significantly less (Fig. 6).

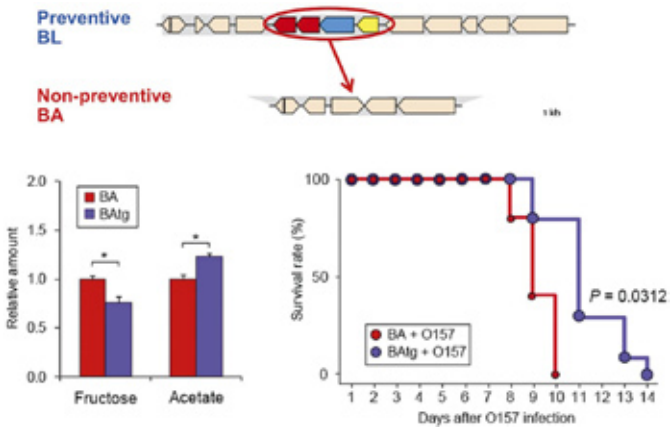

Figure 7: Disruption of the genes encoding probiotic transporter lacks the preventive effect against O157 lethal infection

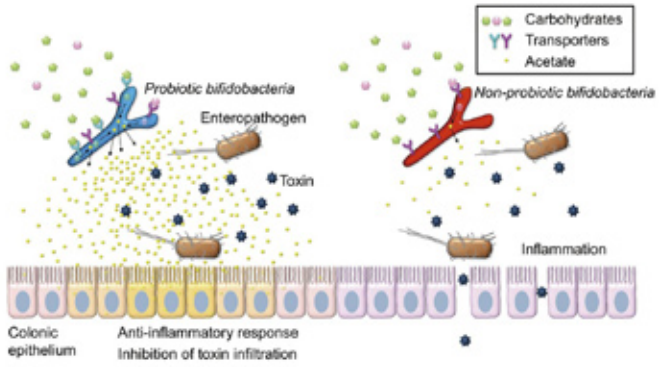

Figure 8: Carbohydrate transporters confer the probiotic effect to bifidobacteria

They also generated a strain of *B. adolescentis* that exogenously expressed the carbohydrate transporters and, when mice were colonized with this strain, they survived longer after infection with *E. coli* O157 (Fig. 7).

Finally, they asked whether simply increasing the amount of acetate in the distal colon could protect mice against *E. coli* O157-induced death. They colonized the mice with *B. adolescentis*, and then fed them a diet supplemented with acetylated starch, which is gradually hydrolyzed, releasing acetate in the intestinal tract. Administration of the acetylated starch significantly increased the amount of acetate in the feces and improved the survival of *B. adolescentis*-associated mice infected with *E. coli* O157, suggesting that the levels of acetate are crucial for this protection. (Fig. 8)

Shinji Fukuda started to work at RIKEN as a researcher in 2006. In collaboration with Prof. Itoh at the University of Tokyo, who had a germ-free facility and had established this enteropathogenic infection model, they discovered these important findings in the subsequent three years from 2006 to 2008. "We submitted the paper to *Nature* in Sep. 2009. After a long, long struggle with *Nature* editors and four reviewers, the paper was accepted finally in November, 2010. It was really tough work for me," Fukuda said at the ceremony. After the publication of the *Nature* paper, many newspapers and TV stations featured their findings, reflecting the lay society's interests in probiotic effects and EHEC O157 infection. "I strongly believe that our findings can open up a new field in the area of mucosal immunology. I will make every effort to progress this research and keep our position as the front runner in this field," said Fukuda.

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Suzuki, K., B. Meek, Y. Doi, T. Honjo and S. Fagarasan. Two distinctive pathways for recruitment of naive and primed IgM+ B cells to the gut lamina propria. ***Proc Natl Acad Sci U S A* 102**: 2482-2486 (2005).

Suzuki, K., B. Meek, Y. Doi, M. Muramatsu, T. Chiba, T. Honjo and S. Fagarasan. Aberrant expansion of segmented filamentous bacteria in IgA-deficient gut. ***Proc Natl Acad Sci U S A* 101**: 1981-1986 (2004).

Tsuji, M., N. Komatsu, S. Kawamoto, K. Suzuki, O. Kanagawa, T. Honjo, S. Hori and S. Fagarasan. Preferential generation of follicular B helper T cells from $Foxp3^{+}$ T cells in gut Peyer's patches. ***Science* 323**: 1488-1492 (2009).

Other Prize Winners 2010

Photo 1: Hiroshi Kawamoto

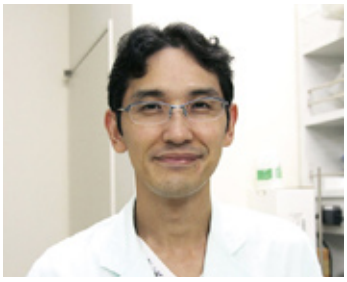


Photo 2: Koji Hase

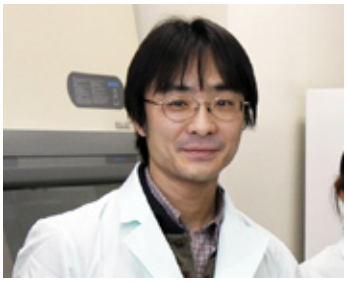


Photo 3: Keiichiro Suzuki

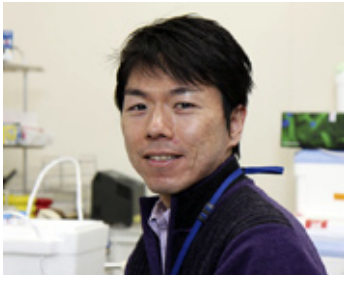


Photo 4: Tomokatsu Ikawa

Photo 5: Hiroshi Watarai

Hiroshi Kawamoto (photo 1), Leader of the Laboratory for Lymphocyte Development, received the 13th Japanese Society for Immunology Award for his validation of a new hematopoietic cell lineage model. Before his discovery, the classical model included a common progenitor cell that would give rise to B lymphocytes or T lymphocytes, but no other hematopoietic cell. However, Kawamoto discovered that no progenitors could differentiate only into B lymphocytes and T lymphocytes, though there were progenitors that would differentiate into T lymphocytes and phagocytes or into B lymphocytes and phagocytes. In his myeloid-based model, he proposed that progenitors retain the ability to differentiate into phagocytes until their fate is sealed by differentiation into erythrocytes, B lymphocytes, or T lymphocytes. He later discovered that the transcription factor Bcl11b regulates the formation of T cells from progenitor cells.

His work was highly recognized to receive the award because of its originality and the fact that it overturned the conventional wisdom. The Japanese Society for Immunology Award is given to a researcher, age of 50 or below, whose contribution to the advancement of immunology was original and extraordinary and from whom further research achievements are expected in the future.

Koji Hase (photo 2), Researcher of the Laboratory for Epithelial Immunobiology, received the Research Encouragement Award of the Japanese Society for Immunology for his elucidation of M-cell functions. He developed a method to screen for genes expressed specifically in M-cells and, by analyzing the molecular functions of these genes, discovered glycoprotein 2 (GP2) as a transcytotic receptor for mucosal antigens. Later, he also discovered a mammalian protein, M-Sec, which induces formation of numerous membrane protrusions extending from the plasma membrane. These protrusions tether onto adjacent cells and subsequently form tunneling nanotubes, which can transmit calcium signals or viruses between cells.

This award is given to young investigators, age 40 or younger, who have performed distinguished immunological research.

RIKEN Research and Technology Incentive Awards 2010 were presented to two RCAI researchers, **Keiichiro Suzuki** (photo 3) and **Tomokatsu Ikawa** (photo 4). Suzuki, Researcher of the Laboratory for Mucosal Immunity, received the award for his study on mechanisms of IgA generation and its functions. Tomokatsu Ikawa, Researcher of the Laboratory for Lymphocyte Development, received the award for his study on molecular mechanisms of T cell lineage developmental decisions.

The RIKEN Research and Technology Incentive Award was established in FY2009 to recognize young researchers and technicians under age 40 who have contributed to furthering RIKEN's ideals by achieving exemplary results in their research or research support activities.

Hiroshi Watarai (photo 5), Researcher of the Laboratory for Immune Regulation, received the Best Researcher Award from the Young Researchers' Study Group of Hematology, headed by Dr. Mineo Kurokawa. The study group selects excellent papers published by Japanese researchers in the journal *Blood* every year, and invites the first authors for discussions. Watarai's presentation "Reprogramming of natural killer T (NKT) cells to induced pluripotent stem cells and their development into functional NKT cells *in vitro*" was awarded among those selected researchers.

Photo 6: Toshiyuki Fukada

Photo 7: Masahiro Kitano

Photo 8: Saya Moriyama

Toshiyuki Fukada (photo 6), Researcher of the Laboratory for Cytokine Signaling, received the excellent poster award at the annual meeting of the Japan Transporter Research Association for his poster presentation on "The loss of function of SLC39A13/ZIP13 cases a novel type of Ehlers-Danlos Syndrome". He also received the Research Encouragement Award at the Frontiers in Oral Medical Science meeting for his oral presentation on "Zinc transporter Zip13: its involvement in tooth development and inherited dental disease." His recent review featured the front cover of *Metallomics*.

Masahiro Kitano (photo 7), Researcher of the Research Unit for Immunodynamics, received an Excellent Poster Award at The 16th Takeda Science Foundation Symposium on Bioscience "Casting Light on Life" for his poster presentation on "BCL6 Expression Dynamics in the Lymph Node during the Antibody Response".

Mariko Okada-Hatakeyama (Laboratory for Cellular Systems Modeling, RCAI), in collaboration with Junya Yamagishi, Noriaki Okimoto, Atsushi Suenaga and Makoto Taiji (RIKEN ASI), received an Excellent Poster Award at the annual meeting of The Chem-Bio Informatics Society for their poster presentation on "Strategy for Structure-Based Peptide Design".

Saya Moriyama (photo 8), Junior Research Associate in the Laboratory for Lymphocyte Differentiation, received a Biology/Medical Science Prize at the RIKEN Noyori Summer School 2010. Her poster presentation "BCL6 Expression Dynamics in the Lymph Node during the Antibody Response" was the fruit from an RCAI internal collaboration with Masahiro Kitano and Takaharu Okada, Research Unit for Immunodynamics.

Seven graduate students studying in RCAI received Young Investigators' Travel Awards from the Japanese Society for Immunology for the 14th International Conference of Immunology. The awardees were **Masashi Ebisawa** (photo 9), **Daisuke Takahashi** (photo 10), **Yuhki Obata** (photo 11) and **Hideaki Shima** (photo 12) of the Laboratory for Epithelial Immunobiology, **Hideaki Takagi** (photo 13) and **Tomohiro Fukaya** (photo 14) of the Laboratory for Dendritic Cell Immunobiology and **Izumi Sasaki** (photo 15) of the Laboratory for Host Defense.

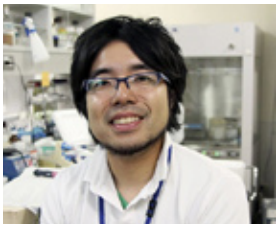


Photo 9: Masashi Ebisawa

Photo 10: Daisuke Takahashi

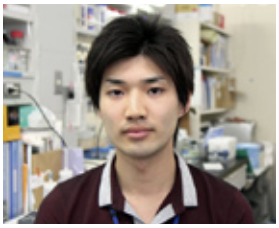


Photo 11: Yuhki Obata

Photo 12: Hideaki Shima

Photo 13: Takagi Hideaki

Photo 14: Tomohiro Fukaya

Photo 15: Izumi Sasaki

Excellent Paper of the Year 2010

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

In 2010, 10 excellent papers were selected from 13 candidates for this award.

Shinji Fukuda and Hiroshi Ohno

Bifidobacteria can protect from enteropathogenic infection through production of acetate
Nature, Vol. 469, 543-547, 2011

Noriko Yumoto, Takeshi Nagashima and Mariko Okada-Hatakeyama

Ligand-Specific c-Fos Expression Emerges from the Spatiotemporal Control of ErbB Network Dynamics
Cell, Vol. 141, 884-896, 2010

Tomokatsu Ikawa and Hiroshi Kawamoto

An essential developmental checkpoint for production of the T cell lineage
Science, Vol. 329, 93-96, 2010

Keiichiro Suzuki and Sidonia Fagarasan

The Sensing of Environmental Stimuli by Follicular Dendritic Cells Promotes Immunoglobulin A Generation in the Gut
Immunity, Vol. 33, 71-83, 2010

Shinya Tanaka, Yasutaka Motomura and Masato Kubo

The enhancer HS2 critically regulates GATA-3-mediated *Il4* transcription in T_H2 cells.
Nature Immunology, Vol. 12, 77-85, 2011

Tadashi Yokosuka and Takashi Saito

Spatiotemporal Basis of CTLA-4 Costimulatory Molecule-Mediated Negative Regulation of T Cell Activation
Immunity, Vol. 33, 326-339, 2010

Kenichi Asano and Masato Tanaka

CD169-positive Macrophages Dominate Anti-tumor Immunity by Crosspresenting Dead Cell-associated Antigens
Immunity, Vol. 34, 85-95, 2011

Yuichi Aiba and Tomohiro Kurosaki

Preferential localization of IgG memory B cells adjacent to contracted germinal centers
Proc Natl Acad Sci USA., Vol.107, 12192-12197, 2010

Hiroshi Watarai, Daisuke Yamada, Shin-ichiro Fujii, Haruhiko Koseki and Masaru Taniguchi

Murine induced pluripotent stem cells can be derived from and differentiate into natural killer T cells
J Clin Invest., Vol.120, 2610-2618, 2010

Tomoharu Yasuda, Kohei Kometani and Tomohiro Kurosaki

Erk kinases control plasma cell differentiation by regulating expression of Blimp-1
Science Signaling, Vol. 4, p. ra25

Tomohiro Fukaya, Hideaki Takagi and Katsuaki Sato

Crucial roles of B7-H1 and B7-DC expressed on mesenteric lymph node dendritic cells in the generation of antigen-specific $CD4^+Foxp3^+$regulatory T cells in the establishment of oral tolerance
Blood, Vol. 116, 2266-2276, 2010

Excellent Poster Award 2010

On Nov. 29th, RCAI held two poster presentation sessions during its retreat meeting in Chiba. Among 104 posters presented, nine posters were selected for “Excellent Poster Award 2010” by vote of laboratory heads.

Awardees of Excellent Poster Award 2010 :

Koji Hase
Lab. for Epithelial Immunobiology

Masahiro Kitano
Research Unit for Immunodynamics

Yu-ichi Fujimura
Lab. for Developmental Genetics

Satoru Yamasaki
Lab. for Cytokine Signaling

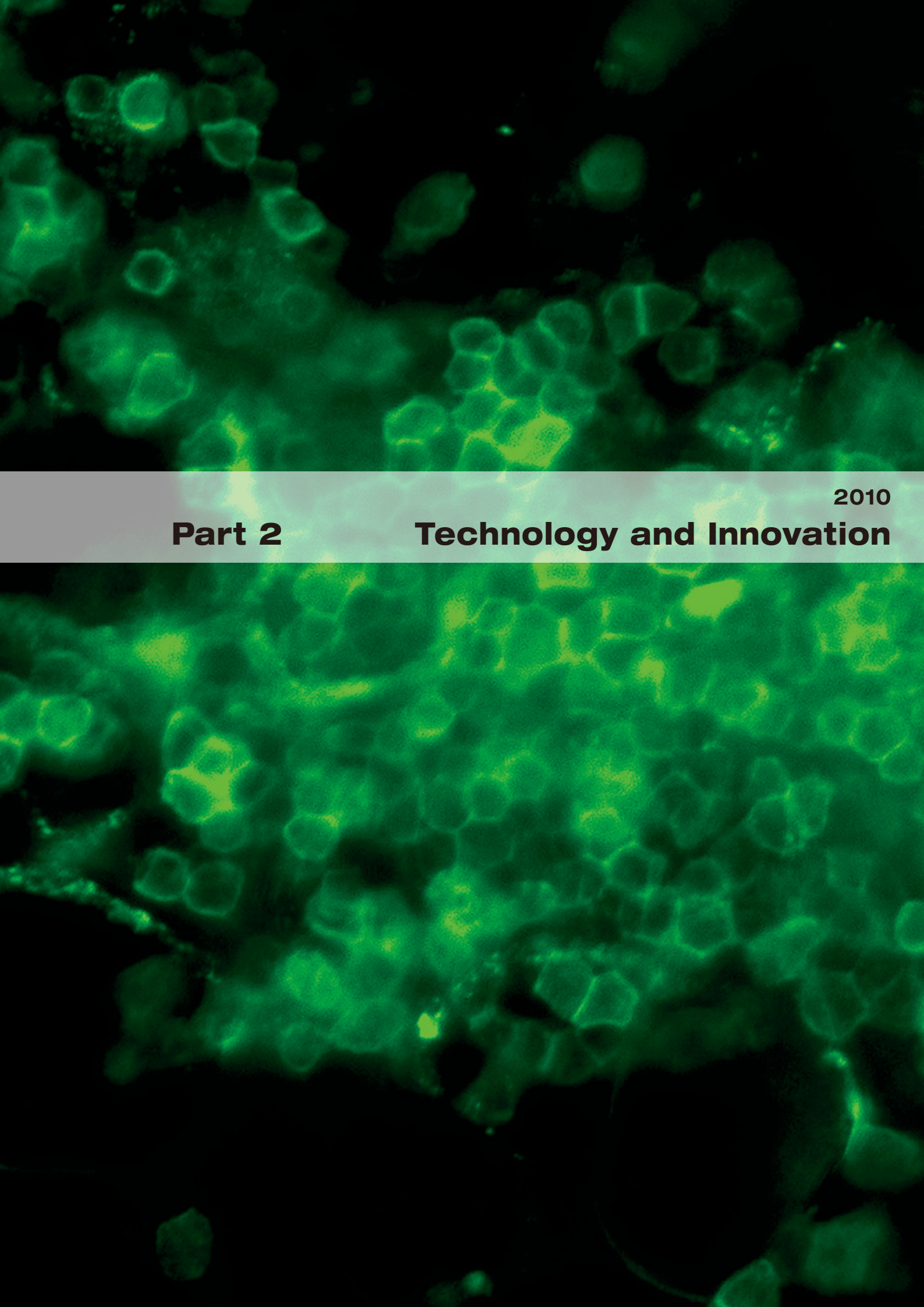
Akiko Hashimoto-Tane
Lab. for Cell Signaling

Masashi Tachibana
Research Unit for Immune Homeostasis

Hideaki Takagi
Lab. for Dendritic Cell Immunobiology

Yu Kato
Lab. for Immunochaperones

Shinji Fukuda
Lab. for Epithelial Immunobiology



2010

Part 2 Technology and Innovation

RCAI and Torii Collaborate for the Allergy Vaccine

Japanese cedar (*Cryptomeria japonica*) pollinosis is a common allergy in Japan, with a prevalence estimated to be 26% in a nationwide survey conducted in 2008. Impaired performance due to pollinosis and medications used for treating pollinosis is thought to be an important cause of the loss of concentration and productivity of patients in the workplace. Antigen-specific immunotherapy (SIT) is considered to be the only curative therapy for allergy; however, SIT has not been a common treatment in Japan due to the risk of side effects and poor clinical outcomes. Cry j 1, a major allergen of Japanese cedar pollen, is one of the candidates for a pollinosis vaccine. Vaccines using allergoids and modified Cry j 1, for example, T cell epitopes, pathogen-related molecular pattern molecule-conjugated allergen, and others, have been developed and used for pre-clinical trials; however, none of them has been commercially available for medical use either due to poor clinical outcomes of late stage clinical trials or the failure to find a cooperative pharmaceutical company to introduce the vaccines into the market. Development of a safe and more effective vaccine is eagerly anticipated by both physicians and patients with Japanese cedar pollinosis. There is a social need to develop a recombinant SIT vaccine with high safety, tolerability and efficacy, and to introduce the vaccine into the Japanese market.

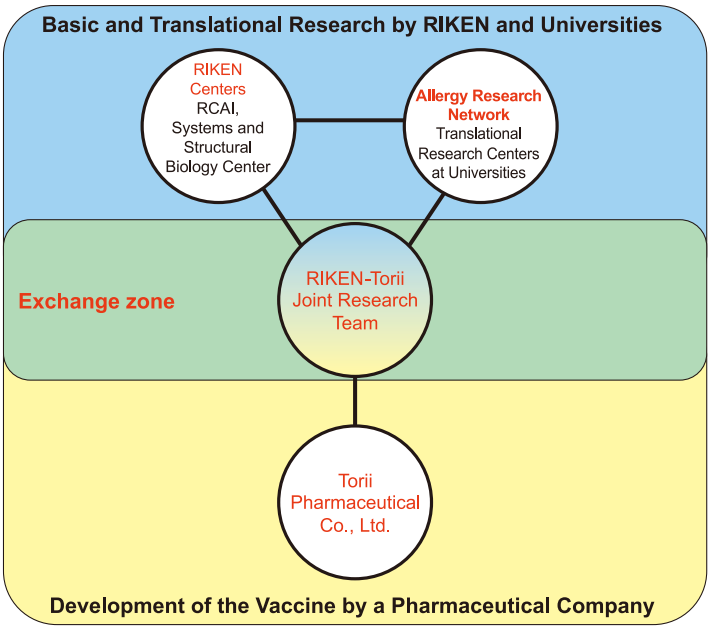


Figure : Overview of the exchange zone to introduce the vaccine into the market.

Establishment of the Joint Research Team

To fill the critical gap between basic research and later stage drug development, RCAI offered a program called the 'exchange zone' where RCAI, universities, hospitals, and pharmaceutical companies work together for drug development, including the allergy vaccine for Japanese cedar pollinosis. The exchange zone is based on the concept that sprinters in a relay race run together in the exchange zone to give/receive the baton firmly and smoothly. The idea here is that RCAI creates the baton (drug candidate) and passes it firmly to the next runner, namely a pharmaceutical company to develop the drug for the market. Based on this program, in December 2009 RIKEN and Torii pharmaceutical Co., Ltd. agreed to collaborate for the development of a vaccine for Japanese cedar pollinosis (Fig.).

Torii and RIKEN set up a joint research laboratory in RCAI in May 2010 and began research and development of the recombinant vaccine considering GLP and GMP guidelines. The vaccine was originally developed at RCAI as safer SIT vaccine, and is less likely to induce dangerous adverse events after injection. For the vaccine, recombinant technology is used to conjugate two major allergens from Japanese cedar pollen, namely Cry j 1 and Cry j 2. The fusion protein is then further modified with polyethylene glycol to prevent binding with immunoglobulin E (IgE). Prophylactic and therapeutic treatment with the vaccine was shown to prevent the increase of serum Cry j 1-specific IgE following challenge of mice with Cry j 1. In order to introduce the vaccine into the market, it is necessary to test the therapeutic efficacy of the vaccine using mouse models of allergic rhinitis and conjunctivitis and to perform research on its mechanisms of action. It is also necessary to develop a simple method for the purification of the recombinant vaccine expressed in prokaryote or eukaryote systems, based on considerations of cost-effectiveness and purity.

Development of a mouse model of Japanese cedar pollinosis

To evaluate the safety and therapeutic effects of the recombinant vaccine, the team is developing a mouse model of allergic rhinitis and conjunctivitis by Cry j 1 sensitization. Their aim is to establish a model where there is high Cry j 1-specific IgE in serum as the primary endpoint and sneezing, conjunctivitis, itching of the nose and/or eyes, or infiltration of inflammatory cells as secondary endpoints. Therefore, they injected Cry j 1 adsorbed with aluminum hydroxide gel

(Alum) into mice intraperitoneally. After this primary sensitization, they challenged the mice with Cry j 1 adsorbed with Alum or Cry j 1 alone intraperitoneally or subcutaneously. The serum Cry j 1-specfic IgE and IgG titers were increased in all cases, and the titer from the mice injected with Cry j 1/Alum intraperitoneally was highest compared to the other groups; however, the individual titers varied widely even within the same treatment group.

To establish a model of allergic rhinitis and conjunctivitis, they first sensitized the mice with Cry j 1 as above, and then challenged them with Cry j 1 via the nose and eye once every day for seven consecutive days. Conjunctivitis and sneezing were not evident in any of the treatment groups; however, after intranasal and ocular challenge with Cry j 1, the mice in the Cry j 1 subcutaneously sensitized group were observed to be scratching their noses. Inflammatory cells such as eosinophils and mast cells infiltrated the nasal mucosa after intranasal challenge with Cry j 1. They will test the therapeutic activity of the vaccine using this mouse model with allergic inflammation.

Members of RIKEN-Torii Joint Research Team

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

Recent publications

1. 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)

2. 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)

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

Open Laboratory for Allergy Research

RCAI has launched an open laboratory for allergy research where researchers from external institutes, universities or hospitals can have access to the Center's resources and conduct collaborative allergy research projects. Taeko Dohi (National Center for Global Health and Medicine) newly joined the open laboratory program in 2010. Four projects are on-going under this program.

1. Masato Kubo (Research Institute for Biological Science, Tokyo University of Science)
"Understanding the principles of IgE production"

2. Noriko Tsuji (Biomedical Research Institute, Advanced Industrial Science and Technology)
"Elucidating mechanisms of oral tolerance"

3. Kenji Matsumoto (National Research Institute for Child Health and Development) and Naoki Shimojo (Chiba University)
"Cohort study of allergy in infants and the generation of a humanized mouse model"

4. Taeko Dohi (National Center for Global Health and Medicine)
"Intestinal flora and epigenomic imprinting"

iPS Technology Development for Anti-cancer Immune Therapy

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

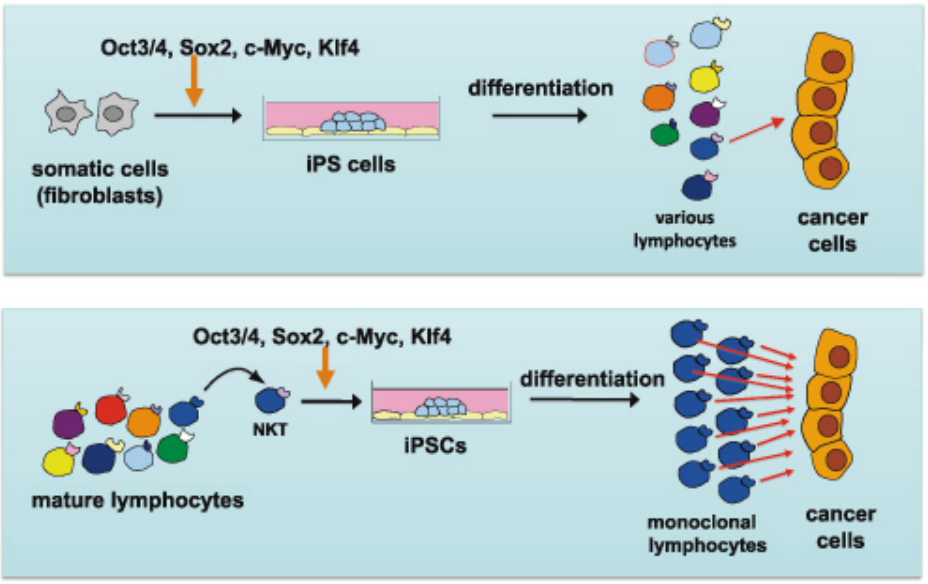


Figure : **Strategy for generating a large number of NKT cells from iPS cells *in vitro* for use in anti-cancer therapy.**

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

Reprogramming of mature lymphocytes to generate iPS cells is a less efficient process compared to that of fibroblasts. The iPS group hypothesized that certain genes in lymphocytes are difficult to fully reprogram. To test this, they established 20 and 5 independent iPSC clones from mature B- and NKT cells, respectively. They have analyzed the gene expression profiles of 6 B cell-derived and 2 NKT cell-derived iPS cell lines and compared them with those of ESCs. They identified a group of genes in lymphocytes that reproducibly failed to be reprogrammed.

Reprogramming of natural killer T (NKT) cells to induced pluripotent stem cells and their development into functional NKT cells *in vitro.* (Watarai et al., 2010)

Although iPS cells hold tremendous potential in cell-replacement therapy, the efficacy of cells with desired functions differentiated *in vitro* from iPSCs has not been rigorously assessed. The iPS group established iPSCs derived from natural killer T (NKT) cell cloned mice (NKT mice), which harbor functional NKT cell-specific rearranged T cell receptor loci in their germline. These iPSCs could be efficiently differentiated into NKT cells *in vitro*, moreover, upon adoptive transfer into NKT cell-deficient mice, these iPSC-derived NKT cells recapitulated the known adjuvant effects of natural NKT cells and suppressed tumor growth *in vivo*. This study showed for the first time that fully functional immune cells can be generated from iPSCs and also demonstrates the feasibility of expanding functionally competent NKT cells via an iPSC phase in a mouse model. These findings point to the clinical potential of this approach for NKT cell-targeted adjuvant therapy in humans.

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

The aim is to induce hematopoietic stem/progenitor cells from human iPS cells. After culturing human iPS cells on OP9 or OP9/DLL1, $hCD45^{-}CD34^{+}$ cells appeared at day 7 and $hCD45^{+}CD34^{+}$ cells appeared at day 14. The iPS group found that the efficiency of inducing $CD34^{+}$ cells from iPSCs is not affected by Notch signaling. This year, they analyzed marrow repopulating capacity in NOD/SCID/IL2rγKO mice, *in vitro* colony forming capacity, and expression of transcription factors by the iPS-derived $CD34^{+}$ cells. These cells were able to generate granulocyte-monocyte and erythroid colonies in methylcellulose semi-solid agar cultures. Through transcriptome analysis, they found that NANOG, OCT3/4, and SOX2 are nicely down-regulated and several HOX genes are up-regulated in the iPS-derived $CD34^{+}$ cells. They are currently trying to transduce HSC-associated signature genes into iPS cells for generating self-renewing human HSCs.

Functional difference between ES and iPS cells revealed by B cell induction *in vitro* (Kawamoto et al., in progress)

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

Members of iPS Group

Leaders	: **Haruhiko Koseki, Hiroshi Kawamoto, Fumihiko Ishikawa, Osamu Ohara**
Senior Research Scientist	: **Hiroshi Watarai**
Research Scientists	: **Daisuke Yamada, Kyoko Masuda**
Technical Staff	: **Momoko Okoshi-Sato, Genta Kitahara, Masako Fujita, Chieko Tezuka, Sakura Sakata, Yuko Nagata, Mariko Tomizawa, Yuri Suzuki**

Recent publications

1. Watarai H, Rybouchkin A, Hongo N, Nagata Y, Sakata S, Sekine E, Dashtsoodol N, Tashiro T, Fujii SI, Shimizu K, Mori K, Masuda K, Kawamoto H, Koseki H, and Taniguchi M. Generation of functional NKT cells in vitro from embryonic stem cells bearing rearranged invariant Vα14-Jα18 TCRα gene. ***Blood*** 115:230-7 (2010)

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

Development of Immune Cell Therapy

In collaboration with Chiba University (Prof. Toshinori Nakayama, Prof. Takehiko Fujisawa and Associate Prof. Shin-ichiro Motohashi), RCAI has been developing a Phase I/IIa clinical study of the application of NKT cell-targeted therapy for advanced lung cancer (stage IIIB, IV or recurrence). α-GalCer-pulsed APCs (1x109/m^2 PBMC-derived DCs) were intravenously administered four times. In this study, the MST (median survival time) of the responder group, which was defined by an increased number of IFN-γ producing NKT cells compared to the non-responder group, was longer than the non-responder group (31.9 months versus 9.7 months). Thus, the increased IFN-γ production by NKT cells upon α-GalCer stimulation showed a significant association with clinical outcome. These phase I/IIa trial results are encouraging and warrant further evaluation of the survival benefit of this immunotherapy.

For the next step, RCAI applied in 2011 for the advanced medical care assessment system established in 2008 by the Japanese Ministry of Health, Labor and Welfare (MHLW), by which the patients' immunotherapy treatment will be covered in part by health insurance. It usually takes approximately 10-12 months to obtain approval by the MHLW review board to use this system. The system was established because advanced medical technologies using medical devices or pharmaceuticals, such as immune cell therapy, that are yet to be 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.

Network for Primary Immunodeficiency Diseases

RIKEN RCAI is establishing a strong presence in the research community of Primary Immunodeficiency Diseases (PID) in Japan and the surrounding region. In keeping with its mission of improving immune health, RIKEN RCAI has been tightening and expanding a collaboration network with the clinical study group for PID from 13 universities and the Kazusa DNA Research Institute (Chiba) to serve as a model demonstrating synergy between basic and clinical research. At the beginning, the approach taken by RCAI was thought to potentially have two main risks: The first was that clinicians would not want to be involved because they could not foresee any long-term benefits for their PID patients; the second risk was that the basic immunologists at RCAI might be too far removed from the PID clinics to participate in such studies of human immunology because they have a tendency to focus on mouse models. It is now evident that RCAI has avoided both risks by properly managing this project. In fact, the number of entries deposited in a PID clinical archive established by RCAI, named PID Japan (PIDJ), has continuously increased and now exceeds 1000 (Fig. 1). The first risk thus proved unfounded, probably because many clinicians, at least in the field of PID research, have been seeking such an opportunity to closely work with genome scientists and basic immunologists. As for the second risk, basic immunologists at RCAI are in general very keen to collaborate with clinicians. This is because they have a strong motivation to demonstrate the relevance of their mouse models to human diseases; a recent RIKEN focus is on “Creation of Social Wisdom” to solve the critical issues that confront humanity today. For example, we have started to examine the functionality of newly characterized immune genes that were first found to be important in mouse models in PID patients. In particular, the RCAI “humanized mouse” project and the immune iPS cell project will offer key technologies to fill the gap between studies of human and mouse immune systems. Such a course of collaboration, extending from basic immunologists to clinicians, has just begun and is expected to bear fruit in the near future on the basis of the PID network.

Initially, there was a serious concern about how to unify a network in which clinicians, genome scientists, and basic immunologists are separated not only by geography but also by disciplines. However, a strong unifying force for the PID network was developed by providing clinicians and basic immunologists with genomic analysis data performed by RCAI and the Kazusa DNA Research Institute. The capacity to share clinical, immunological, and genetic data played a pivotal role in tightening the network. On the other hand, although the number of PID genes is continuously increasing and now exceeds 200, many PID patients still

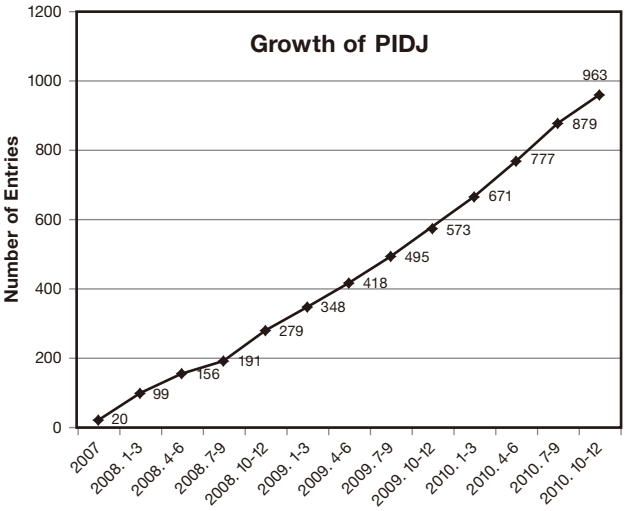


Figure 1: Growth of PIDJ

remain genetically undiagnosed because they do not have mutations in any of the known PID genes. To address this problem, we have tackled common variable immunodeficiency, one of the most common but complex PIDs, through integrating efforts by clinical and basic immunologists and genome scientists. Recent advances in genomics (e.g., next-generation DNA sequencing) have drastically changed the strategy for exploration of genetic causes of human diseases and RCAI is one of the major workhorses for introducing these new technologies into the PID research community.

Another key component to enhance the network activity originates from exploiting the power of the internet and bioinformatics. We have already established PID-related informational platforms such as PIDJ (a secured clinical archive, http://pidj.rcai.riken.jp/), RAPID (an integrated bioinformatics platform for PID, http://rapid.rcai.riken.jp), and Mutation@A Glance (a mutation annotation server, http://rapid.rcai.riken.jp/mutation/). The latter two platforms were developed through a collaboration with the Institute of Bioinformatics in India. Notably, the function of Mutation@A Glance has now been enhanced to cover all known human disease genes. Furthermore, because RIKEN has a wealth of information potentially useful for analyses of human diseases, we have begun to integrate all these lines of information in RIKEN to tackle human diseases. Towards this final goal, we have worked together with the RIKEN Bioinformatics and Systems Engineering division to fully

Figure 2: Poster on the signs of primary immunodeficiency diseases

utilize SciNetS (Scientist's Networking System) in PID. The PID network, including interdisciplinary researchers such as these, is steadily growing.

As an activity of the RIKEN Jeffrey Modell Diagnostic and Research Center for PID, which was launched in 2008, a Japanese-version of the poster "10 Warning Signs of Primary Immunodeficiency" (Jeffrey Modell Foundation) was prepared and distributed to the clinical community (Fig. 2). In addition, we have continued our efforts in networking the PID community in Asia. For this purpose, the 3rd PID meeting was held as a session of the 7th Pediatric Academic Societies and Asian Society for Pediatric Research Joint Meeting in Denver, CO, USA on May 1, 2011.

Development of a Humanized Mouse Model

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

By creating humanized mice carrying the human leukocyte antigen (HLA) through collaboration with the Jackson Laboratory, they showed that the expression of human genes is essential to fully recapitulate human lymphopoiesis in immunodeficient NOD/SCID/IL2rγKO mice. In the humanized mice they created (HLA class I A02 transgenic NOD/SCID/IL2rγKO mice), human cytotoxic T lymphocytes ($CD8^+$ T cells) developed successfully and these $CD8^+$ T cells expressed effector molecules such as granzyme, in accordance with their phenotypic maturation from naïve to central memory and effector memory cells. These human $CD8^+$ T cells were functional in terms of cytokine production and cytotoxicity. They recognized Epstein-Barr virus (EBV)-infected cells and virus-associated peptides, and showed cytotoxicity in an HLA-restricted manner. Moreover, this antiviral cytotoxic T lymphocyte response was inhibited by the addition of anti-HLA class I antibody (Shultz et al., *Proc Natl Acad Sci U S A*, 2010). (Fig.)

The next generation humanized mice is expected to create various models of individual human diseases.

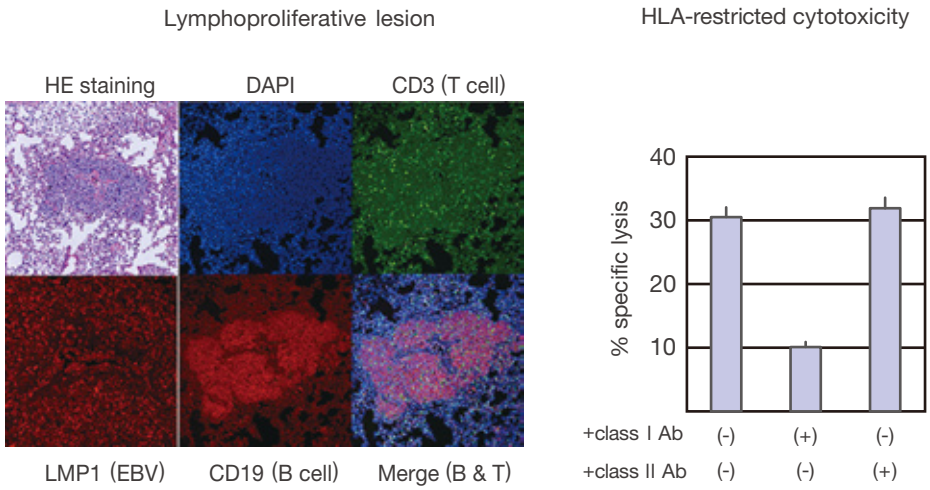


Figure :
At 5-6 weeks post-EBV infection, formation of lympho-proliferative disease was identified in the lung of HLA class I Tg humanized mice (*left*). Human $CD8^+$ T cells developed in the HLA class I expressing humanized mice exhibited HLA-restricted cytotoxicity against EBV-infected cells (*right*).

Drug Discovery Antibody Platform Unit is Established in RCAI

In order to conduct research and development of novel drugs and medical technologies, RIKEN established the Drug and Medical Technology Basic Program (DMP) in December 2009. Since then, DMP has selected drug discovery themes and projects proposed by scientists at RIKEN research centers. According to DMP guidelines, the selected projects focus on addressing intractable diseases.

To achieve effective progress in each of the projects, DMP needs to increase technological excellence and skilled personnel at each research center. Hence, DMP required various technological platform units to manage the program through close cooperation with the Drug Discovery Basic Unit established at each research center. In this context, a Drug Discovery Antibody Platform Unit (APU) was established in RCAI (Unit leader: Toshitada Takemori), in order to develop monoclonal antibodies that can be used as therapeutic drugs for treatment of cancer and other diseases. The APU is a collaboration with the Crystallographic Drug Discovery Platform Unit, which possesses powerful technology for preparing recombinant transmembrane type antigens, for example signaling receptors expressed by tumor cells, for use both as immunogens and screening reagents.

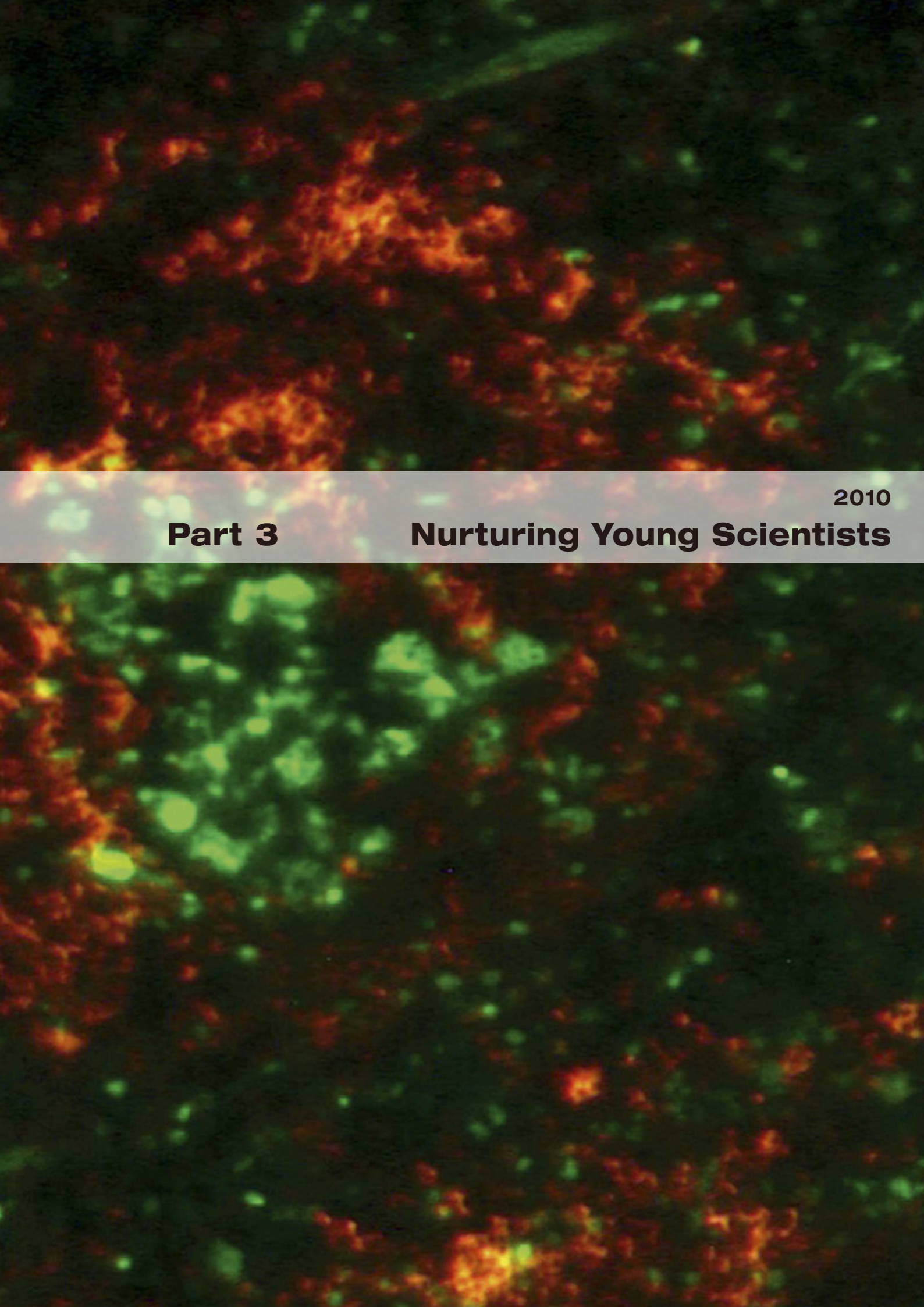
In collaboration with theme leaders, the APU prepares monoclonal antibodies by hybridoma technology and selects antibodies with the desired properties. The APU has attempted to establish a novel approach for preparing high-affinity monoclonal antibodies by use of artificial lymph node technology which was created at RCAI. RCAI has also developed humanized mice, in which the human hematopoietic system is reconstituted into immunocompromised mice. A future plan of the APU is to develop this system to create completely human monoclonal antibodies in mice. The activity of this Unit is supported by RCAI Leaders, Takashi Saito, Hiroshi Kawamoto, and Fumihiko Ishikawa, who are experts in monoclonal antibody production, artificial lymph nodes, and humanized mice, respectively.

RCAI Innovation Projects in Drug Discovery and Medicine

The government changed its science policy recently from “Science and Technology” to “Science, Technology and Innovation”, and RIKEN launched “RIKEN Research Cluster for Innovation” in April, 2010. In this cluster, “platform for drug discovery and medicine” was newly established. Considering this direction, RCAI decided to support limited number of projects with the aim of innovation in drug discovery and medicine.

RCAI launched Innovation Projects in Drug Discovery and Medicine and selected three projects from five applications.

1. Yasuyuki Ishii
   “Monoclonal antibody specific to malignant lymphoma that induces apoptosis ”
2. Shin-ichiro Fujii
   “Development of artificial adjunvant vector cells”
3. Takashi Tanaka
   “Development of a novel treatment for wounds using siRNA against PDLIM2”



2010

Part 3 Nurturing Young Scientists

RCAI International Summer Program August 17-27, 2010

The RCAI International Summer Program (RISP) was held on August 17-27, 2010. This was the fifth annual RISP and was co-sponsored by the Global Center of Excellence at Chiba University. There were 141 applicants to the program and forty-four graduate students and postdoctoral fellows from ten countries were accepted to participate in RISP 2010. The first part of the program was held in Yokohama at RCAI. There were morning oral presentations by the students, and their posters were available for viewing and discussion throughout the week. The afternoon sessions featured lectures by invited experts from RCAI, Japanese universities, and abroad. There was also a presentation by Kevin Da Silva, an editor for *Nature Medicine*, who described the journal's submission and review process. This year's RISP was unique – for the second part of the program the participants traveled to Kobe where they attended the 14th International Congress of Immunology. A prerequisite for their acceptance into RISP was that they present their research in poster or oral format at this meeting. After the Kobe meeting, three of the RISP participants returned to Yokohama for a month-long laboratory internship at RCAI in the laboratories of Takashi Saito, Hiroshi Ohno and Masato Tanaka.

The RISP lectures at RCAI were very diverse. Topics included lymphocyte development differentiation and physiology, as well as the function of microRNAs in these processes (Qi-jing Li, photo1), dendritic cells, epigenetic regulation of gene expression, signaling, tolerance, mucosal immunity, and the innate immune system. Because of their breadth, these lectures provided an expansive overview of the immune system, with approaches ranging from single molecule 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 as well as in more informal settings. The question periods following talks by the invited lecturers and participants were spirited and stimulating. Awards for best RISP posters were presented at a reception held at RCAI the evening before the students departed for Kobe on August 21st. All of the participants expressed their appreciation to the organizers for inviting them to RISP, and many of them established collaborations as a result of the RISP that are likely to be long lasting.

The RISP 2010 students had the opportunity to explore both the Yokohama/Tokyo and Kobe areas. With a small meeting atmosphere at the RCAI venue and a large international meeting flavor at Kobe, this provided an exceptional experience from both scientific and cultural perspectives. In a survey completed after the RISP, 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 period of collaborative research. The success of this unique program was due to the efforts of the Organizing Committee, chaired by Hiroshi Ohno (photo 2) and Tomohiro Kurosaki, and the RISP Secretariat, Mari Kurosaki, Yuko Ochi (photo 3 left), and Sachiko Dohi (photo 3 center), who kept the entire operation running smoothly, as well as to the efforts of the outstanding participants.



Table 1 : Lectures

Lecturer	Title
Paul W. Kincade, Oklahoma Medical Research Foundation, USA	Producing immune effector cells from diverse stem and progenitor cells
Qi-Jing Li, Duke University Medical Center, USA	MicroRNA and its micromanagement during T cell development and antigen responses
Toshinori Nakayama, Chiba University Graduate School of Medicine, JAPAN	Epigenetic regulation of GATA3 expression in Th2 cells
Shigeo Koyasu, Keio University School of Medicine, JAPAN	Natural helper cell: a newly identified Th2 type innate lymphocyte
Balbino Alarcón, Consejo Superior de Investigaciones Científicas, SPAIN	Multiple roles of RRas GTPases in the immune system
Fumihiko Ishikawa, RIKEN RCAI, JAPAN	Investigating normal & diseased human immune systems using the humanized mouse
Masaru Taniguchi, RIKEN RCAI, JAPAN	Discovery of NKT cells with adjuvant activity and their clinical application
Hiroshi Ohno, RIKEN RCAI, JAPAN	Interaction of host, commensal microbiota, and pathogens
Diane Mathis, Harvard Medical School, USA	Immunological tolerance, centrally
Shohei Hori, RIKEN RCAI, JAPAN	Dominant immunological tolerance
Ifor R. Williams, Emory University School of Medicine, USA	The genesis of antigen-sampling intestinal M cells: elucidating the relative contributions of RANKL, the enteric microflora, and B lymphocytes
Klaus Rajewsky, Harvard Medical School, USA	B cell physiology, Epstein-Barr-Virus and immune surveillance of EBV-infected and -transformed B cells
Tsuneyasu Kaisho, RIKEN RCAI, JAPAN	How dendritic cells sense and respond to nucleic acid adjuvants
Ruslan Medzhitov, Yale University School of Medicine, USA	Innate immune system
Kevin Da Silva, Nature Medicine, USA	Publishing in *Nature Medicine*

Table 2 : Participants

Name (country)			
Cindy Banh (USA)	Joseph Barbi (USA)	Tyani Chan (Australia)	Pheh Ping Chang (Malaysia)
Jean-Enno Charton (Germany)	Luisa Cimmino (Australia)	Nicholas Clarkson (UK)	Jonathan Clingan (USA)
Sumit Deswal (India)	David DiLillo (USA)	Georg Gasteiger (Germany)	Eliver Ghosn (Brazil)
Stephanie Grabow (Germany)	Stephan Halle (Germany)	Robert Johnston (USA)	Steven Josefowicz (Canada/ USA)
Justin Killebrew (USA)	Byung-Seok Kim (South Korea)	Kathryn Knoop (USA)	Dmitriy Kolodin (Russia)
Ryan Larson (USA)	Monica Leung (Canada)	Yinming Liang (China)	Jing Shan Lim (Singapore)
Xi Liu (China)	Lisa Ma (China)	Mona Mashayekhi (Iran)	Kendle Maslowski (Australia)
Frederick Masson (France)	Melba Munoz-Roldan (Colombia)	Hae-Young Park (South Korea)	Carolina Prado (Chile)
Priyanka Sathe (Australia)	Olga Schulz (Germany)	Vera Schwierzeck (Germany)	Fernando Sepulveda (Chile)
Alice O. Kamphorst Silva (Brazil/ France)	Santi Suryani (Australia)	Anastasia Tikhonova (Russia)	Annick van de Ven (Netherlands)
Catharina Van Elssen (Netherlands)	Eduardo Villablanca (Chile)	Annie Xin (China)	John Yi (USA)



Harvard Summer School at RCAI June 14-August 27, 2010

RCAI offers a summer internship program for undergraduate students from Harvard University. This program consists of a research internship in the laboratory of an RCAI PI as well as courses in basic immunology and Japanese. The participants receive course credit from Harvard. Three students, Michael Wang, Liang Cheng and Chinwe Madubata, stayed at RCAI from June 14 to August 22, 2010.

Wang conducted his research project in the Research Unit for Human Disease Model, Cheng in the Laboratory for Immunological Memory, and Madubata in the Laboratory for Immune Regulation. During the internship, the students had numerous discussions with RCAI researchers and, at the end of the program, they gave oral presentations describing their projects. In addition to the scientific aspects of the program, there was a cultural exchange at the Science Frontier High School, where the Harvard students learned Shodo (Japanese caligraphy) and Sado (tea ceremony) from the local high school students. At the farewell party, they played beautiful classic ensembles and dedicated the music to Director Masaru Taniguchi.

Photo 1: **Immunology Lecture.** *From left* :Masaru Taniguchi (Director), Chinwe Madubata, Liang Cheng, Michael Wang, and Yoshiro Kawaoka (Guest Lecturer from The Univ. of Tokyo)

Photo 2: **Cultural Exchange at Science Frontier High School**

RCAI Retreat November 29-30, 2010

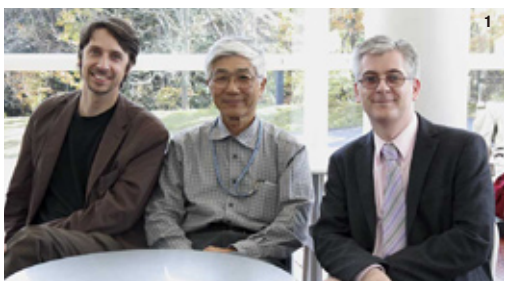
This year's RCAI Retreat was held on Nov. 29-30 at Kazusa Academia Park in Chiba prefecture. The aim of the RCAI Retreat is to provide the RCAI scientists with the opportunity for open communication and in depth discussion of ongoing research projects by gathering the members at a quiet place with few distractions. One hundred and fifty one RCAI members and two editors, John F. Foley of *Science Signaling* and Zoltan Fehervari of *Nature Immunology*, participated in the Retreat. (photo 1 right and left, respectively)

Two poster sessions were held on the first day. One-hundred four posters were presented, and nine posters were selected for awards by vote of the laboratory heads (See Excellent Posters on page 22). Between the sessions, eighty-seven participants took a short walk to the nearby Kazusa DNA Research Institute (KDRI), where Michio Ohishi (photo 2), Director of KDRI, greeted them at the entrance and conducted a tour. The participants were all impressed by the achievements and the state-of-the-art equipment in the institute.

The second day began with talks by the two Editors, which were followed by ten oral presentations by selected young researchers. Their stimulating presentations provoked active discussions.

The great success of this year's retreat was due to the dedicated efforts of the organizers, Ji-Yang Wang and Hisahiro Yoshida (photo 3 right and left, respectively), and the support of their lab members (Lab. for Immune Diversity and Lab. for Immunogenetics) and the secretariats (Mizuki Shimura, Norie Takeuchi and Hiroko Iwamoto). There were several changes from the past Retreats; the organizers decided to hold the meeting in autumn, searched for the best venue on a limited budget, offered a tour of KDRI, and discontinued the on-site duties of the secretariats. Based on responses to the post-retreat questionnaire, more than 80% of the participants had very good impressions and many hoped to join the meeting in Kazusa again.

Wang noted that it was very difficult to select the best posters, and that a common standard for the selection and the decision making process must be reconsidered. Active participation by all researchers is also essential for the success of Retreat. Therefore, this year's efforts for change brought great success and satisfaction, and even inspired further ideas for improvement of future Retreats.



RIKEN Joint Retreat January 31 - February 1, 2011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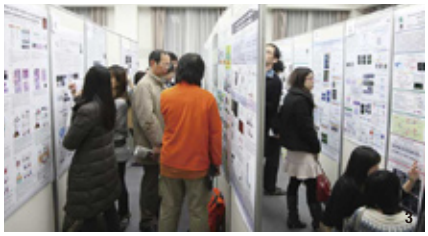
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A RIKEN 2011 Joint Retreat was held at YAMAHA Resort Tsumagoi in Kakegawa, Shizuoka prefecture on Jan. 31 and Feb. 1, 2011. This year's meeting was coordinated by RCAI, and 117 researchers gathered from 7 RIKEN centers: Advance Science Institute (ASI), BioResource Center (BRC), Brain Science Institute (BSI), Center for Developmental Biology (CDB), Omics Science Center (OSC), Research Center for Allergy and Immunology (RCAI) and Spring-8.

This meeting aims to stimulate synergy within RIKEN by gathering and thinking together about common biological topics in a relaxed atmosphere. In the opening remarks, Tomohiro Kurosaki (RCAI) (photo 1), the chief meeting organizer, said "The meeting will provide valuable opportunities to get to know the researchers from other fields and create synergistic collaboration."

The theme of this year's meeting was evolution. Although evolution has been a common theme in biology for a long time, there remain many questions. The recent spectacular advances in molecular sciences and the associated research techniques should provide keys to solve the remaining evolutionary questions, however, not many RIKEN labs really focus on them. During the sessions, participants revisited the evolutionary themes and thought together on the molecules and methods that are common in various biological fields.

The first speaker, Shigeru Kuratani (CDB) (photo 2) introduced his recent discoveries on the formation of the turtle shell (carapace), which is a specialized variation in the more familiar rib growth seen in other vertebrates. Hisaya Kakinuma (BSI) then talked about the evolution of the cerebellum in vertebrates. During development, the midbrain-hindbrain boundary (MHB) region acts as a signaling center for cerebellar development. Specific gene enhancers conserved in jawed vertebrates promote this development, but are absent in the jawless lampreys, which have a very small cerebellum. The third speaker, Shigeharu Moriya (ASI), discussed strategies to understand the evolution of the complex ecosystem. Although phylogenetic analysis has been used as the conventional approach to track the evolutionary trail, a large part of the biosphere is easily rearranged by lateral gene transfer, generation of organelles and /or symbiosis. He introduced a range of omics approaches to analyze the symbiotic protists of termites and then presented an integrated multi-omics analysis of marine microbial communities, which may help us to understand the effect of environmental changes. Hiroshi Ohno (RCAI) then introduced his recent meta-genomics research on gut bifidobacteria. They found that mice colonized by bifidobacterium subspecies *B. longum* survived when fed the pathogenic bacteria *E. coli* O157, and they were able to pinpoint acetate as the protective molecule. The last speaker was Akihiko Nakano (ASI). He discussed evolutionary aspects of post-Golgi and endocytic trafficking. He introduced unique plant molecules involved in membrane trafficking and indicated that land plants have evolved a unique and complex membrane trafficking mechanism.

That evening, 86 posters were presented, giving the participants the opportunity for informal discussions that should ultimately increase communication among the centers. (photo 3)

On the second day, six investigators from various centers introduced the most recent research highlights. In the closing remarks, Carina Hanashima (CDB) said, "this meeting is still in the process of evolution. The next meeting is planned two years from now, and more feedback signals are appreciated."

Thanks to the participants, the organizers, Tomohiro Kurosaki (RCAI), Masato Tanaka (RCAI) (photo 4 right), Satoshi Ishido (RCAI) (photo 4 left), Atsushi Mochizuki (ASI) (photo 5), Yoshihiro Yoshihara (BSI), Carina Hanashima (CDB) (photo 6) and the secretariat office, especially Kazunari Fukushima (Yokohama RIKEN), Yasuaki Murahashi (RCAI), Aiko Iyama (RCAI) and Rie Morita (RCAI), the meeting was constructive and a great success. "It was my first time to participate in this meeting, but it was a great opportunity to get to know researchers from other Centers," said a young postdoc from RCAI.


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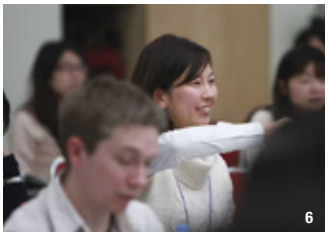
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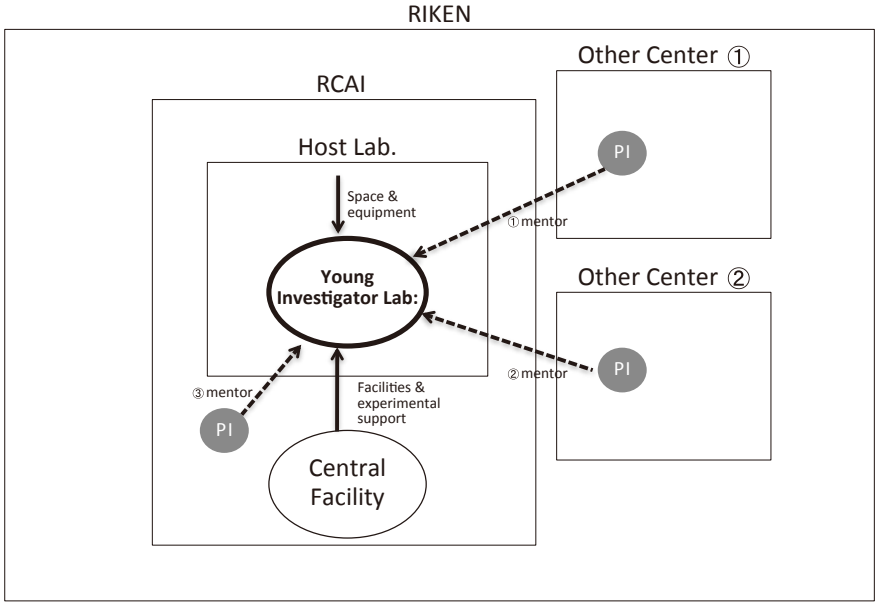
Young Chief Investigator Program

RCAI established a new program, Young Chief Investigator Program, to provide a career path for young investigators who conduct multidisciplinary research that will bridge immunology with other research fields. In this program, the selected Young Chief Investigator (age below 40) will head an independent research laboratory but will have structured 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 laboratory will also share space, equipment and facilities with a host laboratory in RCAI (Groups, Teams or Units). (Fig.)

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 to take a position at another institution or be promoted to another type of position within RCAI.

At the end of FY2010, three researchers were selected for the Young Chief Investigator Program, **Hayato Naka-Kaneda** (stem cells and aging reversal), **Shinji Nakaoka** (development of interface between integrative biology and mathematics), and **Koji Hase** (mucosal flora and epigenetic analysis).



*Other Centers : all the research centers in RIKEN including PSC, CGM, SSBC, OSC, BASE, CRNID, ASI, BSI, CDB, CMIS, BRC, Spring-8, XFEL, Nishina Center, Sendai Facility, Nagoya Facility, Center for Computational and Quantitative Life Science, etc.

Figure: Scheme of Young Chief Investigator Program

RIKEN Foreign Postdoctoral Researcher

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

In 2010, **Jafar Sharif** (Photo) studied in the Lab. for Developmental Genetics as a RIKEN FPR.

RIKEN Special Postdoctoral Researcher (SPDR) Program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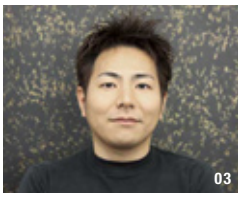
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RIKEN's program for Special Postdoctoral Researchers was instituted to provide young and creative scientists the opportunity to be involved in autonomous and independent research in line with RIKEN objectives and research fields. The positions are 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, eight postdocs conducted their research at RCAI through the SPDR program.

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

RIKEN Joint Graduate School Program International Program Associate


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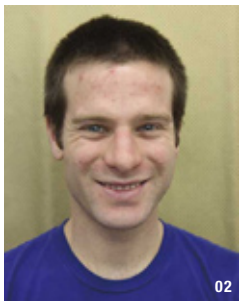
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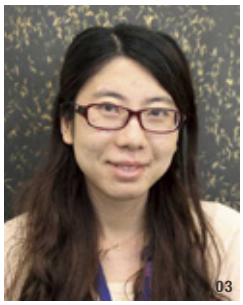
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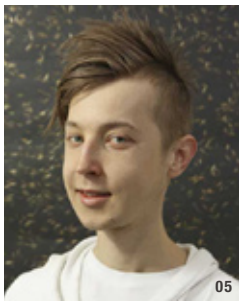
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RCAI accepted four international students as RIKEN International Program Associates (IPA). Under this IPA program, RCAI lab heads host students from collaborating graduate schools and supervise their Ph.D. program as Joint Supervisors. The students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

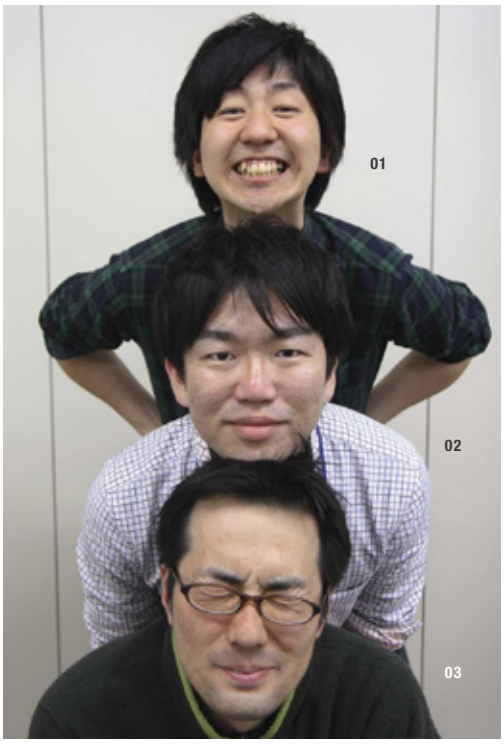
The IPA students studying at RCAI in 2010 were

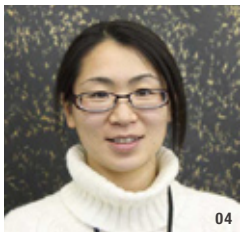
Yingqian Li (Nanjing University, China) studied in the Lab. for Immune Diversity (Photo 1)
Sebastian Nieke (Tubingen Univ, Germany) studied in the Lab. for Transcriptional Regulation (Photo 2)
Shuyin Li (China Agriculture University, China) studied in the Lab. for Immune Diversity (Photo 3)
Yue Ren (Jilin University, China) studied in the Lab. for Immune Regulation (Photo 4)
Felix Reinhard Helmut Jonas (The Univ. of Tokyo) from Germany studied in the Lab. for Cellular Systems Modeling (Photo 5)

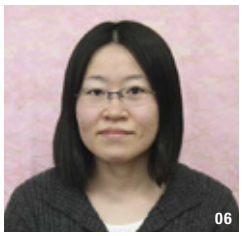
RIKEN Junior Research Associate (JRA) Program

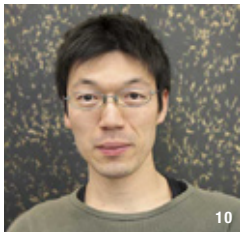
The Junior Research Associate program was launched in 1996 to encourage young scientists with fresh ideas and youthful enthusiasm to collaborate with, and learn from, veteran scientists with years of experience. This program provides part-time positions at RIKEN for young researchers enrolled in university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

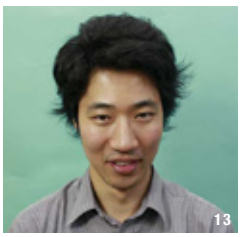
Minoru Sawaguchi (Lab. for Cell Signaling) Photo (01)
Hideaki Takagi (Lab. for Dendritic Cell Immunobiology) Photo (02)
Tomohiro Fukaya (Lab. for Dendritic Cell Immunobiology) Photo (03)
Machiko Sugiyama (Lab. for Immunogenetics) Photo (04)
Izumi Sasaki (Lab. for Host Defense) Photo (05)
Chihiro Yamazaki (Lab. for Host Defense) Photo (06)
Toshi Jinnohara-Yuyama (Lab. for Epithelial Immunobiology) Photo (07)
Shinsuke Takagi (Research Unit for Human Disease Model) Photo (08)
Takanori Sawaguchi (Lab. for Developmental Genetics) Photo (09)
Yuuhou Najima (Research Unit for Human Disease Model) Photo (10)
Masanaka Sugiyama (Lab. for Host Defense) Photo (11)
Yuki Aoki (Research Unit for Human Disease Model) Photo (12)
Beum-Ho Byn (Lab. for Cytokine Signaling) Photo (13)
Nanako Shimura (Lab. for Immunogenomics) Photo (14)
Kazunori Kadokura (Lab. for Epithelial Immunobiology) Photo (15)
Daisuke Takahashi (Lab. for Epithelial Immunobiology) Photo (16)
Saya Moriyama (Lab. for Lymphocyte Differentiation) Photo (17)

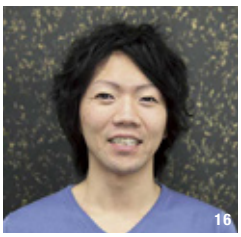








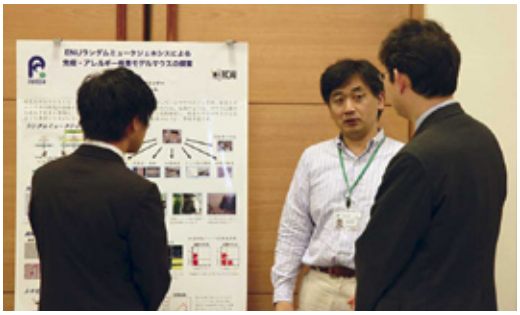




Adjunct Professorship Programs

RCAI accepts graduate students through the mechanism of adjunct professorships at various Japanese universities. RCAI collaborates with and accepts graduate students from nine domestic university graduate schools. The total number of adjunct professors/associate professors in RCAI is now 29 (Table). Fifty-eight students were studying at RCAI in 2010.

On May 23, RCAI held a briefing session on the Adjunct Graduate School Program (AGSP) for potential applicants. Eighteen participants, including 4 from companies, gathered from all over Japan - Kumamoto, Tokushima, Hyogo, Osaka, Toyama, Nagano, Aichi, Shizuoka, Iwate, Kanagawa and Tokyo. The session began with an introduction of RCAI and a summary of the AGSP by the organizer, Hisahiro Yoshida. Next, five RCAI PIs, Haruhiko Koseki, Hiroshi Ohno, Masato Kubo, Hei-ichiro Udono and Katsuaki Sato, briefly introduced their research programs. After the oral session, the individual laboratories held poster presentations and then each participant had the opportunity to visit the laboratory of his/her interests.



All sixteen participants indicated that they are thinking positively about entering the adjunct graduate school. One participant noted that it was very good to get acquainted with the atmosphere of RCAI and that he was impressed by the facilities, but he suggested that this session be held in different places in Japan because it was a long way to come.

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)
	Tsuneyasu Kaisho (Professor)
	Toshiyuki Fukada (Visiting Associate Professor)
Graduate School of Medicine, Chiba University	Takashi Saito (Visiting Professor)
	Haruhiko Koseki (Visiting Professor)
	Hiroshi Ohno (Visiting Professor)
	Shinichiro Fujii (Visiting Associate Professor)
	Yasuyuki Ishii (Visiting Associate Professor)
	Fumihiko Ishikawa (Visiting Associate Professor)
Graduate School of Biomedical Science, Tokyo Medical and Dental University	Takashi Saito (Visiting Professor)
	Tomohiro Kurosaki (Visiting Professor)
	Masato Kubo (Visiting Professor)
	Mariko Okada-Hatakeyama (Visiting Professor)
	Sidonia Fagarasan (Visiting Associate Professor)
International Graduate School of Arts and Sciences, Yokohama City University	Hiroshi Ohno (Visiting Professor)
	Tsuneyasu Kaisho (Visiting Professor)
	Satoshi Ishido (Visiting Associate Professor)
Research Institute of Biological Sciences, Tokyo University of Science	Masato Kubo (Professor)
	Osamu Ohara (Visiting Professor)
	Shohei Hori (Visiting Associate Professor)
	Tadashi Yokosuka (Visiting Associate Professor)
Graduate School of Frontier Sciences, The University of Tokyo	Mariko Okada-Hatakeyama (Visiting Associate Professor)
Graduate School of Medicine, Kyoto University	Fumihiko Ishikawa (Visiting Associate Professor)
Graduate School of Medicine, Kobe University	Masaru Taniguchi (Adjunct Lecturer)

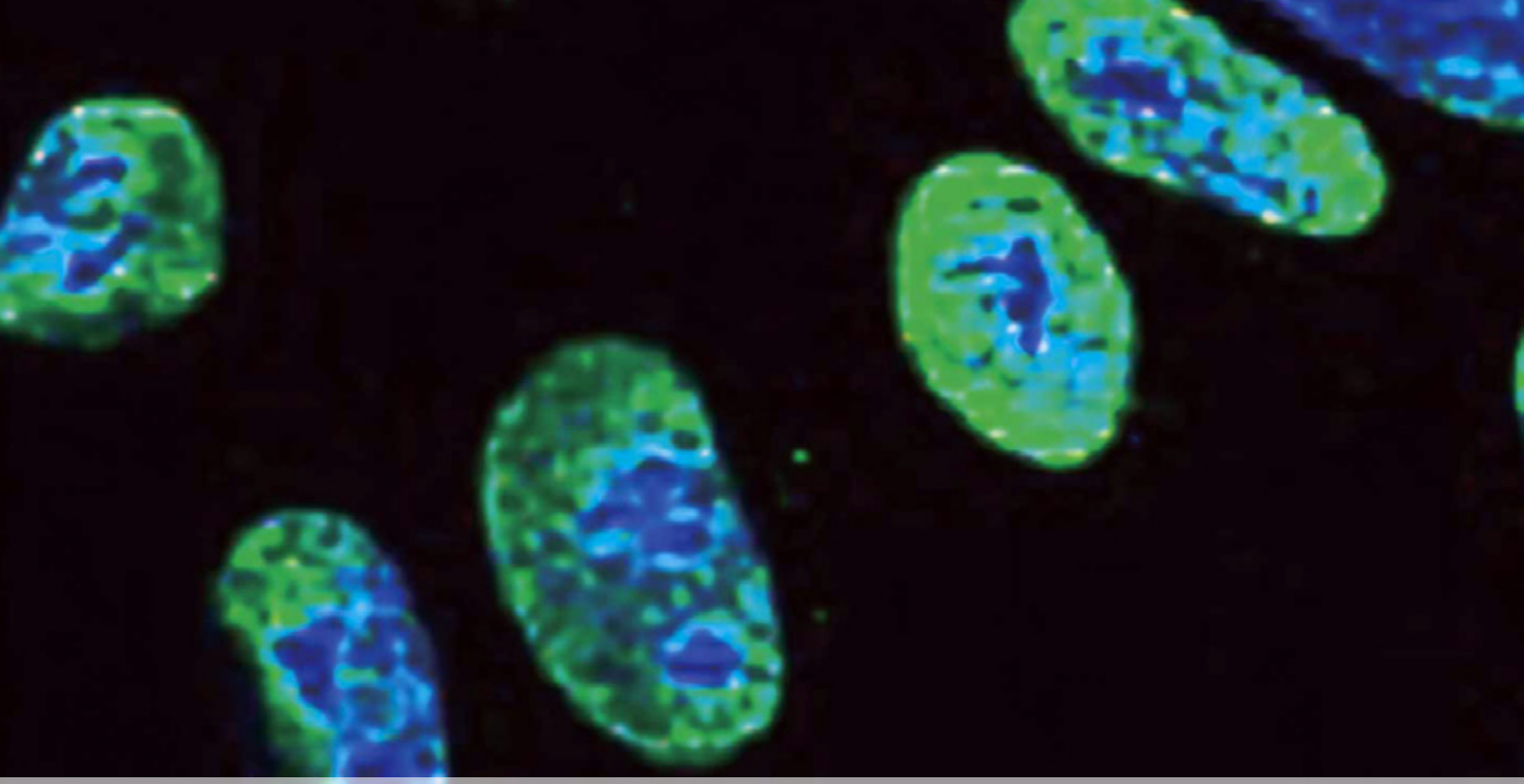
RCAI Administrative Coordination Office

Table : Members of AdmCO

Position	Name
Office Manager	Takashi Saito
Administrative Staff	
Chief Assistants	Hiroko Tanabe
	Hiroko Yamaguchi
	Mari Kurosaki
Assistants	Sachiko Dohi
	Sachiko Haraguchi
	Akiko Imai
	Akiko Imaizumi
	Hiroko Iwamoto
	Aiko Iyama
	Shihoko Kato
	Satomi Law
	Ryoko Moriizumi
	Rie Morita
	Toshiko Nakamura
	Kazuyo Nomura
	Yuko Ochi
	Mizuki Shimura
	Norie Takeuchi
	Yuuki Yamada
	Mio Yoshioka
IT Team	
Technical Scientists	Yasuaki Murahashi
	Miho Izawa
Technical Staff	Aoi Ozawa
Facilities Staff	
Technical Scientist	Toshihiko Ogata

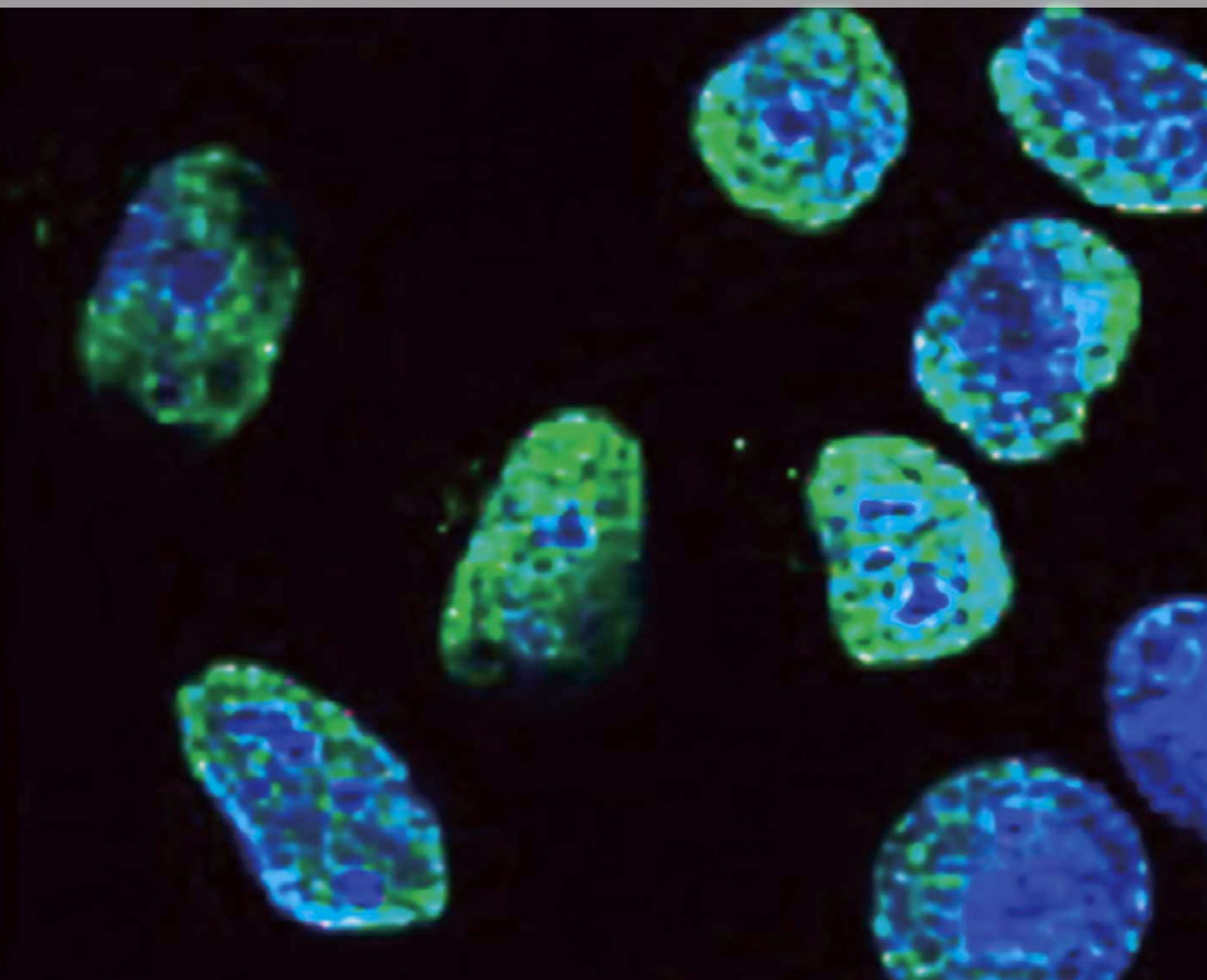
RCAI reorganized the administrative staff and established the Administrative Coordination Office (AdmCO) in April, 2010. The AdmCO, which coordinates and manages a wide range of tasks, consists of not only assistants, but also the IT team and facilities support staff. Assistants support the Center and its laboratories by providing secretarial assistance and serve as Secretariats of the Center's annual events, such as the International Summer Program, Harvard Summer School Program, Retreat, Joint Workshops, Graduate Students Tutorial Meeting, etc. The IT team designs and maintains the RCAI's unique server and databases, fixes PC troubles and supports scientific meetings and conferences both inside and outside RCAI. The facilities staff gives advice on RCAI facilities and arranges construction required in remodeling laboratories and installing large equipment, etc.

Owing to the tremendous efforts by Chief Assistants and AdmCO members, the reorganization has made great progress. A new data sharing system has been installed, which enables each member to share information, creating an environment where they can learn from each other. Using this centralized approach, the AdmCO is now able to provide smooth and efficient administrative support under the same standards to all RCAI laboratories. Among the AdmCO's contribution to the Center, the highlight of this year is the development of an RCAI Order 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. It has proven a useful system to prevent fraud and to manage various budgets simultaneously. The AdmCO, in cooperation with the YRPD, also manages and coordinates the issues between the laboratories and the YRPD. The strong spirit and constant efforts by the AdmCO are now supporting the fundamental base of the RCAI activities.



2010

Part 4 Collaborative Networks



INSERM/Pasteur-RIKEN Joint Workshop August 30-31, 2010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The second INSERM/Pasteur-RIKEN Joint Workshop "Frontier in Immunology – from basic to clinics" was held on August 30-31, 2010 at RCAI. The first of these workshops was held in 2008 at the La Ferme des Vallees (The Farm of the Valleys) in Auffargis, France. Participating scientists at the 2010 meeting were from RIKEN RCAI in Yokohama, the Pasteur Institute in Paris, and several INSERM-affiliated research institutes throughout France. The French scientists had been attending the 14th International Congress of Immunology in Kobe before traveling to Yokohama for this small focused workshop, which was organized by Takashi Saito (RIKEN, RCAI, photo 1), Marc Bonneville (INSERM, Nantes, photo 2), and Bernard Malissen (INSERM, Centre d'Immunologie de Marseille-Luminy (CIML), photo 3).

The goal of these joint workshops is to promote collaboration between Japanese and French researchers and also between investigators in France, all of whom had the opportunity for in depth discussions at RCAI. Many common interests became apparent in each of the different workshop sessions. The first session on "Hemopoiesis and lymphocyte development" featured talks on lymphoid progenitors and lineage commitment at both cellular and molecular levels (Cumano (photo 4), Kawamoto, Taniuchi), and included a spirited discussion on whether the "common" lymphoid progenitor, a cell restricted in its potential to the generation of B and T cells, actually exists. Next was a large session on "Lymphocyte activation and function". The first two presentations dealt with innate type lymphocytes, the NK cell (Vivier) and the γδ T cell (Bonneville). The next series of talks were on various aspects of conventional T cell activation by dendritic cells (Amigorena, Ishido) and the signaling events in the activated T cell (Saito, Malissen). In the last talk in this session, Kurosaki provided new insights into the physical location of memory B cells in spleen, adjacent to contracted germinal centers (GC), their birthplace. The final session of the day focused on regulation of allergic and inflammatory responses by intracellular signaling molecules (Kubo) and an intranuclear ubiquitin E3 ligase, PDLIM2 (T. Tanaka).

The second day of the workshop began with three talks highlighting new developments in the area of the mucosal immune system. IgA is the major mucosal antibody isotype, and Fagarasan (photo 5) described how the follicular dendritic cells in the GC of Peyer's patches are particularly well-equipped to promote IgA class switch-

ing. Eberl (photo 6) described unusual innate lymphoid cells that mirror all shades of conventional helper T cells and are highly enriched in the intestinal lamina propria, where they promote intestinal homeostasis. Ohno has been studying the mechanisms by which so-called probiotic bacteria, in this case *Bifidobacterium longum*, protect against the harmful effects of pathogenic strains.

The final session moved to the "clinics", as promised in the workshop title. Taniguchi (photo 7) described very promising clinical trials of NKT cell-targeted adjuvant therapy for human non-small cell lung cancer as well as efforts to generate NKT cells from iPS cells *in vitro* for potential clinical application. Chatenoud discussed translational studies using antibodies to CD3 to promote immune tolerance in type 1 diabetes, and Fischer described two diseases affecting lymphocyte homeostasis, including new forms of inherited hemophagocytic lymphohistio-cytosis and somatically acquired defects in Fas-mediated apoptosis that can cause autoimmune diseases in some cases. In a strategy combining human and animal models, Ishikawa described the use of "humanized" mice, which can be reconstituted with human immune cells, to study to pathogenesis and potential treatment of acute myeloid leukemia.

The second INSERM/Pasteur-RIKEN Joint Workshop ended with a renewed commitment to fostering collaboration between RCAI and the French institutes, as well as plans to hold a 3rd such workshop in the future.

Table: Program of INSERM/Pasteur-RIKEN Joint Workshop 2010

August 30

Session 1: Hemopoiesis and Lymphocyte development	
Ana Cumano (Pasteur)	Heterogeneity of the common lymphoid progenitors in fetal liver and bone marrow
Hiroshi Kawamoto (RIKEN RCAI)	Termination of myeloid potential in T progenitors: an essential developmental checkpoint for production of the T cell lineage
Ichiro Taniuchi (RIKEN RCAI)	Regulation of thymocyte developments by the silencers
Session 2: Lymphocyte activation and function	
Eric Vivier (INSERM, CIML)	Regulation of natural killer cell function
Marc Bonneville (INSERM, Nantes)	Key role played by a butyrophilin family gene product in human γδ T cell activation
Sebastian Amigorena (INSERM, Institut Curie)	Imaging FoxP3$^+$ regulatory T cells *in vivo*: insights into their mechanisms of action
Satoshi Ishido (RIKEN RCAI)	MARCH-I: a new regulator of dendritic cell function
Takashi Saito (RIKEN RCAI)	Imaging of positive/negative T cell co-stimulation
Bernard Malissen (INSERM, CIML)	How T cells keep protein tyrosine kinase activity in check?
Tomohiro Kurosaki (RIKEN RCAI)	Localization of IgM and IgG memory B cells
Jean Claude Weill (INSERM, Necker)	Multiple layers of B cell memory with different effector functions
Session 3: Immune regulation and inflammation	
Masato Kubo (RIKEN RCAI)	Role of IL-6-STAT3-SOCS3 in psoriatic skin inflammatory disease
Takashi Tanaka (RIKEN RCAI)	PDLIM2, a nuclear ubiquitin E3 ligase, negatively regulates inflammatory responses

August 31

Sidonia Fagarasan (RIKEN RCAI)	New aspects of IgA synthesis in gut Peyer's patches
Gerard Eberl (INSERM/Pasteur)	Symbiotic microbiota and the development of the immune system
Hiroshi Ohno (RIKEN RCAI)	Multi-omics analysis for probiotic effect of bifidobacteria in a gnotobiotic mouse model of O157 infection
Session 4: Immune diseases and therapy	
Masaru Taniguchi (RIKEN RCAI)	NKT cell-mediated adjuvant activity
Lucienne Chatenoud (INSERM, Necker)	Modalities of immune tolerance induction in primed hosts
Alain Fischer (INSERM, Necker)	Inherited diseases of lymphocyte homeostasis
Fumihiko Ishikawa (RIKEN RCAI)	Creating therapeutic strategies targeting human leukemia stem cells


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LIAI-RCAI Joint Workshop November 11-12, 2010


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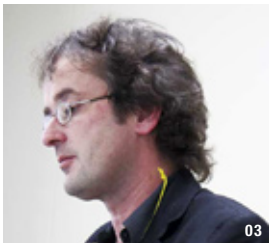
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RCAI and the La Jolla Institute for Allergy and Immunology (LIAI) held a Joint Workshop "New Horizon in Immune Regulation: Towards Disease Intervention" on November 11-12, 2010 at RCAI. RCAI and LIAI established a collaborative agreement in 2004, and since then have held a series of joint workshops to facilitate research collaboration. The first meeting was held at LIAI in 2006 and the second at RCAI in 2008. In this third workshop, eleven researchers from LIAI and nine from RCAI presented their recent findings.

The first day began with the topics of developmental lineages of lymphocytes, transcriptional control and lineage plasticity. Kawamoto (RCAI) explained that reduction of IL-7 triggers upregulation of Bcl11b and enforces transition from DN2 to DN3 stage. Taniuchi (RCAI) discussed transcriptional control of CD4/CD8 lineage choice and described interesting candidates for the regulator of ThPOK silencer. Cheroutre (LIAI, photo 1) explained how gut-specific stimulation reverses ThPOK activity and gives rise to CD4CD8αα T cells in the gut. Hori (RCAI) discussed the lineage stability and plasticity of $Foxp3^+$ T cells, and Rao (LIAI, photo 2) described how DNA domain-swap by FOXP3 dimers is important for suppressor activity. Kurosaki (RCAI) described his studies of memory B cells and suggested that the localization of these cells adjacent to the germinal center (GC) is important for their survival and quick reactivation during a memory response.

The second day started with topics related to allergy. Kawakami (LIAI) reported the development of an atopic dermatitis-like skin inflammation in PLCβ3KO mice. Mast cells were abundant in the skin due to Stat5-dependent IL-3 mediated proliferation of mast cells. SNP analysis revealed significant association of *PLCb3, STAT5A* and *STAT5B* genes with human atopic dermatitis. Croft (LIAI) described how LIGHT, a member of the tumor necrosis factor receptor family, controls asthmatic airway remodeling and Th2 persistence, and Nishida (RCAI) introduced ARF1 as the regulator of granule translocation during mast cell degranulation. The topic then moved to signaling and cellular dynamics. T. Okada (RCAI) and von Herrath (LIAI, photo 3) described their studies using two-photon microscopy. Okada analyzed BCL6 expression in the GC and found that upregulation of BCL6 in pre-GC B cells contributed to their interactions with helper T cells and their entry into GC clusters. After the development of Tfh cells, BCL6 was gradually downmodulated and the IL-7 receptor was upregulated.

von Herrath showed that the IL-21R was required for pancreatic DCs to express CCR7 after antigen uptake and then to provide the antigen, MHCII and CD86 to autoreactive effector cells in draining lymph nodes. Thus he speculated that IL-21R deficiency causes defects in CD4 T cells and antigen presentation, which prevents autoreactive CD8T cells from entering the pancreatic islets and causing type1 diabetes.

During the meeting, Mitchell Kronenberg (photo 4), President of LIAI, talked about how LIAI and RCAI share a few things in common. Both names contain "Allergy and Immunology", and, as the names indicate, they share the same mission. Both institutes are about the same size, and both have an important relationship with Kimishige Ishizaka, who discovered IgE. Ishizaka is the founder of LIAI and the Special Advisor of RCAI. "It is so natural that both institutes collaborate with each other to achieve their same mission," said Kronenberg. Cheroutre and Taniuchi continue a collaboration that began in 2005, when they received RCAI's International Collaboration Award. Aside from them, most researchers from the two institutes already knew each other, and in the resulting friendly atmosphere there were many questions and enjoyable overtime discussions. The joint workshop provided a good occasion for the researchers to expand and deepen their collaborative relationships.

The period of the joint workshop coincidentally overlapped with the Asia-Pacific Economic Cooperation (APEC) meeting in Yokohama. No hotels were available in Yokohama and traffic was under strict control for security reasons. Thanks to the efforts of the organizer (Takashi Saito) and the secretariats (Hiroko Yamaguchi, Toshiko Nakamura, and Sachiko Haraguchi) this joint workshop went smoothly despite this unusual situation.

Table : Program of LIAI-RCAI Joint Workshop 2010

November 11

Session 1. Development & Selection of Lymphocytes	**Chair: Hilde Cheroutre & Shohei Hori**
Hiroshi Kawamoto (RIKEN RCAI)	Retention and termination of myeloid potential in T cell progenitors: An essential developmental checkpoint for production of the T cell
Ichiro Taniuchi (RIKEN RCAI)	Transcriptional control of CD4/CD8 lineage choice
Hilde Cheroutre (LIAI)	Stretching plasticity: mature CD4 effector T cells switch from the Th- to the CTL-lineage
Tomohiro Kurosaki (RIKEN RCAI)	Heterogeneity of memory B cells of different isotypes
Session 2. Immune Homeostasis & Mucosal Immunity	**Chair: Hilde Cheroutre & Shohei Hori**
Shohei Hori (RIKEN RCAI)	On the origin and nature of $Foxp3^+$ T cells that exhibit developmental plasticity
Anjana Rao (LIAI)	Structure of a domain-swapped FOXP3 dimer on DNA and its function in regulatory T cells
Hiroshi Ohno (RIKEN RCAI)	Spontaneous colitis developed in mice deficient in AP-1B, the epithelium-specific polarized sorting factor

November 12

Session 3. Mechanism & Intervention of Allergy	**Chair: Joel Linden & Takashi Saito**
Toshiaki Kawakami (LIAI)	Phospholipase C-β3 deficiency predisposes to atopic dermatitis-like skin inflammation in a mast cell-dependent manner
Michael Croft (LIAI)	LIGHT interactions control asthmatic airway remodeling and Th2 cell survival
Keigo Nishida (RIKEN RCAI)	The adaptor molecule Gab2, via PI-3K, regulates ARF1 in Fc epsilon RI-mediated granule translocation and mast cell degranulation
Session 4. Dynamics & Signaling of Immune Response	**Chair: Joel Linden & Takashi Saito**
Patrick Hogan (LIAI)	Calcium signalling via the CRAC channel and the calcineurin-NFAT pathway
Amnon Altman (LIAI)	The molecular basis for PKCθ function and recruitment to the immunological synapse: A novel drug target opportunity?
Takaharu Okada (RIKEN RCAI)	BCL6 protein expression shapes pre-germinal center B cell dynamics and follicular helper T cell heterogeneity
Klaus Ley (LIAI)	New macrophage phenotypes
Session 5. Innate & Infection Immunity	**Chair: Chris Benedict & Masato Tanaka**
Chris Benedict (LIAI)	Cytomegalovirus suppression of TRAIL death receptors attenuates persistent infection
Tsuneyasu Kaisho (RIKEN RCAI)	Molecular mechanisms for dendritic cell responses against nucleic acid adjuvants
Mitchell Kronenberg (LIAI)	Recognition of microbial and environmental antigens by invariant Natural Killer T cells
Session 6. Disease Regulation	**Chair: Chris Benedict & Masato Tanaka**
Matthias von Herrath (LIAI)	IL-21R deficiency causes defects in CD4 T cells and antigen presentation preventing autoreactive CD8 T cells from entering pancreatic islets and causing type 1 diabetes
Mariko Okada-Hatakeyama (RIKEN RCAI)	Modeling cellular signaling functions for cell differentiation
Joel Linden (LIAI)	Engagement of A_{2A} adenosine receptors suppresses innate and adaptive immune responses

New Zealand-RIKEN-CHIBA Joint Workshop June 14-16, 2010


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Immunity is one of our fundamental physiological systems whose normal task is to combat pathogens (e.g. viruses, bacteria and fungi) and maintain our health. On the other hand, dysregulated or excessive immunity may cause serious immune-mediated diseases (e.g. allergy and autoimmune disease). Therefore, it is very important to understand how the immune system is regulated, and this knowledge will help us to develop strategies for the treatment of diseases, including vaccines for infectious disease. All countries share this vision for the future direction of medical science. In order to strengthen the relationship between immunologists of Japan and New Zealand (NZ) and thus hasten the realization of this goal, RIKEN, Chiba University and the NZ Ministry of Research, Science and Technology (MoRST) held the NZ-RIKEN-CHIBA Joint Workshop on June 14-16 at RCAI and Chiba University. The goals of the meeting were threefold: 1) to bring together leading experts in immunology and translational research to exchange current state-of-the-art research findings, 2) to identify difficulties in terms of applying current basic knowledge to the clinic and discuss how they can be overcome, and 3) to identify opportunities for interactions and collaborations at the level of individual investigators.

In the first session, the initial step of many immune responses, i.e. the interaction between T cells and antigen-presenting cells (APC), was discussed. Since dendritic cells (DCs) are potent antigen-presenting cells, it is important to understand how their development and function are regulated. RCAI members, Sato and Kaisho described their studies on the molecular basis of DC development, the importance of DC-mediated immune regulation and possible clinical application of DCs. Specifically, they presented data about the discovery of a new transcription factor important for DC development, novel immune regulation by specific subset of DCs and establishment of DC-mediated immunotherapy for cancers. T cells are stimulated not only by DCs but also by macrophages. Macrophage-mediated immune regulation was discussed by M. Tanaka (RCAI) and La Flamme (NZ, photo 1). The discussions on APCs were extended to T cell-mediated immune regulation by Painter, Hermans and Bogle (NZ); how the function of T cells can be regulated and what is the most effective strategy for T cell regulation by Rod Dunbar (NZ, photo 2). From the clinical point of view, the molecular basis and possible treatment of allergic disorders were intensively discussed by Nakayama (Chiba Univ., photo 3), Okamoto (Chiba

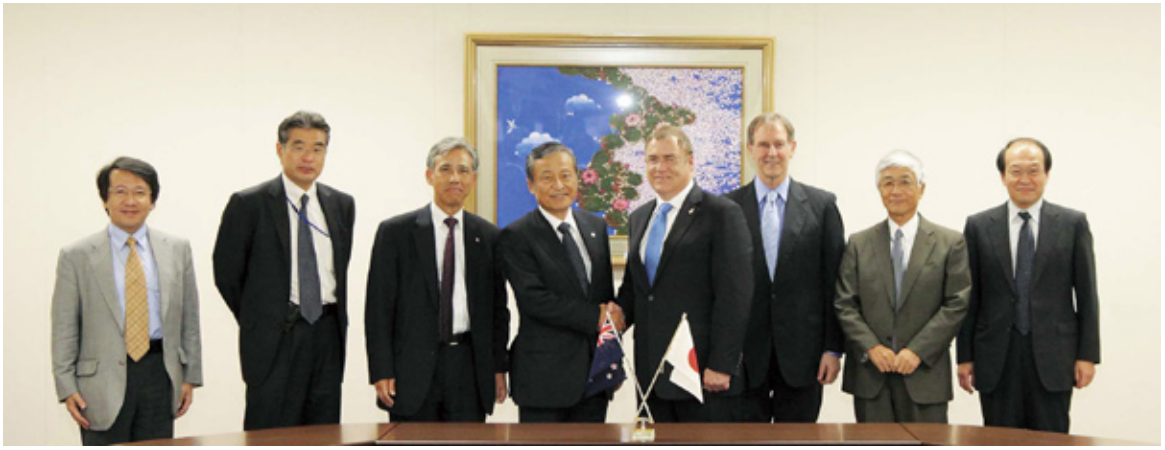

Photo 4: ***From left;*** **Shigeyuki Yokoyama (Director, RIKEN Systems and Structural Biology Center), Hiroshi Ikukawa (Director, RIKEN Yokohama Institute Promotion Division), Kenji Okuma (Director, RIKEN Yokohama Institute), Kenji Takeda (RIKEN Executive Director), Hon. Dr. Wayne Mapp (Minister for Research Science and Technology, New Zealand), Ian Kennedy (New Zealand Ambassador to Japan), Masaru Taniguchi (Director, RIKEN RCAI) and Takashi Saito (Deputy Director, RIKEN RCAI)**

Univ.) and Fujii (RCAI). Last, on the agenda was a discussion of bacterial pathogenesis and vaccine development.

During this meeting, there were constructive and valuable exchanges on both sides. Based on this interaction, Hook (NZ) initiated the preparation of collaborative studies with Ishii (RCAI). In addition, Hon. Dr. Mapp, Minister for NZ MoRST (photo 4) visited RIKEN Yokohama Institute and RCAI on Oct. 13, 2010. Thus, this joint meeting successfully fulfilled its aims, and hopefully the collaborations between NZ and Japan will result in great contributions to basic science and translational research in immunology.

Table: Program of New Zealand-RIKEN-Chiba Joint Workshop

I: Immune activation and regulation	**Chair: Takashi Saito**
Gavin F. Painter, Industrial Research Limited	Forodesine: a rationally designed enzyme inhibitor blocking human T-cell proliferation
Ian F. Hermans, Malaghan Institute of Medical Research	Strategies to enhance $CD8^+$ T cell responses to vaccination
Gib Bogle, Auckland Bioengineering Institute	Mobilizing the T cell army - agent-based simulation of T cell activation and proliferation in a lymph node
Katsuaki Sato, RIKEN RCAI	Crucial role of plasmacytoid dendritic cells for the regulation of inflammation and T cell immunity *in vivo*
Hiroshi Nakajima, Chiba University	Regulation of IL-21 production in $CD4^+$ T cells
II: Infection, autoimmune, allergy and vaccine	**Chair: Tsuneyasu Kaisho**
Anne Camille La Flamme, Victoria University of Wellington	Regulating inflammation by altering macrophage activation
Sarah Hook, University of Otago	Modifying immune responses to vaccines through formulation
Tsuneyasu Kaisho, RIKEN RCAI	Molecular mechanisms for dendritic cell heterogeneity
Masato Tanaka, RIKEN RCAI	Immune regulation by apoptotic cell clearance
Masafumi Arima, Chiba University	A critical role of the IL-4 intron enhancer in chromatin remodeling of Th2 cytokine gene loci and allergic response
III: Immunotherapy	**Chair: Toshinori Nakayama**
John Fraser, University of Auckland	Mechanisms of staphylococcal virulence and pathogenicity
Rod Dunbar, University of Auckland	Programming human T cells
Toshinori Nakayama, Chiba University	iNKT cell-based immunotherapy for cancer
Yoshitaka Okamoto, Chiba University	Development of effective mucosal immunotherapy for allergic rhinitis
Shin-ichiro Fujii, RIKEN RCAI	iNKT cell-triggered *in vivo* DC targeting immunotherapy

The 17th International RUNX Workshop July 11-14, 2010


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The 17th International RUNX Workshop was held at The Oriental Hotel Hiroshima, Hiroshima City in Japan on July 11-14, 2010. The meeting was very successful, with about 100 attendees including 61 scientists from 12 overseas countries (Israel, France, Germany, United Kingdom, Scotland, USA, India, Thailand, Singapore, China, Taiwan and Korea).

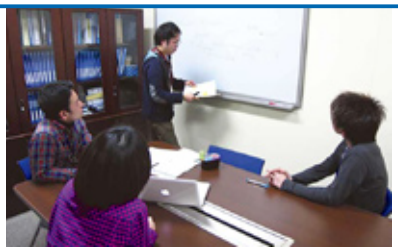
The RUNX gene family was identified in 1993 based on studies from multiple fields including virology, oncology and developmental biology. This brief history of discovery already indicates the variety and complexity of the biological functions of the RUNX proteins, a situation that stimulated researchers in the field to set up a joint interdisciplinary RUNX workshop. The 1st RUNX workshop was held in 1994, with just a few participating laboratories, and has been held annually since then. The International RUNX Workshop has become well recognized all over the world because of the rapid growth of this research area as well as a meeting spirit of sharing unpublished results, which has led to several epoch-making presentations at these workshops .

The International RUNX Workshop came back to Japan this year, for the first time since 2000, when Yoshiaki Ito, Kyoto University organized the 7th RUNX workshop in Kyoto. Ichiro Taniuchi (photo 1) from RIKEN, Research Center for Allergy and Immunology, who is a president of The 17th International RUNX Workshop, selected Hiroshima as the meeting venue. Hiroshima suffered the disaster of the Atomic Bomb attack in 1945 and the people of Hiroshima have been afflicted with many diseases, mainly leukemias. Considering that one of the major topics in the RUNX workshop was leukemogenesis by RUNX1/AML1 dysfunction, as well as in keeping with the contemporary international movement for the abolition of nuclear weapons, Taniuchi decided to have the meeting at Hiroshima. Indeed, almost all participants visited the Hiroshima Peace Memorial Museum during the meeting.

The meeting program started with a talk by Yoshiaki Ito (photo 2), who is recognized as “a father of RUNX” for his pioneering studies on the function of RUNX3 in preventing gastrointestinal tract (GIT) epithelium carcinogenesis. Other topics discussed during the meeting included hematopoietic stem cell generation, lymphocyte development, bone and cartilage formation, neuronal development, and leukemogenesis. As in other fields, genome wide analyses incorporating novel technologies such as ‘omics’ studies, ChIP-sequence and RNAi library based screening, are widely used in the RUNX field. Reflecting the close relationships that have developed among regular participants in this workshop, which has been ongoing for nearly two decades, many issues were deeply and, to some extent emotionally, discussed again in Hiroshima. Historically, this workshop has highlighted different model organisms, from *C. elegans*, *Drosophila*, all the way to mouse. This year, the octopus was shown by a chairperson of the model animal session (photo 3), but it soon turned out to be an expression of sympathy for two disappointed attendees from the Netherlands. The final of the FIFA World Cup, in which Spain defeated the Netherlands after extra time, fell on the second day of the workshop and the octopus on the screen was the world-renowned psychic octopus Paul, who successfully predicted the winners of multiple soccer matches.

The 18th international RUNX Workshop will be held next in San Diego, USA.

Systems Biology Open Laboratory



Systems biology is a newly emerging field of biology and thus its definition still remains multivalent. However, immunologists consider that the immune system is one of the most attractive fields in biology from a systems biological viewpoint because of its high complexity and dynamics. To uncover the secrets of the immune systems, we think that multidisciplinary approaches beyond conventional immunology will be indispensable. With this in mind, the Systems Biology Open Lab has been launched to function as a gateway for researchers outside of immunology. At the outset, researchers in mathematics and systems engineering have been invited to join the Open Lab. However, it is not generally straightforward to exchange ideas among multidisciplinary researchers (e.g., mathematicians and immunologists) mainly because the vocabulary used in their discussions is not shared at all, even when describing the same biological events. To solve this problem, we invited young researchers in mathematics and systems engineering to RCAI and set up opportunities for free discussion with them about some current problems in immunology (Photo). Three main topics were discussed in this framework: Modeling of CD4 T cell proliferation and differentiation, granulopoiesis, and the ecology of gut microbiota and the immune system. These discussions with mathematicians have given us valuable lessons in how to make interdisciplinary collaboration work. In this regard, collaboration with mathematicians and systems engineers marks only the beginning of the systems biology initiative at RCAI and is just a part of what will become the “systems immunology” collaborative network. Given that the mission of RCAI is to prevent and/or regulate immune diseases, the direction of the systems biology initiative at RCAI should be the same as its mission. To borrow a phrase from the systems biological lexicon, what we ultimately expect from the systems biology initiative is the ability to fix a global immune system by applying a local action. This capacity requires knowledge of how the entire system operates, which is very difficult to obtain using strictly reductive scientific approaches. While the systems biology initiative at RCAI has just begun, the Systems Biology Open Lab will keep serving as an incubator for multidisciplinary collaboration, not only for theoretical/mathematical approaches but also in the development of quantitative measurement technology to grapple with the problems of complex human immune diseases.

Multidisciplinary Research Projects

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

In FY2010, eight projects were selected and received the support.

Table : Awardees of RCAI Multidisciplinary Research Projects 2010

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

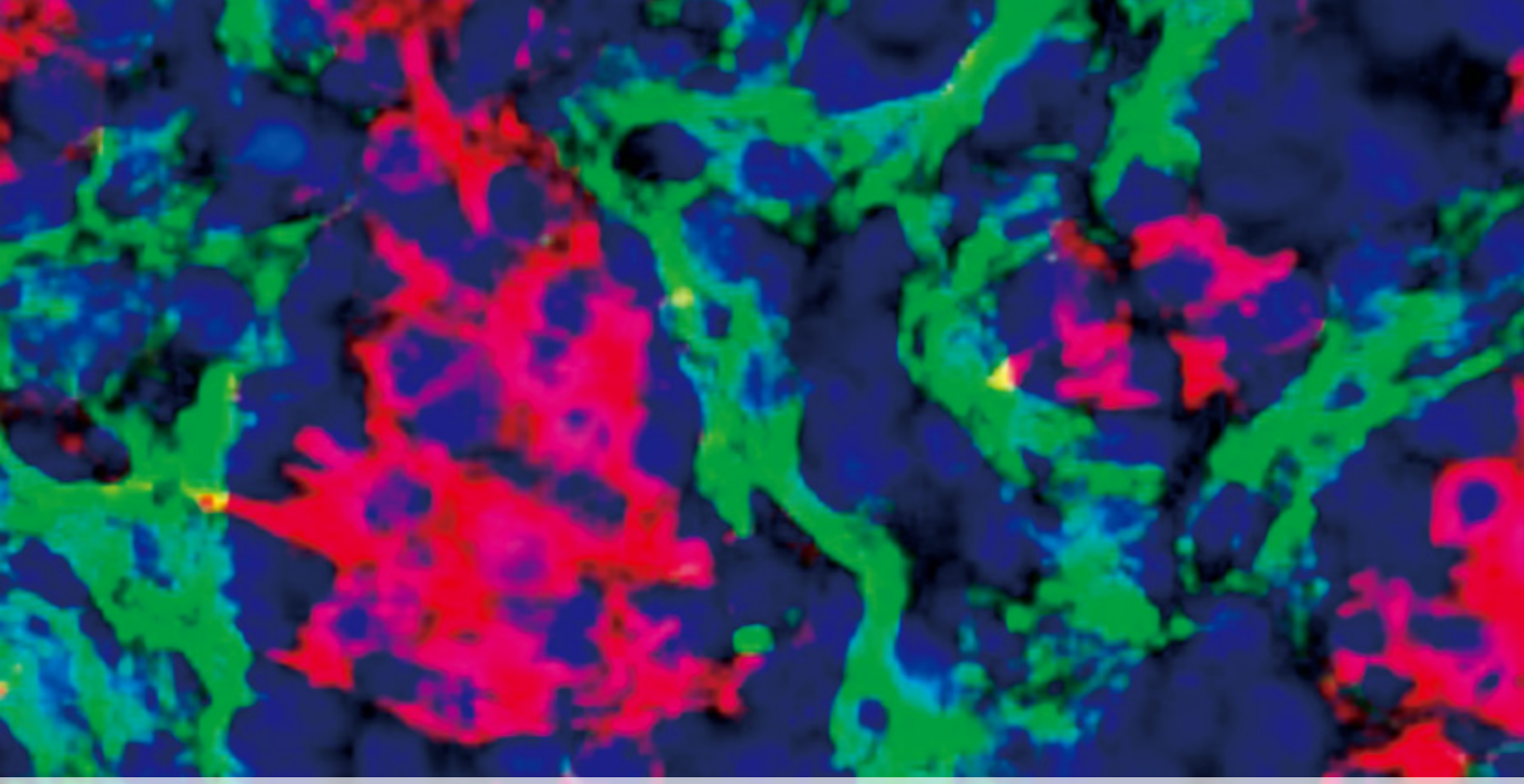
International Research Collaboration Award

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, 15 projects have been funded (Table) and the collaborations have resulted in several important papers. 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). Dustin and Saito published their work on T cell microclusters in *Nat. Immunol*. (2005) and *Immunity* (2006). Ewijk and Kawamoto's work on thymic progenitor cells resulted in three papers in *Development* (2006) and *Mol. Immunol*. (2009 and 2010). Bix and Kubo published their work on the IL4 repressor in *Nat. Immunol* (2009), and Ellemeier and Taniuchi published their study on transcription factor network for CD4/CD8 lineage choice in *Nat. Immunol*. (2010).

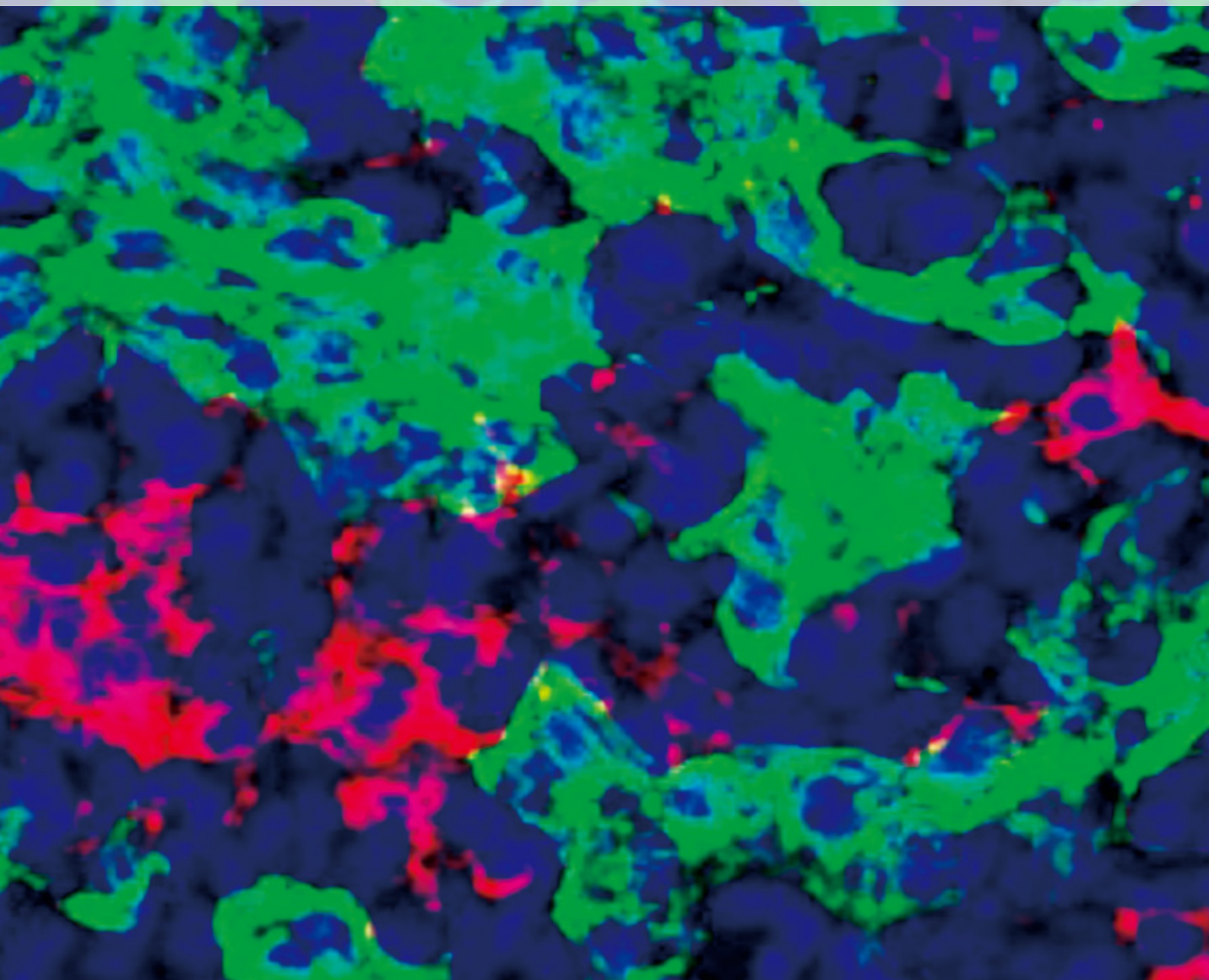
Table : Awardees of RCAI International Collaboration Award Program

Year	Host Lab.	Title of Research	Awardee
2004-2006	**Takashi Saito**	Analysis of dynamism and function of immunological synapse using planar membrane and knock-in T cells	**Dr. Michael DUSTIN** New York University School of Medicine
2004-2006	**Hiroshi Kawamoto**	Regulatory role of lymphoid progenitors during development of thymic microenvironments	**Dr. Willem van EWIJK** Leiden University Medical Center
2004-2006	**Haruhiko Koseki**	Genomic and functional analysis of the role of the Polycomb Ring1 genes in B-cell development	**Dr. Miguel VIDAL** Centro de Investigaciones Biologicas, CSIC
2004-2005	**Masaru Taniguchi**	Role of NKT cells in TSLP-mediated allergic inflammation	**Dr. Steven 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-	**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



2010

Part 5 Outreach Activities



RIKEN Yokohama Open Campus July 3, 2010

RIKEN Yokohama Institute Open Campus was held on July 3, 2010. There were 2,629 visitors, including 1,757 adults and 872 children. Fumihiko Ishikawa, Leader of the Research Unit for Human Disease Model, gave a seminar "Humanized Mouse: Challenges for the New Medical Care and the Drug Development." He first introduced what humanized mice are, and then described how these mice can contribute to new medical care for incurable diseases. The audience spanned many generations, students from high schools and universities, young couples, families and retired folks, and they were surprised to learn that such new technology is becoming a reality. The seminar can be viewed on the RIKEN Channel of YouTube (in Japanese only) <http://www.youtube.com/watch?v=Qrq-g230M0o>.

Twenty RCAI teams exhibited posters and the researchers explained their projects to the visitors. Hiroshi Kawamoto and the members of the Laboratory for Lymphocyte Development gave hands-on practice to isolate lymphocytes using the fluorescence activated cell sorter. After learning the basics of lymphocyte development and the principles of the cell sorter, the twenty participants were divided into groups and isolated $CD4^+CD8^+$T cells from mice. "I took this course before, but came back again. To me, this is one of the highlights of this Open Campus," one participant explained.

Tokyo Metropolitan Assembly Members Visited RCAI July 15, 2010

Cedar pollinosis afflicts an estimated more than 20% of the Japanese population, and it is said to cause serious economic losses every year. Twenty-five members of the Tokyo Metropolitan Assembly, mainly members of the Committee for Countermeasures against Pollinosis, visited RCAI on July 15, 2010. After hearing the presentation about RCAI's strategies for allergy research by Masaru Taniguchi, Director of RCAI, they had many questions and active discussions on the development of cedar pollinosis vaccines. Because RCAI is the only research institute for immunology and allergy in Japan, there is a tremendous social expectation for the development of allergy vaccines (See also page 24 in the Technology and Innovation section).

Immunology Workshop for High School Students July 30, 2010

Every year, RCAI holds one-day workshop on immunology for high school students in Kanagawa prefecture. The program includes lectures by RCAI researchers, hands-on experiments, group discussions and presentations. The sixth of these workshops was held on July 30, 2010. Twenty-three students and four teachers participated from Yokohama Science Frontier High School, Hakuyo High School, Yokosuka High School and Kibogaoka High

School. In the morning session, there were two lectures, "Influenza virus and immunity" by Tomohiro Kurosaki and "Basics of immunology" by Hisahiro Yoshida. After they enjoyed lunch together with RCAI researchers, the students experienced hands-on practice of mouse dissections in the afternoon. Then the students were divided into four groups and given a discussion question "Imagine that a virus pandemic occurred in Asia. What kind of virus is that, and how should we react?" Each group made a short presentation on the question, and the first and second place winners were announced at the end of the program. Nine researchers, technicians and graduate students from RCAI volunteered to help with the program this year. "It was very difficult to trim each organ. I'd like to thank the staff for teaching me so well during the dissection. I could understand well by dissecting real organs, and I also realized the importance of life," a student said. "It was a stimulating day and I am now really thinking of my future path," another student said.

Science Frontier Seminar
August 8, 2010

RIKEN RCAI, OSC (Omics Science Center) and GSC (Genome Science Center) jointly held a public seminar at Yokoyama Science Frontier High School, a public school nearby the RIKEN Yokohama Institute. This seminar was one of a series of events planned to celebrate the 10th anniversary of the RIKEN Yokohama Institute, and there were 52 participants. Such a joint outreach activity by the RIKEN Centers was the first of its kind apart from the annual open campus. The first talk by Yoichi Gondo (GSC) was entitled "Mouse tells the genome: What are the genome, DNA and genes?" He explained the importance of genome

research in an easily understood manner. The second talk was "The day we were defeated by dinosaurs" by Harukazu Suzuki (OSC). He described how his gene transcription-network project was published in *Cell*, but the news did not appear in newspapers or on TV, because, unfortunately on the same day, a discovery concerning the extinction of dinosaurs was published in *Science*. He discussed how outreach activity is becoming important in order to facilitate the people's understanding of scientific research. The third talk was "Allergy and Immunology" by Yasuyuki Ishii (RCAI). He explained the current status of allergy therapeutics and his allergy vaccine project. After the presentations, emcee (Haruka Iwano, RCAI) led the panel discussion by the three speakers, who answered questions from the floor. "I could ask my long-time questions. I hope this kind of event will be repeated," one participant commented.

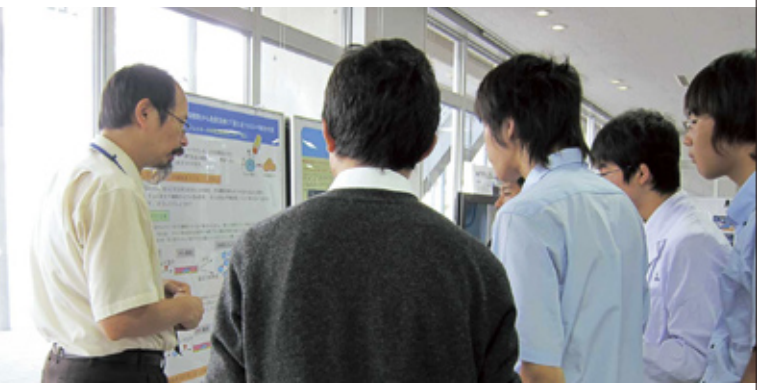
Science Café
September 4, 2010

RCAI held a Science Café on Saturday, Sep. 4 at Kanagawa Prefectural Kawasaki Library. Hiroshi Kawamoto (Leader, Laboratory for Lymphocyte Development) introduced a story “Making iPS cells from lymphocytes.” Fifty-five participants, from high school students to retired folks, learned about iPS cells that Kawamoto and colleagues generated from NKT cells and their possible use for cancer therapies. Kawamoto’s original illustrations, a video of his amateur rock band “Negative Selection,” and lots of humorous stories helped the participants understand the topic easily and feel more familiar with researchers. A high school girl said that she was worrying about what she should major in at the university, and Kawamoto told her that she

doesn't have to be worried too much because immunology is related to various scientific fields, and he invited her to visit his laboratory.

This was the first Science Café organized by RCAI with the support of the RIKEN Yokohama Institute. It was planned as one of a series of events to celebrate the 10th anniversary of the RIKEN Yokohama Institute, but because of its success, the Yokohama Institute is considering holding these kinds of outreach events continuously.

RCAI Participated in the Annual Festival of the Yokohama Science Frontier High School Oct. 2-3, 2010



The Yokohama Science Frontier High School is a new public high school established in 2009, and located in Tsurumi-ono near the RIKEN Yokohama Institute. The school emphasizes the teaching of advanced science and technology, and RIKEN Yokohama Institute closely provides support for the students' education. Akiyoshi Wada, the first Director of the RIKEN Genome Science Center, is now the Super Advisor of the high school, and RCAI's Hiroshi Kawamoto (Leader, Laboratory for Lymphocyte Development) is also an advisor there.

On Saturday and Sunday, Oct. 2nd and 3rd, Kawamoto participated in the high school's annual festival and used a poster to explain the generation of NKT-iPS cells for cancer therapy. There were 4,586 visitors to the entire school festival, mainly high school students, teachers, neighbors, junior high school students who are interested in entering the school and their families. Other research institutes and companies also prepared booths at the festival.

"It was good to talk with researchers on this occasion and get to know RIKEN's activities," one student said. "The number of visitors was nearly twice that of RIKEN Yokohama Institute's Open Campus. This may be a good occasion to communicate with young students and help them to develop interests in science," a RIKEN staff member noted.

Celebration of RIKEN Yokohama Institute 10th Anniversary

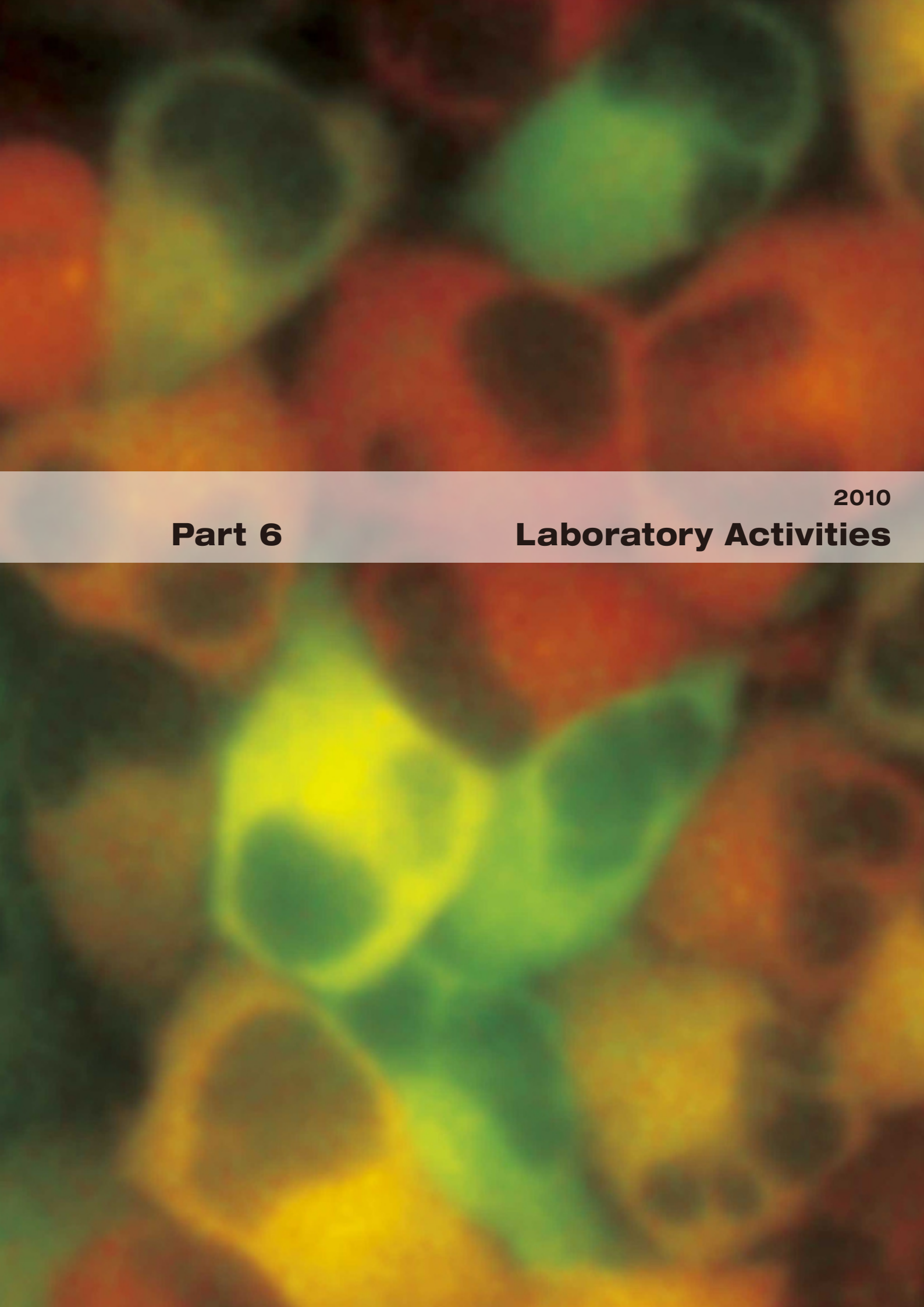
Nov. 25, 2010

The RIKEN Yokohama Institute celebrated the 10th anniversary of its establishment on Nov. 25 at the Yokohama Kaikou Memorial Museum. There were 270 participants including 93 invited guests. After the opening remarks by RIKEN President Noyori and speeches by the guests, there was a two-hour panel discussion on "Science and Society". The discussants were Mitsuru Miyata (chief editor of Nikkei BP publishing), Yuji Kagohashi (Torii pharmaceutical), Satoshi Kawakami (DOWA eco-system), Yoshifumi Nishimura (Yokohama City University) and Directors of RIKEN Yokohama Institutes; Masaru Taniguchi (RCAI), Kazuo Shinozaki (PSC), Naoyuki Kamatani (CGM), Yoshihide Hayashizaki (OSC), Shigeyuki Yokoyama (SSBC), Tetsuro Toyoda (BASE) and Yoshiyuki Nagai (CRNID).

During 2010, RIKEN Yokohama Institute researchers had continued a series of brainstorming discussions about the future direction of the institute and how they can collaborate together. From these discussions, a common keyword "Life and Environment" emerged, and they started a consideration of actual collaboration schemes. To expand this discussion, they decided to use the occasion of the anniversary event to reconsider the relationship between science and the society, and the functions of RIKEN.

According to the new policy drafted by the Council for Science and Technology Policy, the government will shift their emphasis from "Science and Technology" to "Science, Technology and Innovation." Social contributions resulting from scientific research are now more expected by the society. The participants discussed the crucial importance of basic science for true innovation, and how a bridging framework can be built between basic science and solution science.

The Science and Society discussion provided the people an opportunity to think about the future direction of science in Japan. "Foundations for creative research is the key, I thought. Japan will be respected by other countries in that context, and it will be our pride," a participant said. "Preventive medicine will be important for the future Japan. I am impressed that therapeutic medicine is only one aspect of medical sciences," another remarked.



2010

Part 6 Laboratory Activities

Laboratory for Developmental Genetics

Group Director **Haruhiko Koseki**

Research Scientists : **Yaman-Deveci, Yixin Dong, Mitsuhiro Endo, Yûichi Fujimura, Kyôichi Isono, Osamu Masui, Daisuke Yamada, Yuki Takada (SPDR), Jafar Sharif (SPDR), Nayuta Yakushiji (SPDR)**

Technical Staff : **Hiroshi Kajita, Iyo Kataoka, Kayoko Katsuyama, Fuyuko Kezuka, Yôko Koseki, Tamie Morisawa, Naoko Ohnaga, Naomi Otsuka, Rie Suzuki**

Student Trainee : **Takanori Sawaguchi (JRA)**

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

The role of epigenetic regulators during development and differentiation

PcG proteins mediate heritable silencing of developmental regulators by forming two distinct multimeric protein complexes, Polycomb repressive complexes-1 (PRC1) and -2 (PRC2), during development and differentiation. PRC2 is shown to mediate histone H3K27 trimethylation (H3K27me3) while PRC1 recognizes H3K27me3 and mediates monoubiquitination of histone H2A (HaAub1). We have been investigating how PcG proteins regulate cellular differentiation. A significant part of our study is focused on embryonic stem cells (ESCs) and trophectodermal stem cells (TSCs), both of which represent the first cell lineage diversification that occurs during pre-implantation development. We have reported that PcG proteins Ring1A/B are indispensable for suppressing ESC differentiation programs (Endoh et al., 2008). We have recently demonstrated an essential requirement of H2Aub1 for Polycomb-mediated silencing, which is mediated by the convergence of PRC2-dependent and -independent pathways (Endoh et al., submitted). In parallel, surprisingly, we found that Ring1A/B were dispensable for maintaining TSCs, whilst their differentiation into trophectoblastic giant cells (TGCs) required Ring1A/B by repressing key transcription factors including *Cdx2, Eomes* and *Sox2*, in a differentiation-dependent manner. These observations indicate the critical involvement of PcG proteins in cell lineage choice and progressive differentiation. During the last

Recent publications

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

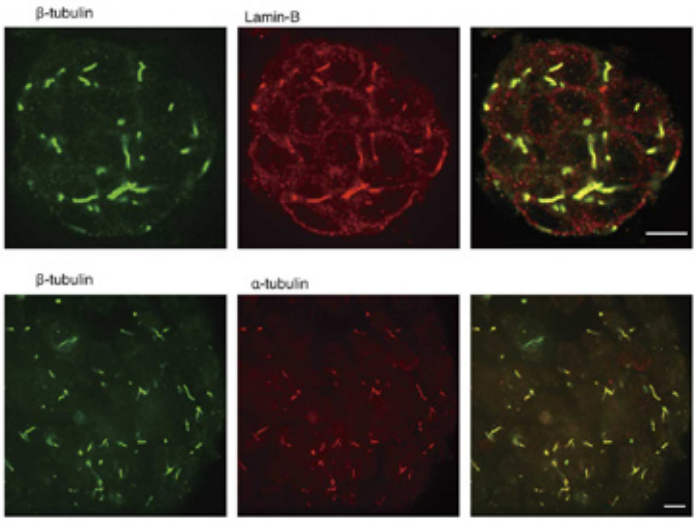


Figure : Subnuclear colocalization of α and β tubulin with Lamin-B in mouse ES cells.

few years, we have expanded this study to other cell lineages and have revealed a critical requirement of PcG proteins for neural differentiation, lymphocyte development, spermatogenesis, and limb development. Studies to elucidate the molecular functions of PcG proteins in these lineages are currently under way.

Our previous studies have also suggested functional interactions between PcG proteins and regulatory mechanisms for DNA methylation, and identified the SRA protein Np95, which interacts with PcG proteins and is also a key component of the DNA methylation maintenance machinery (Sharif et al., 2007). We are focusing on the mechanisms underlying repression of the endogenous retrotransposon associated IAP (intercisternal A particles) loci, at which Dnmt1, Np95 and PcG-related proteins are converged. Our results suggest that Np95 may have a basal activity to induce IAP transcription in WT cells, which can then be much more amplified or suppressed upon receiving developmental/epigenetic cues. At present we are working to elucidate the molecular mechanisms.

Regulation of large scale chromatin structures by epigenetic regulators

Compartmentalization in eukaryotic nuclei is important for division and coordination of various events that proceed simultaneously in a single nucleus. We have shown that PRC1 components exhibit focal accumulations, which are designated the “PcG body”, in primary cells and in developing embryos and identified a role for PRC1 in condensing the *Hoxb* cluster into a single Polycomb body to mediate repression. We further identified the role of SAM domains of polyhometic homologues to mediate Polycomb body formation by linking multiple PRC1 molecules via homophilic interactions. Polycomb body formation was further suggested to be a process to enhance recruitment of PRC1 to polycomb target genes. This study therefore identified an additional layer of Polycomb repression, and potentially provides new insight into epigenetic regulation of cellular differentiation.

We have previously reported the interaction of PRC1 with various components of the spliceosome and their regulation of Polycomb repression. We have identified a critical region for this interaction within the AT-hook domain of Cbx2. We further found that this region was essential for *Hox* repression by using an AT-hook domain point mutant allele. Notably, we have found that *Hox*-derepression in this mutant was not accompanied by defects in local recruitment of PRC1. We are currently investigating the interaction of PRC1 with the spliceosome in this mutant.

In the process of affinity purification of PRC1, we have repeatedly co-purified α and β-tubulins, proteins know historically as primal components of microtubules. By immunofluorescence analysis of ESCs after extraction by detergents and high concentration of salt, we identified a fraction of tubulins that persistently associated to the nuclear lamina and exhibited a filamentous structure. ChIP-chip analysis for tubulins and Lamin-B, a major component of nuclear lamina, revealed significant co-occupancy of their target genes in ESCs and thus confirmed the interaction of tubulins with the nuclear lamina. By using Vinblastin, which depolymerizes tubulins, we found that this is a highly polymerized form of tubulins. Immuno-FISH analysis for several genes that were bound by both tubulins and Lamin-B, showed their relocalization from the nuclear periphery to the nuclear interior. This was accompanied by dissociation of these genes from Lamin-B as shown by ChIP. These observations indicated the role of nuclear tubulins is to link their target to the nuclear lamina. Interestingly, tubulins extensively colocalized with HDACs at nuclear lamina and this accumulation of HDACs depends on tubulin polymerization. Our data suggest that tubulins act as a scaffold for the convergence of chromatin-HDAC-nuclear lamina, and are necessary for maintaining the hypoacetylated status of genes that associate with the nuclear lamina.

3. Harada M, Murakami H, Okawa A, Okimoto N, Hiraoka S, Nakahara T, Akasaka R, Shiraishi Y, Futatsugi N, Mizutani-Koseki Y, Kuroiwa A, Shirouzu M, Yokoyama S, Taiji M, Iseki S, Ornitz DM, Koseki H.. FGF9 monomer-dimer equilibrium regulates extracellular matrix affinity and tissue diffusion. ***Nat Genet***. 41, 289-298 (2009)

4. Endoh M., Endo T.A., Endoh T., Fujimura Y., Ohara O., Toyoda T., Otte A.P., Okano M., Brockdorff N., Vidal M., Koseki H. Polycomb group proteins Ring1A/B are functionally linked to the core transcriptional regulatory circuitry to maintain ES cell identity. ***Development*** 135,1513-1524 (2008)

Laboratory for Lymphocyte Development

Team Leader **Hiroshi Kawamoto**

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

Research Associate : **Rumi Satoh**

Technical Staff : **Raul Vizcardo Sakoda**
Asako Shibano
Chiho Matsuura

Visiting Scientists : **Nagahiro Minato**
Yoshimoto Katsura

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

Lineage commitment of hematopoietic stem cells progressing toward the unipotent T cell progenitor stage

We have recently revealed that early thymic T cell progenitors that have lost B cell potential are still able to generate myeloid cells (Wada et al, Nature, 2008). Therefore, the most critical step for construction of the T cell lineage is now thought to be at the point where myeloid potential is terminated. We have also shown that such T cell lineage determination occurs in the midst of the intrathymic DN2 stage.

We then addressed the issue of whether such a determination step serves as a developmental checkpoint. Developmental checkpoints can be visualized when progenitors exhibit developmental arrest at a certain stage under particular culture conditions or as a result of genetic manipulation.

We recently found that when murine hematopoietic progenitors were cultured on immobilized Notch ligand DLL4 protein in the presence of a cocktail of cytokines including interleukin-7, progenitors developing toward T cells were arrested and the arrested cells entered a self-renewal cycle, while maintaining myeloid potential. Reducing the concentration of interleukin-7 promoted T cell lineage determination. A similar arrest and self-renewal of progenitors were observed in thymocytes of mice deficient in the transcription factor Bcl11b. Our study thus identifies the earliest checkpoint during T cell development and shows that it is Bcl11b-dependent (Ikawa et al, *Science*, 2010).

Recent publications

1. Ikawa, T, S Hirose, K Masuda, K Kakugawa, R Satoh, A Shibano-Satoh, R Kominami, Y Katsura, H Kawamoto. An essential developmental checkpoint for production of the T cell lineage. ***Science***. 329: 93-96, (2010).
2. Kawamoto H, T Ikawa, K Masuda. H Wada, Y Katsura. A map for lineage restriction of progenitors during hematopoiesis: the essence of the myeloid-based model. ***Immunol. Rev***. 238: 23-36, (2010).
3. Moro K., T. Yamada, M. Tanabe, T. Takeuchi, T. Ikawa, H. Kawamoto, J-I. Furusawa, M. Ohtani, H. Fujii, and S. Koyasu. Innate production of Th2 cytokines by adipose tissue-associated c-Kit+Sca-1+ lymphocytes. ***Nature*** 463: 540-544, (2010).
4. Kakugawa K., T. Yasuda, I. Miura, A. Kobayashi, H. Fukiage, R. Satoh, M. Matsuda, H. Koseki, S. Wakana, H. Kawamoto*, H. Yoshida*. (*correspondence) A novel gene essential for the development of single positive thymocytes. ***Mol. Cell. Biol***. 29:5128-5135, (2009).

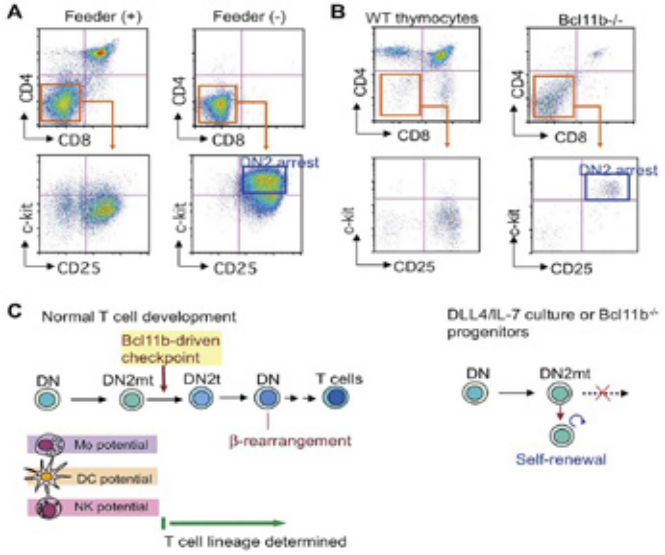


Figure1: An essential developmental checkpoint for production of the T cell lineage

A. Immobilized DLL4 with a combination of cytokines induces self-renewing expansion of immature thymocytes. Lin$^-$ckit$^+$Sca1$^+$ (LKS) progenitor cells (200 cells) from 13 dpc murine fetal liver mice were cultured on TSt-4/DLL4 cells [feeder (+)] or immobilized Fc-DLL4 supplemented with 10 ng/ml of SCF, IL-7 and Flt3L [feeder (-)] for 14 days. Profiles for CD4 vs CD8 and c-kit vs CD25 gated on Lin$^-$ cells are shown. The cells are arrested at the c-kit$^+$CD25$^+$ (DN2) stage in the feeder-free culture.

B. Bcl11b is essential for T lineage determination. Fetal liver cells from Bcl11b$^{+/+}$ or Bcl11b$^{-/-}$ mice (Ly5.2) were transferred into lethally irradiated mice (Ly5.1). Adult thymocytes derived from Bcl11b$^{-/-}$ progenitors exhibited a developmental arrest at the DN2 stage.

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

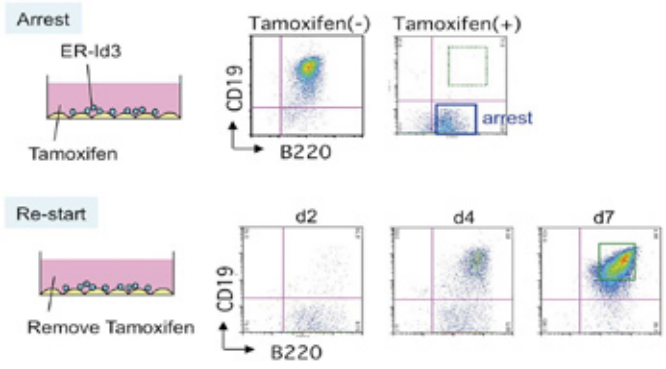


Figure2: Arrest-restart culture system of the B cell lineage determination step

A. LKS cells from murine fetal liver were transduced with ER-Id3 and cultured under B cell inducing conditions in the presence or absence of Tamoxifen. Cells are arrested at the B220$^+$CD19$^-$ prepro-B cell stage in the Tamoxifen cultures.

B. When Tamoxifen is removed from the cultures, cells restart B cell differentiation.

Arrest-restart *in vitro* system of cell-differentiation capable of manipulating lineage commitment of hematopoietic progenitors

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

In this context, the culture system in which the T-lineage determination step can be controlled will be a useful method for further study of the mechanisms of T cell lineage determination.

We also applied this system in the study of early B cell development. It has been known that the developmental arrest of cells at the multipotent pre-proB cell stage, as well as the self-renewal of arrested progenitors, occurs in E2A deficient progenitors. We expanded on this observation to establish a controllable culture system in which Id protein, a natural dominant negative factor for E2A, was conjugated with the estrogen receptor (ER) and retrovirally over-expressed in murine hematopoietic progenitors. By adding Tamoxifen to the culture medium, we were able to expand multipotent progenitors retaining T, B and myeloid potential, and then to restart differentiation by withdrawing Tamoxifen. This system can be used not only for the study of B cell development, but also for other hematooietic cells.

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

Laboratory for Transcriptional Regulation

Team Leader **Ichiro Taniuchi**

Research Scientists : **Hirokazu Tanaka**, **Taku Naito**, **Wooseok Seo**, **Mari Tenno**
Technical Staff : **Sawako Muroi**, **Chizuko Miyamoto**, **Risa Chihara**
Student Trainees : **Sebastian Nieke (IPA)**, **Hisashi Wada**

One of the major questions in developmental biology is how the fate of progenitor cells differentiating into divergent lineages is determined. Even as we learn more about cell fate determination, other questions arise, namely, how genetic programming after lineage specification functions to establish cell identity and then how cell identity, once established, is maintained in differentiated cells. Research projects in my laboratory are directed toward understanding (a) how progenitor cells sense external or intrinsic stimuli and turn on a genetic program for regulating lineage choice and (b) how a specific gene expression pattern is established during the commitment process to become fully differentiated cells. We are addressing these questions by studying transcription factor networks regulating T lymphocyte development. In particular, we have been studying the transcriptional regulation of lineage choice by $CD4^+CD8^+$ double-positive (DP) thymocytes differentiating into either $CD4^+$ helper- or $CD8^+$ cytotoxic-lineage T cells. Expression of the ThPOK transcription factor is essential for development of helper-lineage cells. Our previous studies have identified a transcriptional silencer (the *ThPOK* silencer) in the *ThPOK* locus and have shown that this silencer is essential to restrict *ThPOK* gene expression in cells expressing an MHC class II-restricted TCR, and thereby is necessary to induce a cytotoxic fate in MHC class I-restricted cells. We have shown that the Runx1/Cbfβ transcription factor complexes are essential to activate silencers in both the *ThPOK* and the *Cd4* loci, and that ThPOK has an antagonistic function against these silencers.

We are expanding our findings to understand how the activity of these silencers is regulated at a molecular level and how Runx complexes are involved in immune system development.

Mechanism regulating *ThPOK* gene expression

Both CD4/CD8 co-receptor expression and the specificity of TCRs for MHC molecules correlate well with the lineage decision outcome of $CD4^+CD8^+$ DP thymocytes. Cells expressing MHC class I-restricted TCR differentiate into the cytotoxic-lineage and terminate *Cd4* gene expression, whereas cells expressing class II-restricted TCR give rise to the $CD4^+$ helper-lineage and lose *Cd8* gene expression. However, it remains unclear how differences in TCR signals are sensed and integrated into developmental programming in the cell nucleus of post-selection thymocytes. It has been assumed that factors regulating *Cd4/Cd8* gene expression must also be involved in regulation of lineage choice. The BTB/POZ family transcription factor MAZR has been identi-

Recent publications

1. Sakaguchi S., Hombauer M., Bilic I., Naoe Y., Schebesta A., Taniuchi I. Ellmeier W. The zinc finger protein MAZR is part of the transcription factor network controlling CD4/CD8 cell fate decision of DP thymocytes. ***Nat. Immunol***. 11, 442-48 (2010).
2. Setoguchi R., Tachibana M., Kitoh A., Ono M., Naoe Y., Ohkura N., Yamaguchi T., Yaguchi H., Kitabayashi I., Tsukada T., Nomura T., Miyachi Y., Taniuchi I. Sakaguchi S. Indispensable role of the Runx1-Cbfβ transcription complex for in vivo-suppressive function of $FoxP3^+$ regulatory T cells. ***Immunity*** 3, 609-20 (2009).
3. Collins A., Littman D.R., Taniuchi I. RUNX proteins in transcription factor networks that regulate T-cell lineage choice. ***Nat. Rev. Immunol***. 9, 106-15 (2009)

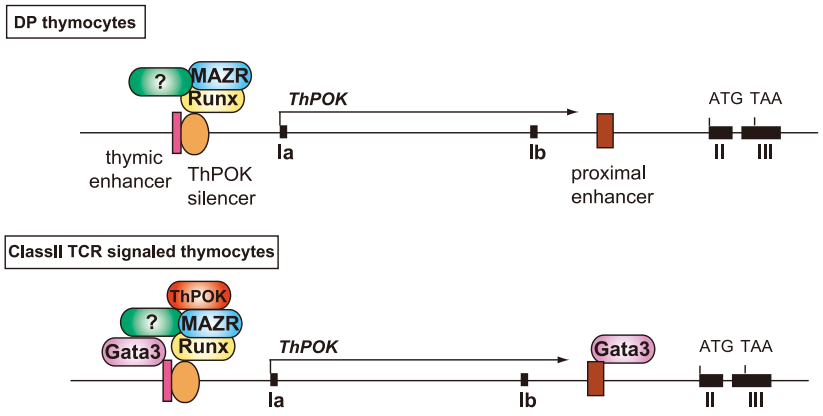


Figure 1 : *Cis*-regulatory elements and *trans*-acting factors regulating expression of the *ThPOK* gene. The expression of the *ThPOK* gene during T cell development is regulated mainly by combined activation and inactivation of the two enhancers and the silencer. Binding of Runx and MAZR to the silencer is essential to exert silencer activity. Gata3 functions as an upstream factor for ThPOK expression by activating enhancers.

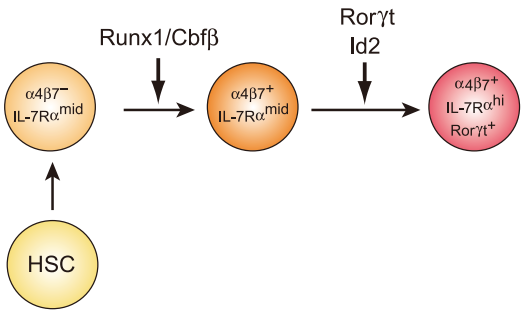


Figure 2 : Model for possible developmental pathway of LTi cells in fetal liver. Runx1/Cbfβ2 complexes play important roles in regulating differentiation of α4β7⁻IL-7Rα^{mid} cells into α4β7⁺IL-7Rα^{mid} cells. This is followed by Id2 and Rorγt dependent developmental program that directs α4β7⁺IL-7Rα^{mid} cells to become α4β7⁺IL-7Rα^{high} LTi cells.

fied as an important regulator of *Cd8* expression. By analyzing gene targeted mice, we have shown that loss of MAZR led to partial redirection of MHC class I-restricted thymocytes into CD4⁺ helper-like T cells, a response that correlated with de-repression of *ThPOK*. MAZR binds to the ThPOK silencer and interacts with Runx1 protein. Thus, we demonstrated that MAZR is part of the transcription factor network that regulates CD8 cytotoxic lineage differentiation of DP thymocytes (Figure 1).

However, we still do not understand how lineage specific activity of the *ThPOK* silencer is regulated. In order to find crucial molecule(s) or mechanisms that act as a switch to reverse the *ThPOK* silencer activity for inducing *ThPOK* expression upon engagement of class II-restricted TCRs, we are isolating novel proteins that bind to the *ThPOK* silencer.

In addition to neutralization of the *ThPOK* silencer, activation of positive regulatory elements, such as enhancers and promoters, is required for efficient induction of *ThPOK* expression. Previously, we have identified a proximal enhancer that is necessary to maintain *ThPOK* expression at later developmental stages. Recently we have mapped another enhancer in the 5' region, in close proximity to the silencer. Our results indicate that this enhancer, designated a thymic enhancer, plays an essential role in an efficient induction of *ThPOK* expression, in part via erasing preexisting repressive epigenetic marks.

Further studies on the molecular control of these regulatory elements, as well as epigenetic regulation at the *ThPOK* locus, will advance our understanding of how cellular signaling initiated by external stimuli is converted into genetic programs in the cell nucleus and is imprinted for stabilizing cell identity.

Role of Runx complexes in lymphoid organogenesis

Lymphoid tissue inducer (LTi) cells are essential for the development of secondary lymphoid tissues such as lymph nodes and Peyer's patches. It remains unknown how specification toward an LTi-lineage is programmed in multipotent hematopoietic progenitors. By using mice in which the function of Runx1/Cbfβ complexes was attenuated by loss of either the P1-Runx1 or Cbfβ2 variant protein, we found that Runx1/Cbfβ2 complexes are necessary for development of early LTi precursors in fetal liver. In addition, loss of Cbfβ2, but not P1-Runx1, resulted in an inefficient upregulation of Rorγt in residual LTi cells in the secondary lymphoid tissue anlagen. Our results thus revealed that Runx1/Cbfβ2 complexes regulate the differentiation of LTi cells at two stages, an early specification toward the LTi-lineage in hematopoietic progenitors and subsequent activation of the Rorγt gene in the anlagen (Figure 2).

4. 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)

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

Laboratory for Cell Signaling

Group Director **Takashi Saito**

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Research Scientists : **Takayuki Imanishi**, **Arata Takeuchi**, **Akiko Hashimoto-Tane**, **Shin-ichi Tsukumo**, **Zijin Guo**
Technical Staff : **Wakana Kobayashi**, **Machie Sakuma**, **Masako Takamatsu**, **Akiko Takumi**, **Midori Unno**
Student Trainee : **Minoru Sawaguchi (JRA)**
Visiting Scientists : **Ikuo Ishige**, **Noriko M. Tsuji**, **Yasutaka Wakabayashi**, **Sho Yamasaki**, **Hu Zeng**
RCAI Central Facility FACS Laboratory : **Hanae Fujimoto**, **Yukiko Hachiman**
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RCAI Central Facility mAb Laboratory : **Tomomi Aoyama**, **Mayuko Matsuda**

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

Dynamic regulation of T cell activation and co-stimulation

We have studied the dynamic movement of signaling molecules in the process of the formation of the immunological synapse (IS) and T cell activation upon antigen recognition at the single-cell level. Using a combination of imaging techniques of model planar bilayers and TIRF, we could visualize the behavior of signaling molecules during the dynamic process of T cell activation. We have identified TCR-microclusters (MCs) as the signalsome to assemble signaling molecules and induce activation signals, which translocate into the center of the IS to generate the cSMAC.

In addition, we have examined the dynamic features of co-stimulation signals by analyzing 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.

Recent publications

1. 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)
2. Hashimoto-Tane A., Yokosuka T., Ishihara C., Sakuma M., Kobayashi W. and Saito T. TCR-microclusters critical for T-cell activation are formed independently of lipid raft clustering. ***Mol. Cel. Biol***. 30, 3421-3429 (2010)
3. Takeuchi A., Itoh Y., Takumi A., Ishihara C., Arase N., Yokosuka T., Koseki H., Yamasaki S., Takai Y., Miyoshi J., Ogasawara K., and Saito T. CRTAM confers late-stage activation of CD8+ T cells to regulate retention within lymph node. ***J. Immunol***. 183, 4220-4228 (2009)
4. Yokosuka T. and Saito T. Dynamic regulation of T-cell costimulation through TCR-CD28 microclusters ***Immunol. Rev***. 229, 27-40 (2009)

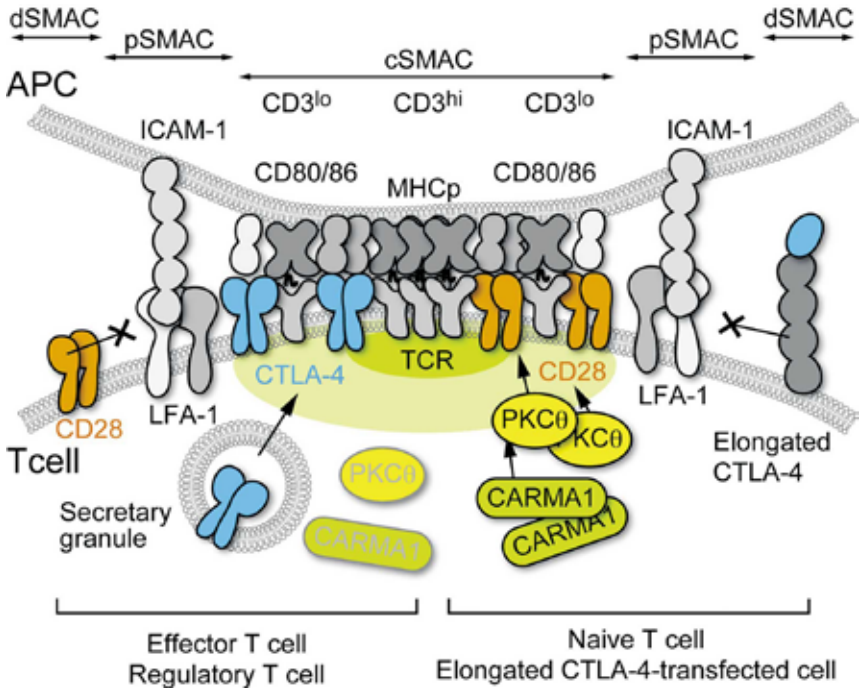


Figure : **Spatially distinct regions of the cSMAC for positive and negative co-stimulation**

Upon interaction with an antigen-presenting cell, a T cell generates TCR-microclusters (MC), which contain signaling molecules to induce T cell activation. TCR-MCs move to the center of the interface to form the cSMAC (*green*). The co-stimulatory receptor CD28 accumulates in a sub-region of the cSMAC (*light green*; signaling cSMAC) together with PKCθ and CARMA1 to induce sustained co-stimulation signals. Later after the initial activation, the negative co-stimulatory receptor CTLA4, already present in secretary granules, moves toward the membrane and accumulates in the same region as CD28-PKCθ, where it competes with CD28 for binding to their common APC ligand, CD80/86. CD28 is then displaced from the cSMAC and T cell activation ceases (*leftmost*). On the other hand, CTLA-4 chimera that cannot enter into the cSMAC due to its large size (*rightmost*), fails to mediate suppression, indicating that the localization of CTLA-4 at the cSMAC is necessary for inhibition of T cell activation.

Later on, CTLA-4 also accumulates in the same area of the cSMAC as CD28, where it competes with CD28 for ligand-binding. Thus, we have clarified the spatio-temporal dynamic regulation of positive/negative co-stimulation and how they are quantitatively fine-tuned.

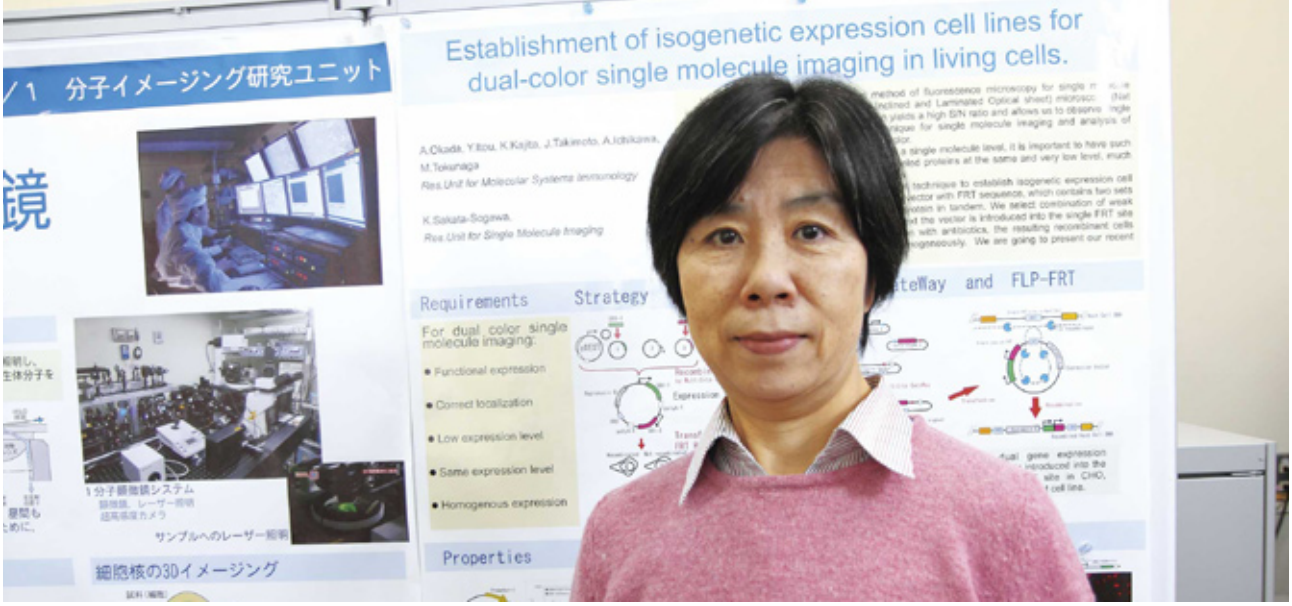
Cytoskeletal regulation of T cell activation

TCR-MCs generated at the periphery of the IS move to the center to form the cSMAC. This translocation of TCR-MCs thus regulates TCR activation by reducing the number of functional TCR-MCs through their degradation at the cSMAC. We analyzed the mechanism of TCR-MC translocation and found that the minus-end microtubule-associated motor protein, dynein complex is co-localized with TCR-MCs and co-precipitated with the TCR complex. We have shown that TCR-MCs are translocated along microtubules to form the cSMAC. As predicted by our model, reduction of the amount of the dynein complex or its function resulted in enhanced T cell signals and activation.

Regulation of T cell responses by innate signals

We have analyzed the interconnected activity of signaling molecules of the innate and acquired immune systems. Particularly, after we identified the critical roles of IRAK4 in T cell activation, we analyzed the function of TLRs expressed by T cells and found that TLR2/3/9 mediate co-stimulatory functions whereas TLR2 directly activates Th1 cells to induce cytokines independently of TCR stimulation. T cell co-stimulation through TLR2 is completely MyD88-dependent, similar to situation in innate cells, however, surprisingly, the co-stimulation function of TLR3 and TLR9 was found to be independent of MyD88/TRIF. We have identified the optimal structure of nucleic acids for this T cell co-stimulatory activity, but the recognition of these nucleic acids appears to be mediated by an as yet unknown novel T cell-specific sensor.

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



Research Unit for
Single Molecule Imaging

Unit Leader **Kumiko Sakata-Sogawa**

Technical Staff : **Yoshiyuki Sasaki**

The focus of our research is to understand immune responses and signaling processes using the technique of single molecule imaging and quantification. Single molecule approaches enable us to capture transient intermediates and heterogeneous behavior, avoiding ensemble averaging. This ability is powerful in elucidating mechanisms of cellular functions at multiple levels: 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. To this end we installed a microscope system and we have focused on the development of a novel type of fluorescence microscopy (HILO) for use in single cell/single molecule studies. HILO has an advantage for making observations inside cells, with a high signal to noise ratio of 7.6. Further, this microscope system has been optimized to observe immunological responses ranging from the plasma membrane to the nucleus by introducing a system for computer control of optical devices.

For quantitative analysis of the interaction of signaling proteins, it is important to observe multiple molecules simultaneously. It is also necessary to prepare many different cell lines harboring target genes for high throughput data collection. To this end, we have established methods to obtain isogenic cell lines expressing two fluorescence tagged proteins at a homogenous and low level, which is essential for this type of analysis. We can expand this technique for multicolor imaging of single molecules.

NF-κB activation mechanism regulated by IκBα

NF-κB is an important transcription factor that activates expression of inflammatory genes in response to a variety of stimuli. In resting cells, NF-κB associates with IκBα and is sequestered in the cytoplasm. Stimulation leads to phosphorylation of Ser residues of IκBα and its subsequent ubiquitination and proteasomal degradation. The liberated NF-κB translocates to the nucleus and activates expression of multiple target genes, one of which is the IκBα gene. The nascent IκBα binds to nuclear NF-κB and exports it back into the cytoplasm, resulting in negative feedback loop.

We aim to directly confirm molecular mechanism of the multiple functions of IκBα by single molecule fluorescence

Recent publications

1. 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 (3) 326-339 (2010)
2. Miletic AV., Graham DB. Sakata-Sogawa K., Hiroshima M., Hamann HJ., Cemerski S., Kloeppel T., Billadearu DD., Kanagawa O., Tokunaga M. and Swat, W: Vav Links the T Cell Antigen Receptor to the Actin Cytoskelton and T Cell Activation Independently of Intrinsic Guanine Nucleotide Exchange Activity. ***PLoS One***. 4 (8), e6599 (2009)
3. Yokosuka T., Kobayashi W., Sakata-Sogawa K., Takamatsu M., Hashimoto-Tane A., Dustin ML., Tokunaga M., and Saito T.: Spatiotemporal Regulation of T Cell Costimulation by TCR-CD28 Microclusters and Protein Kinase C θ Translocation. ***Immunity*** 29, 589-601 (2008)
4. Tokunaga, M., Imamoto, N., Sakata-Sogawa, K.: Highly inclined thin illumination enables clear single-molecule imaging in cells. ***Nat Methods***. 5, 159-161 (2008)

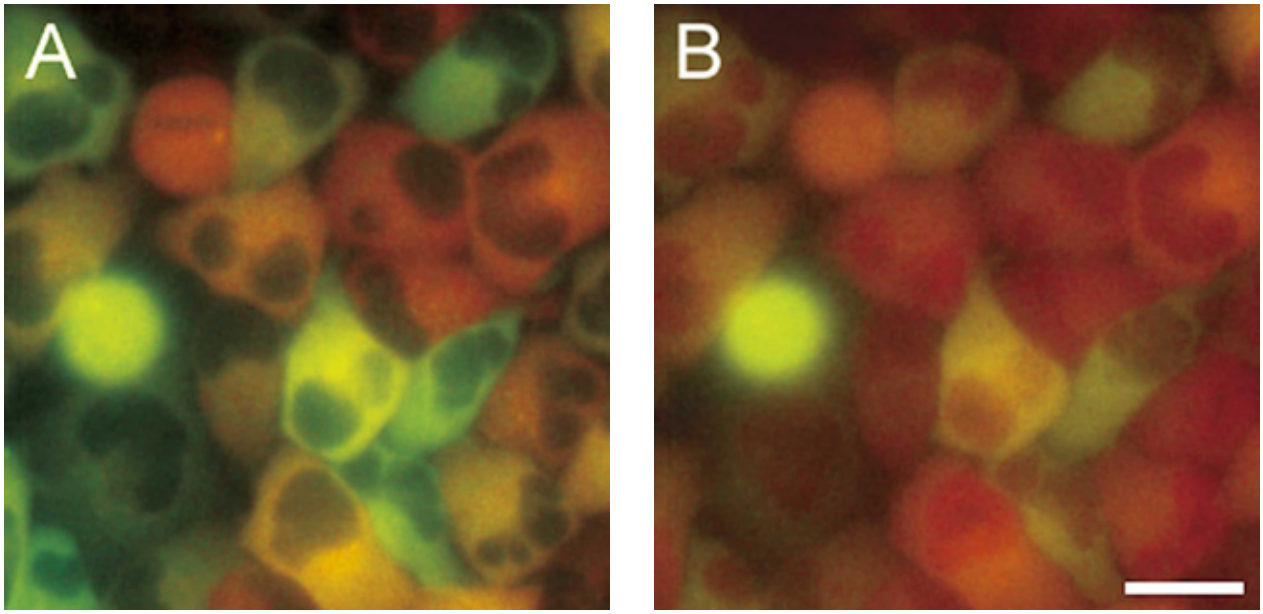


Figure : Response of NF-κB and IκBα after TNF-α stimulation.
HeLa cells expressing both NF-κB-RFP and IκBα-GFP were stimulated with hTNF-α. (A) Before stimulation. (B) 30 min after stimulation. Bar: 20μm.

microscopy. For this purpose, we established HeLa cell lines expressing GFP- IκBα and RFP-NF-κB (p65 subunit) fusion proteins and confirmed their co-localization in the cytoplasm. We visualized single molecule movements of IκBα both in the cytoplasm and nucleus, suggesting the shuttling of NF-κB/IκBα complexes. After stimulation with TNFα, IκBα remained in the cytoplasm and disappeared, whereas p65 translocated into the nucleus. We also tested a dominant negative IκBα mutant that lacks the phosphorylated Ser residues. In this case there was no IκBα degradation or p65 nuclear translocation. We are currently investigating the movements of IκBα molecules in the cytoplasm using single molecule imaging analysis and comparing the behavior of IκBα wild type and mutant proteins.

Dynamics of spatial-temporal regulation of NF-κB inactivation

Inflammatory response-mediated NF-κB activation plays an important role in the immune system. As excessive inflammatory responses cause massive damage to host cells, the regulation of NF-κB activation is indispensable. Recently Dr. Tanaka and Dr. Kaisho in RCAI found that the E3 ubiquitin ligase PDLIM2 terminates NF-κB activation by intranuclear sequestration and degradation. However detailed mechanisms of PDLIM2 function remain unclear.

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

Laboratory for Lymphocyte Differentiation

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Hisaaki Shinohara
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Masanori Matsumoto
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Technical Staff : **Yoko Fujii**
Shiori Maeda
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Student Trainee : **Saya Moriyama (JRA)**

B cells play an essential role in the regulation of immune responses. Upon encountering their cognate antigen, B cells assume multiple functions including antibody production, antigen-presentation, and induction of T cell differentiation. In addition to protective roles against pathogens, B cells also have regulatory roles, e.g. serving as negative regulators of autoimmunity by secreting anti-inflammatory cytokines such as interleukin-10 (IL-10) and transforming growth factor-β (TGF-β). In order to exert such multiple B cell functions, signals propagated through the B cell receptor(BCR), Toll-like receptors (TLRs), TNF-family receptors, cytokine receptors, and chemokine receptors are important. The quality and quantity of the signals are dictated by multiple factors including the receptors themselves and intracellular signaling molecules and transcription factors. Our laboratory has focused on a molecular understanding of the signaling pathways that lead to crucial B cell fate decisions such as memory versus plasma cell differentiation.

Function of calcium sensors Stim1 and Stim2 in B cells

The calcium flux triggered by BCR engagement occurs in two waves, first a rapid but transient release of Ca^{2+} from endoplasmic reticulum (ER) stores, and then a sustained influx of extracellular Ca^{2+} across the plasma membrane (PM). Initiation of this second wave is thought to result directly from the emptying of ER Ca^{2+} stores, which activates Ca^{2+} channels located in the PM, a process referred to as store-operated Ca^{2+} (SOC) influx. Until recently, the mechanism of SOC influx was a mystery, but the identification of several molecules including Stim1 (sensor stromal interaction molecule 1) has provided long-awaited insight.

Calcium signaling is thought to be critical for multiple B cell functions; however, supportive *in vivo* evidence has been lacking. Thus, discovery of Stim1 and a sister molecule, Stim2, has allowed us to approach this question by taking a genetic approach. We generated mice with B cell-specific deletions of both Stim1 and Stim2 and found that, although both molecules are critically required for BCR-induced SOC influx and proliferation *in vitro*, they were dispensable for B

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2. Aiba, Y., Kometani, K., Hamadate, M., Moriyama, S., Sakaue-Sawano, A., Tomura, M., Luche, H., Fehling, H.J., Casella, R., Kanagawa, O., Miyawaki, A., and Kurosaki, T. Preferential localization of IgG memory B cells adjacent to contracted germinal centers. ***Proc. Natl. Acad. Sci. U.S.A.*** 107, 12192-7 (2010)
3. Hawkins, B.J., Irrinki, K.M., Lien, Y-C., Wang, Y., Bhanumathy, C.D., Ramasamy, S., Mallilankaraman, K., Ritchie, M.F., Soboloff, J., Baba, Y., Kurosaki, T., Joseph, S.K., Gill, D.L. and Madesh M. S-glutahionylation activates STIM1 and alters mitochondrial homeostaisis. ***J. Cell Biol.*** 190(3):391-405 (2010)
4. Kurosaki, T., Shinohara, H., and Baba, Y. B Cell Signaling and Fate Decision. ***Annu. Rev. Immunol***. 28, 21-55 (2010)

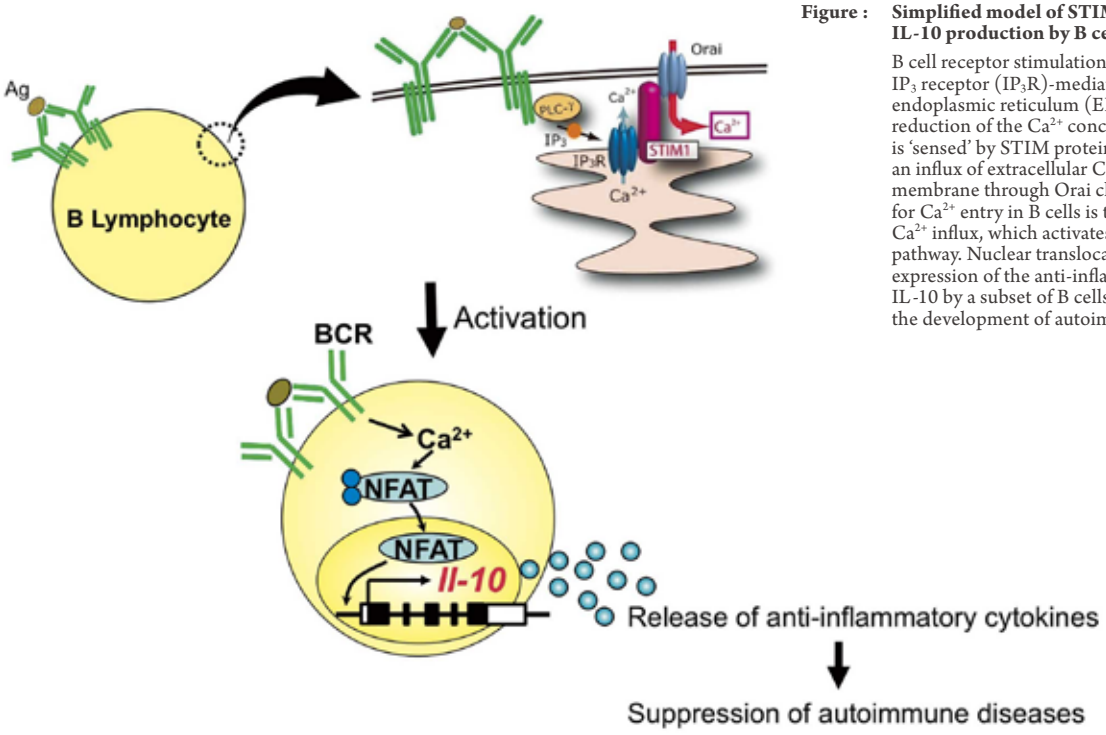

Figure : Simplified model of STIM-dependent IL-10 production by B cells

B cell receptor stimulation causes PLC-γ/IP_3/IP_3 receptor (IP_3R)-mediated Ca^{2+} release from endoplasmic reticulum (ER) Ca^{2+} stores. A reduction of the Ca^{2+} concentration within the ER is 'sensed' by STIM proteins, which in turn induce an influx of extracellular Ca^{2+} across the plasma membrane through Orai channels. The main source for Ca^{2+} entry in B cells is the STIM-dependent Ca^{2+} influx, which activates the calcineurin-NFAT pathway. Nuclear translocation of NFAT promotes expression of the anti-inflammatory cytokine IL-10 by a subset of B cells and thereby suppresses the development of autoimmune diseases.

cell development and antibody responses *in vivo*. However, the ablation of Stim1 and Stim2 in B cells caused defects in NFAT activation, B cell intrinsic IL-10 production, and suppression of an EAE model of autoimmune diseases. Thus our results provide genetic evidence for the significance of Stim-dependent Ca^{2+} signaling in B cell regulatory functions.

CIN85 regulates the canonical NF-κB pathway in B cells

By recruiting signaling proteins into complexes, adaptor molecules create nodes of regulation and activity. CD2AP, the founding member of the CD2AP/CIN85 family of adaptor proteins, was initially isolated in a yeast interaction screen as a binding partner of CD2 expressed on T cells. Subsequently, its mammalian homologue CIN85 (Cbl interacting protein of 85 KDa) was identified as a partner of the E3 ubiquitin ligase Cbl. Based on coimmunoprecipitation experiments, colocalization studies, and *in vitro* protein-protein interaction assays using fibroblasts, it has been proposed that CIN85 primarily functions in endocytosis to down-regulate receptor tyrosine kinase activity. According to this model, CIN85 constitutively associates with endophilin and, on stimulation with growth factors such as epidermal growth factor (EGF), complexes with Cbl to mediate receptor down-regulation.

The same mechanism also appears to operate in mast cells. CIN85 overexpression in the RBL-2H3 rat mast cell line accelerated the distribution of engaged FcεRI complexes, their sorting into early endosomes, and their delivery to a lysosomal compartment for degradation. In contrast to observations in mast cell lines, a positive role for CIN85 in pre-TCR signaling has been recently suggested. Thus, CIN85 may mediate distinct biological outcomes, which depend on the type and developmental stage of each immune cell. To determine the role of CIN85 in B cells, we have used the mb1-Cre line to generate mice with a B cell-specific deletion of CIN85. These mice manifested impaired T-independent type II antibody responses *in vivo* and diminished IKKβ activation and cellular responses to BCR cross-linking *in vitro*. Furthermore, introduction of a constitutively active IKKβ corrected the defective antibody responses as well as the cellular responses in the mutant mice. Thus, our results suggest that CIN85 participates in linking BCR to IKKβ activation, thereby contributing to T-independent immune responses.

5. Kurosaki, T., Aiba, Y., Kometani, K., Moriyama, S., and Takahashi, Y. Unique properties of memory B cells of different isotypes. ***Immunol. Rev.*** 237;104-16 (2010)

Laboratory for Immune Diversity

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Student Trainees : **Chie Kano**, **Yingqian Li** (IPA), **Shuyin Li** (IPA)

Technical Staff : **Hiromi Mori**

During an adaptive immune response, resting B cells can be activated by a variety of external stimuli including antigen stimulation, CD40 ligation and cytokine stimulation. The activated B cells form germinal centers (GC) where they expand enormously. These rapidly dividing GC B cells undergo somatic hypermutation (SHM) and class switch recombination (CSR) of their immunoglobulin (Ig) genes and ultimately differentiate into antibody-producing plasma cells or memory B cells. Abnormalities in B cell activation and terminal differentiation can lead to immunodeficiency, autoimmune diseases and B cell malignancies. My laboratory focuses on the molecular mechanisms that control B cell activation and differentiation. Specifically, we have two research aims. The first is to understand how somatic mutations at A:T base pairs are induced in the Ig V genes of GC B cells. The second is to identify genes highly expressed in B and GC B cells and elucidate their physiological function by genetic approaches.

Mechanism of A:T mutations in the GC B cells

The activation-induced cytidine deaminase (AID) initiates SHM by catalyzing the deamination of cytosine (C) to uracil (U), which generates a U:G lesion. Yet half of the somatic mutations are induced at A:T base pairs, which are not a direct target of AID. Using a sensitive *lac*Z-transgenic system to detect spontaneous genome mutations, we have shown that GC B cells have an intrinsic propensity to generate A:T mutations. Moreover, using an *in vitro* mutagenesis system that closely mirrors SHM in GC B cells, we found that the induction of A:T mutations is dependent on the GC B cell environment but independent of the target gene. Studies thus far indicate that A:T mutations are generated by an atypical "error prone" mismatch repair (MMR) pathway that utilizes the low-fidelity DNA polymerase η (POLH) for repair synthesis. We are now addressing the following three non-exclusive hypotheses to explain the efficient induction of A:T mutations in GC B cells. 1) Rapid cell division may predispose GC B cells to utilize POLH for repair synthesis as the high-fidelity POLδ is fully devoted to DNA replication. 2). GC B cells express a specific factor(s) that function to actively recruit POLH during DNA repair. 3) MMR components and POLH undergo specific modifications in GC B cells, resulting in an atypical repair process not observed in other cell types. Preliminary results suggest that rapid cell

Recent publications

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2. Kano, C., Ouchida, R., Kokubo, T. and Wang J.-Y. Rapid cell division contributes to efficient induction of A/T mutations during Ig gene hypermutation. ***Mol. Immunol.*** 15-16: 1993-1999 (2011).
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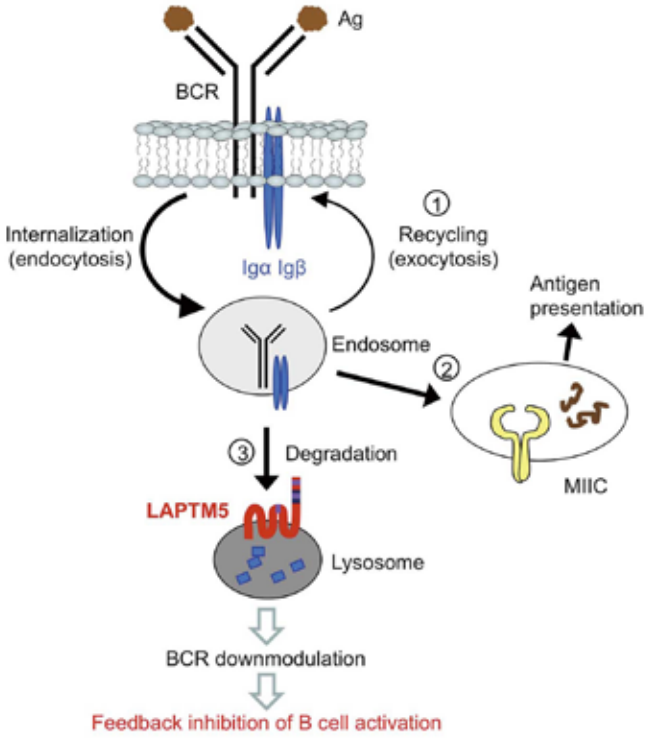

Figure 1: Model of LAPTM5-mediated BCR degradation. Upon antigen (Ag) stimulation, surface BCR is rapidly internalized into the endosomes. The internalized BCR/Ag complex can be ① recycled back to the surface, ② translocated to the specialized late endosomal compartment rich in MHC class II (MIIC) for Ag presentation, or ③ transported to the lysosomes for degradation. Our results indicate that LAPTM5 functions to promote the lysosomal degradation of the internalzied BCR and prevent its recycling to the cell surface.

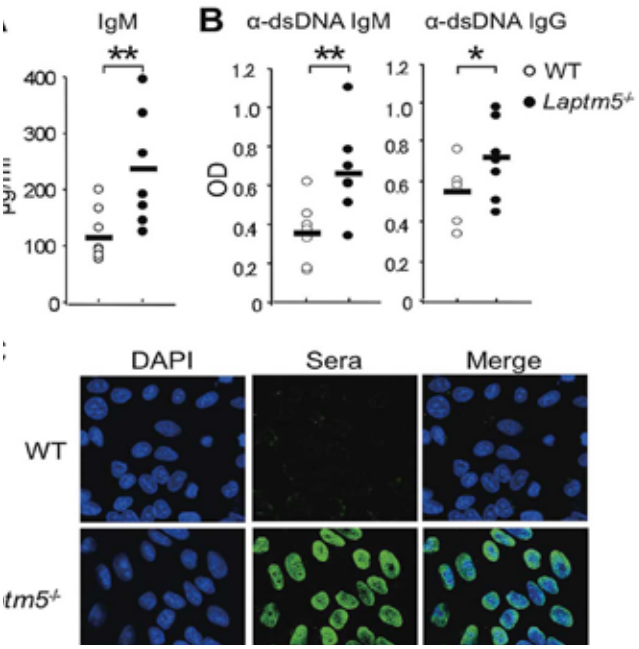

Figure 2: Increased levels of serum IgM and autoantibodies in aged *Laptm5*$^{-/-}$ mice. **A**, Levels of serum IgM and **B** anti-dsDNA IgM and IgG. $*p < 0.05$; $**p < 0.005$ (unpaired t-test). **C**, Presence of anti-nuclear antibodies in the sera of *Laptm5*$^{-/-}$ mice. Hep2 cells were stained with sera (100-fold dilution) from WT or *Laptm5*$^{-/-}$ mice.

division is one of the conditions that promote efficient induction of A:T mutations.

Identification and characterization of genes highly expressed in B and GC B cells

To better understand the diverse and complex processes regulating B cell activation and differentiation, we have focused on genes that are highly expressed in resting B and GC B cells. LAPTM5 is a lysosomal protein highly expressed in resting B cells but downregulated in GC B cells. We found that LAPTM5 negatively regulates cell surface expression of the B cell antigen receptor (BCR) by promoting its lysosomal degradation (Fig. 1). The absence of LAPTM5 leads to increased levels of serum IgM and autoantibody production in aged mice (Fig. 2). BCP1 is an intracellular molecule that is specifically expressed in B cells and upregulated in GC B cells. Analysis of BCP1-deficient B cells revealed that this molecule negatively regulates signaling through CD19, an important co-stimulatory molecule for B cell activation. More recently, in collaboration with other groups, we are analyzing the function of the Fc receptor for IgM, which is exclusively expressed in B and GC B cells in mice. Through further analysis of these mice, we aim to uncover new pathways that control the activation and terminal differentiation of B cells during adaptive immune responses.

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Laboratory for Immunological Memory

Group Director **Toshitada Takemori**

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Natsumi Yoneda
Natsuki Kobayashi
Aika Sawada

Guest Scientists : **Yoshimasa Takahashi**
Masaki Hikida

Student Trainee : **Naoka Itoh**

Memory B cells are antigen (Ag) experienced cells that are sustained for a long period after immunization in the absence of the immunizing Ag. These memory cells respond to antigen stimulation more rapidly than naive B cells, and possess efficient APC activity to antigen-primed helper T cells (unpublished data). After a secondary challenge, memory cell-derived plasma cells produce large amounts of high affinity antibodies, implying that GC B cells are precursors to high-affinity memory B cells. However, class-switched memory B cells develop by both GC-independent and –dependent pathways (Kaji et al. submitted).

Our recent analyses suggest that the orphan receptor, EBV-induced molecule 2 (EBI2), guides activated B cells to interfollicular and outer follicular niche(s), where EBI2 ligand is expressed. Once Bcl6 is upregulated in B cells after activation, GC-committed B cells repress the expression of EBI2 and move to the center of the follicle where the GC reaction takes place, generating high-affinity memory B cells and plasma cells (Reviewed by Pereira et al. *Int. Immunol.*). We observed that activated B cells also differentiate into IgG memory B cells early after stimulation, prior to GC formation (Kaji et al. submitted). Consistently, single cell PCR revealed that EBI2 was highly expressed in most of these GC-independent memory B cells, but was barely detected in GC B cells. This GC-independent memory pathway requires T cell dependent B cell activation by antigen and subsequent proliferative expansion of the cells. The cells entering this pathway undergo class-switch recombination (CSR), but not somatic hypermutation (SHM).

Thus, B cell memory sustains two classes of antibody repertoire, one with V genes in a germline configuration and the other accumulating SHM as a consequence of selection in the GC. The generation of high-affinity somatic mutants is advantageous for the system to rapidly and efficiently exclude the initiating pathogen, although, in the process, this narrows-down cross-reactivity to any variants or related pathogens. Of importance, the germ line encoded antibody memory repertoire, with its cross-reacting specificities, may provide a rapid response to both the initial pathogen and its variants, followed by production of large amount of antibodies that are useful as a first-line of defense against common pathogens.

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2. 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***. 2010, 185(1):211-9.
3. Hikida M, Casola S, Takahashi N, Kaji T, Takemori T, Rajewsky K, Kurosaki T. PLC-gamma2 is essential for formation and maintenance of memory B cells. ***J Exp Med***. 2009 206(3):681-9.

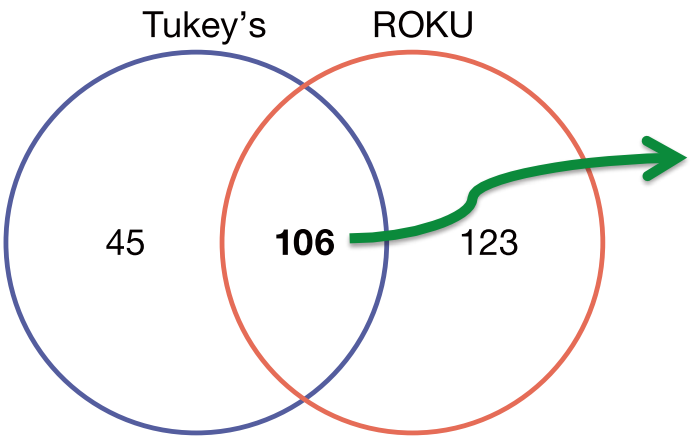


Figure : IgG memory B cell-specific genes

We compared the gene expression profiles in three replicates of day 7 and day 40 NP-specific/IgG1^{+} memory B cells, GC B cells, plasma cells, and naïve B cells with or without multiple types of *in vitro* stimulation. Ninety-four genes were identified as memory B cell-specific using two distinct statistical methods, Tukey's multiple comparison test and ROKU. We identified 106 probe sets corresponding to 94 genes that are highly expressed in the IgG1 memory B cells.

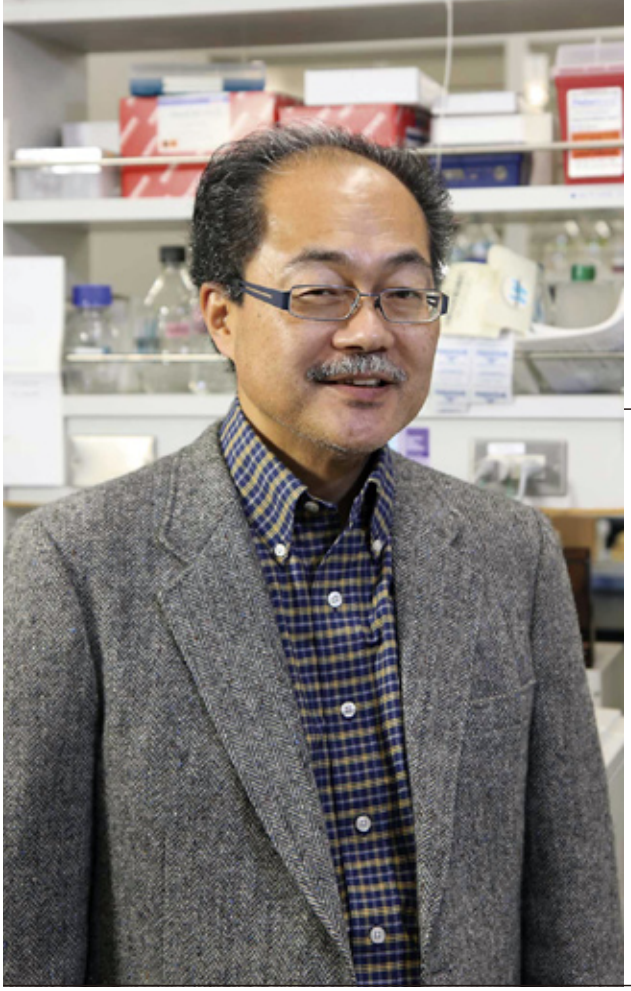
In the context of the role of the immune system in defense against infection, it seems reasonable to propose that the germ line encoded antibody repertoire is constantly evolving to provide optimal protection.

Because the secondary response is characterized by high-affinity antibody production, affinity selection in the GC reaction has been suggested to determine the fate of B cells to differentiate into memory B cells. However, the existence of GC-independent memory B cells excludes this as a possibility. We do not know the intrinsic signals that elicit memory B cell formation, perhaps influenced by signals received from local niche cells, and regulate the quality and quantity of the memory cells, as distinct from other types of B cells. To address this issue, we utilized Affymetrix GenChip technology and determined the intrinsic differences that distinguish memory and other types of B cells by comparison of the transcriptional profiles of highly purified populations of these cell types isolated from immunized mice. We purified NP-specific memory B cells from WT mice seven days after immunization (mostly GC-independent memory B cells) and at day 40 (GC-independent and -dependent memory B cells) and from immunized mice without formation of GC by *in vitro* treatment with anti-CD40L mAb.

After qRT-PCR validation of the microarray results, we identified 30 genes that are highly expressed in NP-specific/IgG1^{+} memory B cells compared to other type of B cells. Nine genes encode transcriptional activators or repressors, five encode receptors, and the rest cytoplasmic proteins involved in cell signaling and protein sorting. GC-independent day 7 and day 40 memory B cells shared the expression of a group of these transcripts, whereas the level of certain transcripts increased or decreased in GC-independent memory B cells over time. These results suggest kinetic changes of gene expression in GC-independent memory cells, perhaps in response to environmental cues.

To understand the role of these candidate genes in memory B cell development and function, we first established a screening system prior to the gene targeting step. We knocked down the candidate genes in purified B cells from B1-8^{high} mice (CD45.1^{+}), in which the frequency of NP specific naïve B cells is greatly increase through the knock-in of a rearranged, mutant V186.2 gene segment (Shih et al. 2002). After gene knockdown, B cells are transferred into syngeneic CD45.2 recipients. Immunization of recipient mice with NP-CG causes synchronous activation of many NP-specific donor B cells, allowing us to dissect their early response *in vivo*. Several pilot experiments haven shown that this system works well.

Up until now, by using gene targeting technology we have identified two genes that play roles in memory B cell development in mucosal tissues and their differentiation into plasma cells during the secondary antibody response (unpublished).



Laboratory for Epithelial Immunobiology

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

Identification of CCR6^{hi}CD11c^{int} B cells as a promoter of M-cell differentiation in the Peyer's patch

M cells are atypical epithelial cells specialized for uptake of luminal antigens, including the phagocytosis of viruses and bacteria, to deliver them to dendritic cells that accumulate beneath the FAE. More than three decades after their initial description, however, molecular mechanisms of M-cell differentiation are not well understood. The differentiation of M cells is thought to be induced by interactions between FAE and PP cells; however, the identity of the types of immune cells that function as M-cell inducers has remained elusive. Therefore, we attempted to identify the cells that serve as an M-cell inducer(s) in PP. To this end, we focused on the mice deficient in CCR6, the sole receptor for a chemokine CCL20. We previously found that CCL20 is constitutively expressed by FAE, thus implicating its involvement in FAE-PP cell-interactions. We found that a unique B-cell subset, characterized as CCR6^{hi}CD11c^{int}, resided in the subepithelial dome

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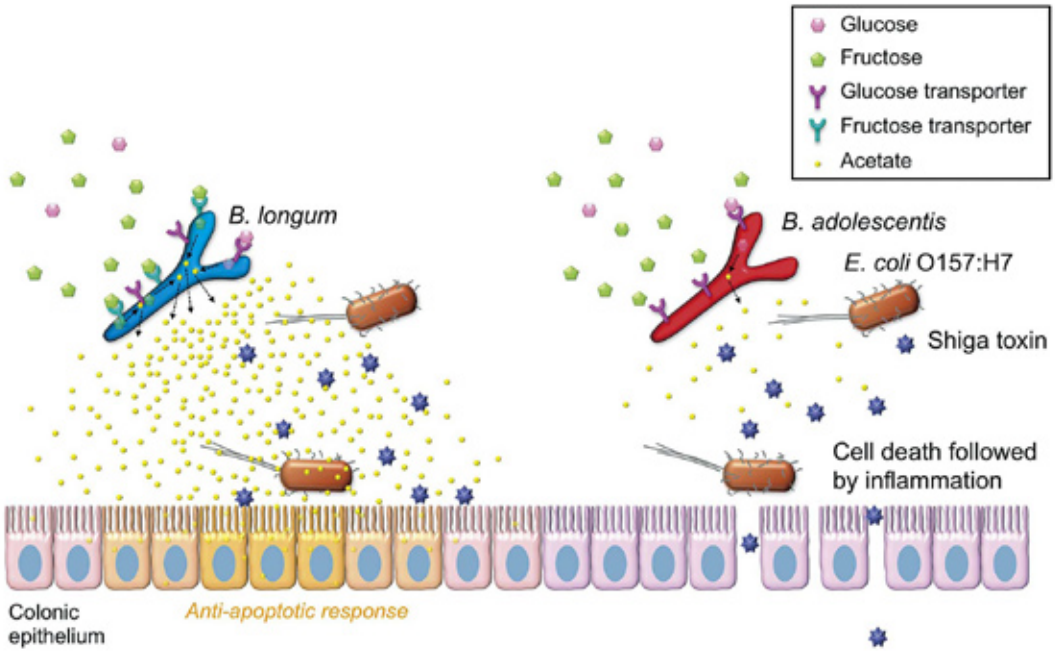

Figure : **Schematic representation of the mechanisms of O157 lethal infection and protection by bifidobacteria.** Both *B. longum* (BL) and *B. adrescentis* (BA) possess glucose transporter(s) and can produce acetate from glucose in the proximal colon, where glucose is thought to be present; this acetate exerts an anti-apoptotic effect on colonic epithelium. In the distal colon, by contrast, only fructose is available and thus only BL, with its fructose transporters, is able to produce acetate in this location. BA lacks the fructose transporters, and thus fails to produce enough acetate to prevent epithelial apoptosis by O157. This results in translocation of Shiga toxin into the bloodstream, leading to death of the mice.

(SED) of the mouse PP. CCR6^{hi}CD11c^{int} B cells showed chemotactic migration in response to CCL20. Furthermore, this unique B-cell subset was substantially decreased in the PP of CCR6-deficient mice, indicating that the SED localization of CCR6^{hi}CD11c^{int} B cells is most likely regulated by the CCL20-CCR6 system. Concomitantly, CCR6 deficiency caused a remarkable decrement of M cells. Moreover, adoptive transfer of CCR6^{hi}CD11c^{int} B cells from wild-type mice partially restored the M-cell defect in the CCR6-deficient mice. Collectively, our data indicate that CCR6^{hi}CD11c^{int} B cells possess an M-cell inducing capacity, and that the spatial regulation of CCR6^{hi}CD11c^{int} B cells via the CCL20-CCR6 system may play a vital role in M-cell differentiation in mice.

Bifidobacteria protect from enteropathogenic *Escherichia coli* infection through production of acetate: Importance of a sugar transporter

Intestinal microbiota impact 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 investigate these mechanisms, we used a simplified model of lethal infection with enterohaemorrhagic *Escherichia coli* O157:H7 (O157) of mice associated with certain bifidobacterial strains, together with an integrated 'omics' approach. We showed that genes encoding an ATP-binding-cassette (ABC)-type carbohydrate transporter present in certain bifidobacteria contribute to protecting mice against death induced by O157. We found that this effect was attributed, at least in part, to increased production of acetate. Our data strongly suggest that acetate produced in large amounts by the bifidobacteria possessing the genes encoding the ABC-type transporter exerts its action on the colonic epithelium by inducing anti-inflammatory and/or anti-apoptotic effects, which prevents translocation of the O157 Shiga toxin from the gut lumen into the blood. We therefore propose that acetate produced by protective bifidobacteria improves intestinal defense mediated by epithelial cells and thereby protects the host against lethal infection.

initiates mucosal immune response. ***Nature*** 462, 226-230 (2009)

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Laboratory for Mucosal Immunity

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Student Trainee : **Shimpei Kawamoto** (received PhD from Kyoto University in December 2010)

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

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

A unique gut signature provided by vitamin A and pattern-recognition receptors that modulates the adaptive immune response

Secretion of immunoglobulin A (IgA) is critical for immune homeostasis. We have provided evidence that mucosal IgA secretions need to be continuously produced in order to maintain a highly diverse bacterial community in the gut (Fagarasan et al, *Science*, 2002, Suzuki et al, *PNAS*, 2004). The presence of highly mutated IgA is apparently required to prevent expansion of certain bacteria species (i.e. segmented filamentous bacteria, SFB) that can cause over-activation of the mucosal and systemic immune systems. Intestinal synthesis of highly mutated IgAs occurs mainly in gut lymphoid structures, such as Peyer's patches. More specifically, most of the intestinal IgAs are generated in specialized microenvironments of germinal centers (GC) in the presence of follicular helper T cells (T_{FH}) (Tsuji et al, *Science*, 2009).

GC formation requires the presence of special stromal cells called follicular dendritic cells (FDCs). These radioresistant cells are well known for their ability to retain immune complexes. Although FDCs play a central role in GC development and function, their role in the specialized function of PP to produce high amounts of IgA was not known. In other words, whether the FDC may help partition mucosal and systemic immune compartments was unresolved.

We demonstrate that FDCs are equipped with surface, cytoplasmic and nuclear receptors capable of recognizing

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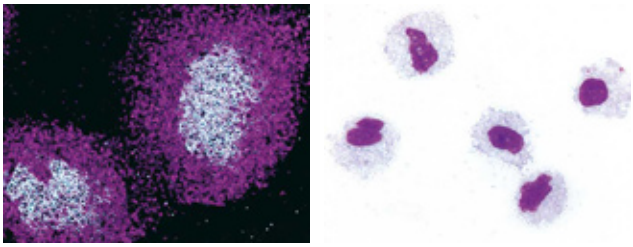

Figure1: *In vivo* and *ex vivo* images of FDCs

Left panel: A horizontal section of the Peyer's patches showing a B cell follicle (B220 showed in *magenta*) built on FDCs (B-cell chemoattractant CXCL13 produced by FDC stained in *blue*). *Right panel*: A micrograph of FDCs sorted from the Peyer's patches, spun on a glass slide and stained with May-Grunwald Giemsa.

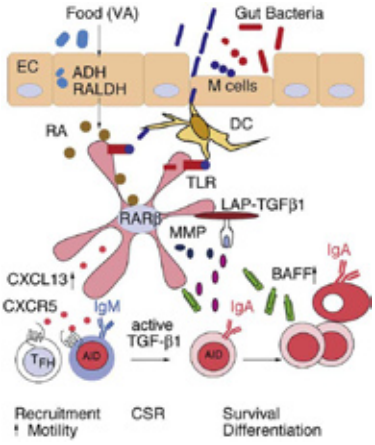

Figure 2 : FDCs promote IgA generation in PPs upon deciphering the gut environmental code.

FDCs express surface and cytoplasmic TLRs and retinoic acid receptors (RARs). Among RARs, RARb is highly expressed in PP FDCs and is induced by RA. Continuous and combined stimulation by bacterial components (delivered by subepithelia dome DCs) through TLR and by RA [produced mainly by the epithelial cells (ECs))] through RARs, up-regulate the expression of chemokines (i.e.CXCL13), adhesion molecules (i.e. VCAM-1, ICAM-1), survival factors (i.e. BAFF) and molecules involved in activation of TGF-b1 (i.e. MMPs, LTBPs). Latent TGF-β1 (LAP- TGF-β1) present on the surface of FDCs can be produced by FDCs or by other local cells including ECs. However, FDCs have the capacity to activate TGF-β1. The factors secreted by PP FDCs facilitate recruitment of B and T cells to the GCs, direct preferential class switching of B cells from IgM to IgA, enhance survival of highly proliferating AID-expressing cells or those recently switched IgA^+ B cells and help differentiation of IgA^+ B cells into plasmablasts that will home to the LP.

gut signal profiles such as vitamin A and bacteria. Furthermore, FDCs convert such signals into effector molecules (CXCL13, BAFF, clusterin, MadCAM-1, matrix modulating and TGF-β1 activating proteins) that are then conveyed to immune cells. Hence, in the GC microenvironment, immune cells are educated by FDCs to respond in a 'mucosal manner', namely to preferential switch to the production of IgA (instead of IgG). (Suzuki K. et al, Immunity. 2010, Comment by Deshane J and Chaplin DD., *Immunity*, 2010)

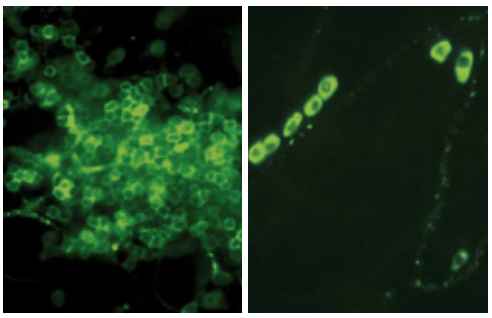

Figure 3 : Whole mount staining of adipose tissues associated with peritoneum. Photomicrographs showing peritoneal B1 cells stained for IgM present in the omentum 'milky' spot (*left*) and B1 cell -derived plasmablasts along the mesenteric vessels (*right*).

The role of dietary vitamin A in bridging the innate and adaptive immune responses

Adaptive immune responses require days to weeks for activation, expansion and differentiation of conventional B2 cells and T cells into effector cells. The optimal transition between early innate responses and the adaptive immune responses is made by special subsets of B cells called B1 cells. These innate-like cells produce the first wave of antibodies required for antigenic clearance. Peritoneal B1 cells are also known to be the source of many gut IgA plasma cells. The mucosal IgA response to commensal bacteria by B1 cells is regarded as part of a primitive mechanism that bridges innate and adaptive immunity in the gut.

We have previously shown that the sensing of bacteria by peritoneal B1 cells (through PRRs) induces complex changes that lead to re-localization of B1 cells to the gut and generation of IgA plasma cells (Ha et al, *J.Exp.Med*, 2006).

Whether peritoneal B1 cells sense other environmental cues from the gut and how these gut signals would impact on the B1 cell biology remained unresolved.

We found that B1 cells are equipped not only with germline encoded and adaptable receptors but also with cytoplasmic and nuclear factors able to detect site-specific metabolic profiles. Thus, B1 cells express high levels of retinoic acid receptors (RAR) and sense the retinoic acid (RA) produced by peritoneum associated adipose tissues, such as omentum and mesentery. Signaling through RAR is essential for regulation of the nuclear factor of activated T cells (NFAT) c1 (through activation of the constitutive promoter), which in turn, is critical for the survival, proliferation and antibody production by B1 cells. In mice, a vitamin-A deficient diet results in reduction of NFATc1 expression in B1 cells, and the almost complete loss of innate-like memory B1 cells. Hence, vitamin A-deficient mice are unable to mount appropriate immune responses to bacterial antigens.

Such environmental regulation of B1 cell biology may be a part of a complex and fine-tuned regulatory pathway involved in immune homeostasis.

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Laboratory for Host Defense

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Both innate and adaptive immunity contribute to host defense in higher organisms. Innate immune receptors function as relatively invariant pathogen sensors and are involved in first-line eradication of pathogens. Furthermore, innate immunity also contributes to the establishment of adaptive immunity, in which lymphocytes use highly diverse receptors to deal with pathogens that evade the innate immune response. Dendritic cells (DCs) are antigen presenting cells critically involved in the sequence of these immune responses. DCs sense various pathogen-derived molecular components and exert their immunostimulatory functions by producing inflammatory cytokines or upregulating expression of costimulatory molecules. Those components are termed immune adjuvants because of their ability to activate DCs. Immune adjuvants are recognized by various pattern recognition receptors including Toll-like receptors (TLRs). Identification of new types of immune adjuvants and characterization of the mechanisms by which they activate DCs should contribute to the development of novel immunoregulatory strategies. We are attempting to clarify how DCs are activated through pattern recognition receptors in order to devise strategies for effective manipulation of the immune response. Various immune adjuvants, including TLR ligands, and gene targeted mice are important tools for this purpose.

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

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

The plasmacytoid DCs (PDC) form a unique DC subset that expresses the nucleic acid sensing TLRs, TLR7 and TLR9, and can produce type I IFN, especially IFN-α, in response to signaling through these receptors. TLR7 and TLR9 are quite similar in their amino acid sequence and can activate similar signaling pathways, which depend on the

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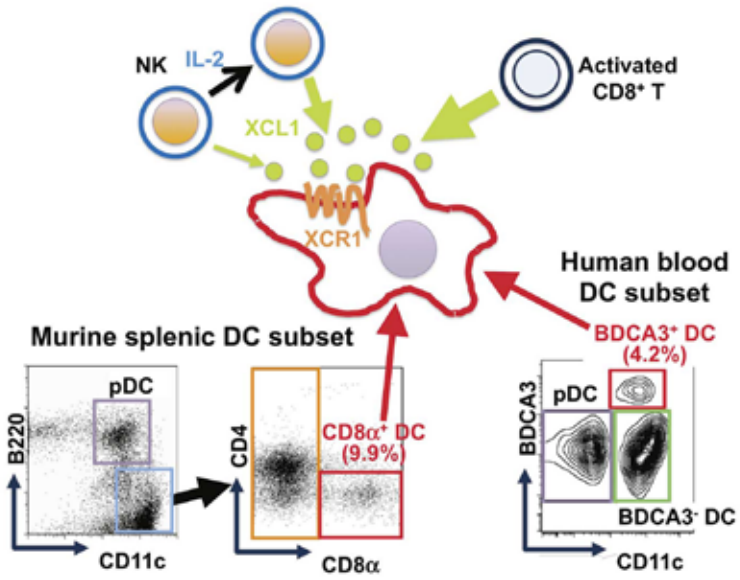

Figure : **Conservation of the XCR1 chemokine receptor and its ligand XCL1 in humans and mice.**

In mouse splenic DC subsets, XCR1 is expressed specifically in the CD8α⁺ cells. An XCR1 ligand, XCL1, is constitutively expressed by NK cells, which increase XCL1 production upon IL-2 stimulation. Activated CD8⁺, but not CD4⁺, T cells produce large amounts of XCL1. In humans, XCR1 is expressed dominantly on BDCA3⁺ DCs, which are the equivalent of murine CD8α⁺ DCs. The human XCL1 expression profile is also similar to the murine profile. Thickness of the green arrows represents relative amounts of XCL1 chemokine production.

cytoplasmic adapter molecule, MyD88. Downstream of MyD88, the signaling pathways become bifurcated into NF-κB and IRF-7 activation pathways, leading to the induction of inflammatory cytokines and type I IFNs, respectively. We have demonstrated that the IκB kinase-α (IKKα), serine threonine kinase, is critically involved in this type I IFN induction pathway in PDC (Hoshino et al. Nature 440:949, 2006). IKKα associates with and phosphorylates IRF-7, which is critical for type I IFN gene expression in response to TLR7/9 signaling. A critical role of IKKα was also elucidated through the analysis on DOCK2-deficient mice (Gotoh et al. JEM 207:721,2010). DOCK2 is a Rac activator and involved in chemokine receptor signaling. DOCK2-deficient mice showed defects in TLR7/9-induced type I IFN production. Furthermore, IRF-7 activation by TLR7/9 was impaired in DOCK2-deficient pDC. Activation of IKKα, but not of any other signaling molecules was defective, indicating that DOCK2 is critical for IKKα activation, which is required for type I IFN induction by TLR7/9 signaling.

We have further tested how IKK is involved in human pDC function. The IKK inhibitor impaired type I IFN, but not TNF-α, production by human pDC activated by TLR7/9 signaling. TLR7/9-induced IRF-7 activation was also impaired by IKK inhibitor. These findings indicate that the IKK-IRF-7 axis should also function in human pDC (Arth. Res. Therap., 12:R87,2010).

Regulatory mechanisms for a TLR3-expressing DC subset

TLR3 is a sensor for dsRNA that is specifically expressed in CD8α⁺ DC among murine splenic DC subsets. The CD8α⁺ DCs are uniquely specialized for crosspresentation of exogenous antigens to CD8⁺ T cells, thereby playing key roles in anti-viral and anti-tumor immunity. TLR3 signaling can augment the crosspresenting activity of CD8α⁺ DC. In order to clarify the molecular mechanisms for regulating CD8α⁺ DC functions, we have first analyzed gene expression profiles of DC subsets. A chemokine receptor, XC chemokine receptor 1 (XCR1), was highly and exclusively expressed in CD8α⁺ DCs (Yamazaki et al. BBRC 397:756,2010). The*Xcr1* gene was not expressed in the other splenic cells such as T cells, B cells or NK cells. An XCR1 ligand, XCL1, was constitutively expressed only by NK cells. XCL1 was detected at 0.5-1.0 ng/ml in the serum and NK cell depletion decreased the serum XCL1 level. NK cells *in vitro* increased XCL1 production in response to IL-2, but not to IL-12, IL-15, IL-18 or IFN-α. CD8⁺ T cells did not constitutively express XCL1, but produced high amounts upon activation with anti-CD3/28, whereas CD4⁺ T cells failed to express XCL1 in either unstimulated and activated conditions.

We have then analyzed XCR1 and XCL1 expression in human cells. Human XCR1 was functionally expressed by BDCA3⁺ DCs, which are an equivalent of murine CD8α⁺ DCs. Furthermore, XCL1 expression was high in NK cells and activated CD8⁺ T cells. Thus, the expression pattern of XCR1 and its ligand is conserved both in mouse and human. This system should thus play a critical role in innate and adaptive cytotoxic immunity in many species (Figure).

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Laboratory for Infectious Immunity

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We have demonstrated that the expression level of MHC class II (MHC II) is regulated through MARCH-I-mediated ubiquitination of the MHC II β chain and have been intensively examining the physiological role of this process. In this regard, last year, we have found that MHC II ubiquitination is required for the maintenance of dendritic cells (DCs). Therefore, one important project of my lab is to reveal the molecular basis of DC maintenance by MHC II ubiquitination. Once we know that molecular mechanism, we can formulate specific strategies for our final goal. In addition, we have started collaborative projects with several groups to understand how antigen-presentation is regulated by MHC II ubiquitination. This information should also provide insight into the physiological role of MARCH-1.

MARCH-I belongs to a family of membrane-bound E3 ubiquitin ligases, named MIR. MIR family members (MIRs) recognize different membrane molecules (e.g. MHC class I, ICAM-1). However, it is still unclear how MIRs recognize their substrates and how MIR-mediated ubiquitination induces lysosomal degradation. To examine these issues, we have generated a Tet-on expression system for MIR E3 ligases. This experimental system was generated by using B cells and HeLa cells, and enables us to analyze the detailed relationship between the status of ubiquitination and the trafficking of substrate molecules. Ubiquitination-mediated regulation of transmembrane proteins (e.g. cytokine receptors) contributes to critical cell signaling events, thus, using this experimental model we should be able to provide important insight into fields beyond immunology.

MHC II is constitutively ubiquitinated in the steady state. However, MHC II ubiquitination is quickly down-regulated upon DC activation/maturation. Interestingly, MARCH-I expression is also down-regulated by DC activation/maturation. Given that MARCH-I ubiquitinates only the peptide-bound form of MHC II, which is able to stimulate cognate CD4 T cells, down-regulation of MARCH-I is thought to be an important initiation signal for immune responses. To test this hypothesis, we examined whether MARCH-I-deficient DCs efficiently present model antigens through MHC II. As we expected, MARCH-I-deficient DCs efficiently presented HEL and I-E alpha through MHC II, as judged by specific antibody responses. In addition, the half life of peptide-pre-

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4. Goto, E., M. Mito-Yoshida, M. Uematsu, Y. M. Aoki, Matsuki, M. Ohmura-Hoshino, M. Miyagishi, H. Hotta, and S. Ishido. An excellent monitoring system for surface ubiquitination-induced internalization in mammals. ***PLoS ONE***, 2008, 30;3(1):e1490.

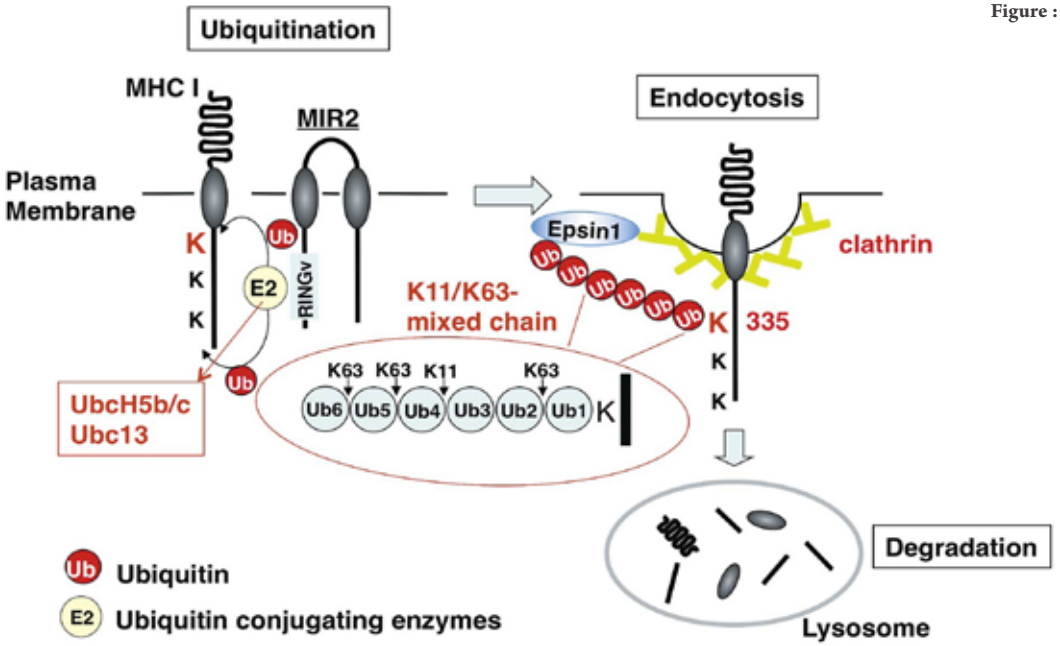

Figure : Hypothetical model of down-regulation by MIR2

Based on our current understanding, the most logical model at this point may be that Epsin binds to K63-linked di-ubiquitin and longer chains, and that the presence of K11 (either as a mixed chain, as a cap or as a side branch) facilitates some events related to Ub-dependent endocytosis. The K11 linkages might support the extension of the polyubiquitin chain required for efficient internalization, or these linkages might generate a suitable chain topology for the association between K63 linkages and epsin1 *in vivo*. In addition, K11 linkages may interact with a second ubiquitin-interacting protein that is needed for endocytosis and/or subsequent sorting.

senting MHC II was extended in MARCH-I-deficient DCs. Thus, loss of MHC II ubiquitination by down-regulation of MARCH-I enhances antigen-presentation. Currently, we are examining how enhanced antigen-presentation alters immune responses (e.g. Th1, Th2, Th17 development).

As second project, we are examining how MIR E3s recognize their substrates and regulate the trafficking of substrates. During this analysis, we found that a novel type of polyubiquitin chain can function as an internalization signal. Polyubiquitination is the sequential reaction between lysine residues on ubiquitin moieties, catalyzed by E3 ubiquitin ligase (E3), and generates the polyubiquitin chain on substrate proteins. All seven lysine residues (K6, K11, K27, K29, K33, K48 and K63) on ubiquitin can be utilized for the formation of polyubiquitin chains. Among them, K48 and K63 were classically reported as the acceptor lysine residues for the generation of polyubiquitin chains. The K48-linked polyubiquitin chain and the K63-linked chain are well-established inducers of proteasome-dependent degradation and signal transduction, respectively. However, the other linkages are not clearly established.

We previously generated a tetracycline-inducible (Tet-on) system for a member of the MIR family. MIR family members are membrane-bound E3 ubiquitin ligases and have been reported to induce ubiquitination-dependent internalization *in vivo*. This Tet-on system could be used to visualize the detailed status of the polyubiquitin chain, which induces the *in vivo* internalization. Recently, the Gygi research group has also successfully developed a detection system for polyubiquitin chains generated *in vivo* using mass spectrometry. By using the Tet-on expression system for MIR2 and quantitative mass spectrometry, we have addressed the issue of how the polyubiquitin chain contributes to receptor internalization. We have found that the polyubiquitin chain generated by MIR2 is a K11- and K63-mixed linkage chain and that this mixed polyubiquitin chain is required for efficient epsin1-dependent internalization of MHC I (Figure). Although further studies are required, our working hypothesis is the K11 and K63 mixed polyubiquitin chain is a common internalization signal utilized by MIR family members. These results indicate that the structure of a functional polyubiquitin chain is not simple, as had been thought. Therefore, it will be important examine the detailed structure of ubiquitin chains that contribute to biological events other than internalization/trafficking, e.g., protein degradation and gene transcription, and to reveal the molecular basis of how such complex chains are generated *in vivo*.

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Laboratory for Innate Cellular Immunity

Team Leader **Masato Tanaka**

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

Technical Staff : **Ai Kurita**
Minako Aihara

Student Trainee : **Kazunori Karasawa**

Every type of cell has its own life span and aged cells undergo apoptosis for their turnover. The immune system eliminates harmful cells, including virus-infected cells or cancer cells, by inducing their apoptosis. Although a substantial number of cells constantly undergo apoptosis even in physiological conditions, it is very difficult to detect such cells, primarily because they are swiftly eliminated by phagocyte cells, such as macrophages and dendritic cells (DCs). Phosphatidylserine (PS) is exposed on the apoptotic cell surface, and most phagocytes recognize this lipid through specific receptors or soluble molecules. A defect in this clearance process has been shown to cause unremitting inflammation in acute injury models. In addition to the resolution of inflammation, apoptotic cell clearance by phagocytes plays a crucial role in maintenance of self tolerance. In the periphery, tissue-resident dendritic cells (DCs) constantly phagocytose apoptotic cells generated during normal tissue turnover, and migrate to draining lymph nodes where they present antigens derived from the apoptotic cells. This presentation of self-antigens leads to deletion or anergy of any self-reactive T cells, thereby providing another mechanism to maintain T cell tolerance. Failure of apoptotic cell clearance results in autoimmune disorders. The Laboratory for Innate Cellular Immunity is investigating the molecular mechanisms for recognition and phagocytosis of dying cells by phagocytes, and the pathological relevance of impaired phagocytosis to inflammatory disorders including autoimmune diseases.

Role of CD169-positve sinus macrophages in anti-tumor Immunity

The generation of tumor antigen-specific cytotoxic T cells (CTLs) is considered crucial for the induction of anti-tumor immunity. To generate and activate T cell immunity against tumor antigens, antigen presenting cells (APCs) must initially acquire tumor cell-associated antigens, and one of the major sources of tumor antigens is dead tumor cells. Massive tumor cell death is induced by anti-cancer therapy, and particulate matter from the dead tumor cells provides large amounts of tumor-associated antigens. However, previous studies have been unable to clarify the mechanism by which APCs in the lymph node (LN) internalize and crosspresent

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these types of antigens to $CD8^+$ T cells. Antigen presentation in peripheral LNs is thought to be coordinately performed by migratory DCs that enter the LN from peripheral tissues via the lymphatics, and LN-resident APCs. However, little is known about the role of different APCs in the clearance of dead cells and the presentation of dead cell-associated antigens in peripheral tissue or LNs.

We recently demonstrated a critical role of $CD169^+$ macrophages located in the LN sinus in induction of anti-tumor immunity by dead tumor cells. Subcutaneous immunization with irradiated tumor cells induces activation of tumor antigen-specific CD8 T cells, and consequently protects mice from a syngeneic tumor. Subcutaneously injected dead cells reach LNs via lymphatic flow and are mainly taken up by $CD169^+$ macrophages. Tumor antigen-specific $CD8^+$ T cell activation and subsequent anti-tumor immunity are severely impaired in mice depleted of $CD169^+$ macrophages. $CD11c^+$, $CD169^+$ macrophages phagocytose dead tumor cells transported to the LN and directly crosspresent cell-associated antigens to stimulate $CD8^+$ T cell proliferation. By contrast, neither migratory dendritic cells (DCs) nor lymph node-resident conventional DCs are essential for the crosspresentation of tumor antigens. Thus, we have identified $CD169^+$ macrophages as lymph node-resident APCs dominating early activation of tumor antigen-specific $CD8^+$ T cells.

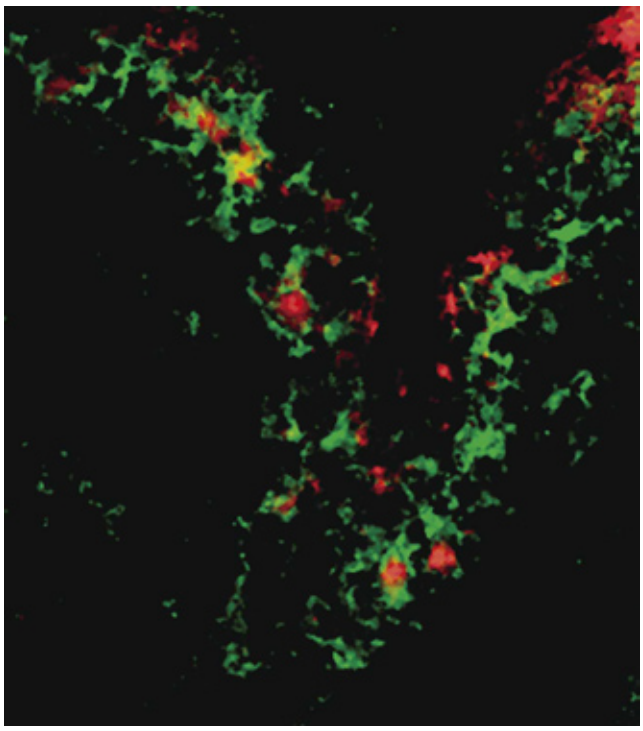

Figure : CD169-positive macrophages (*Green*) are the gate keepers of the lymph node. They reside in the lymph node sinus and make initial contact with particles in the lymphatic fluid. Dead tumor cells (*Red*) that flow into the lymph node are phagocytosed by $CD169^+$ macrophages.

xCT is required for survival of activated macrophages and protection from tumorigenesis

Macrophages are key players in the initiation and regulation of inflammation. Macrophages infiltrating into an inflammatory site, e.g., an acute injury or local infection, recognize invading microorganisms or endogenous adjuvants released by injured cells. In response to this recognition, macrophages produce inflammatory cytokines and chemokines. Although the precise mechanisms of inflammation-induced tumorigenesis remain unclear, cytokines or growth factors persistently produced at the chronic inflammatory site may stimulate the proliferation of DNA-damaged cells.

During the course of inflammation and its resolution, macrophages are exposed to various cytotoxic substances, including reactive oxygen species. Thus, macrophages require a mechanism for protection against oxidative stress to survive at the inflammatory site. We recently showed that xCT, a component of the anionic amino acid transport system x_c^-, was significantly upregulated in activated infiltrating cells, including macrophages and neutrophils, at the inflammatory site. System x_c^- mediates the uptake of extracellular L-cystine and is consequently responsible for maintenance of intracellular glutathione levels. We established an xCT loss-of-function mouse mutant line by N-ethyl-N-nitrosourea mutagenesis. Macrophages from $xCT^{mu/mu}$ mice exhibited cell death in association with the excessive release of High Mobility Group Box Chromosomal Protein 1 upon stimulation with LPS, suggesting that xCT deficiency causes unremitting inflammation due to the impaired survival of activated macrophages at the inflammatory site. Subcutaneous injection of 3-methylcholanthrene (3-MCA) induces the generation of fibrosarcomas in association with inflammation. When 3-MCA was injected subcutaneously into mice, xCT mRNA was upregulated *in situ*. In $xCT^{mu/mu}$ mice, inflammatory cytokines such as IL-1β and TNFα were overexpressed and the generation of 3-MCA-induced fibrosarcomas was accelerated. These results clearly indicate that the defect of the oxidative stress protective system impaired survival of activated macrophages and subsequently enhanced tumorigenesis.

4. Qiu C. H., Miyake Y., Kaise H., Kitamura H., Ohara O., and Tanaka M. Novel Subset of CD8α+ Dendritic Cells Localized in the Marginal Zone is Responsible for Tolerance to Cell-associated Antigens. ***J. Immunol.*** 182, 4127-4136 (2009)
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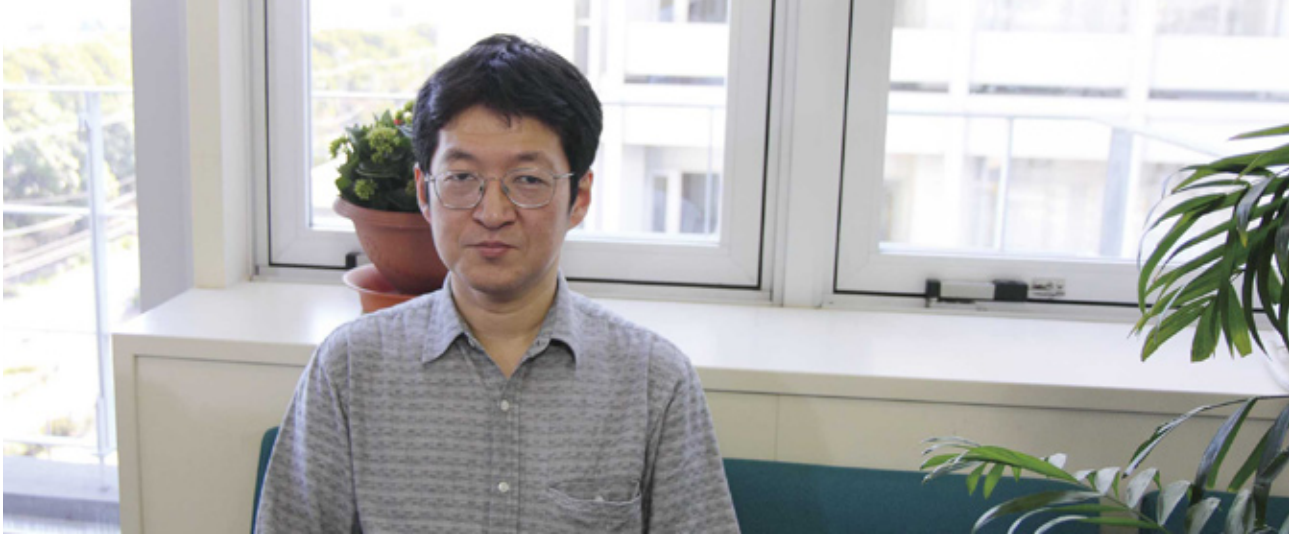

Research Unit for Inflammatory Regulation

Unit Leader **Takashi Tanaka**

Technical Staff : **Emiri Haga**

The inflammatory response is an important host defense mechanism to sense and eliminate invading microbial pathogens. Innate immune cells, such as macrophages and dendritic cells, first detect pathogens by their sensors of pathogen associated molecular patterns (e.g. Toll-like receptors [TLR]). TLR signaling then leads to 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. Depending on their nature, these inflammatory responses can then direct T-helper (Th) lymphocyte differentiation into distinct subsets, such as Th1, Th2 and Th17 cells, to combat various pathogens. These initially helpful inflammatory responses must be terminated at appropriate time points; otherwise an overblown response can damage normal tissue and may lead to 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.

Molecular mechanisms to regulate PDLIM2-mediated termination of NF-κB activation

PDLIM2, also known as SLIM (STAT-interacting protein), is a nuclear protein that contains both PDZ and LIM domains and belongs to a large family of LIM proteins. PDLIM2 was originally isolated in CD4+ T cells as a nuclear ubiquitin E3 ligase for the STAT4 transcription factor, thereby suppressing Th1 cell differentiation (Tanaka T et al, Immunity, 2005). We have also demonstrated that PDLIM2 negatively regulates 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 T et al, Nat. Immunol., 2007). PDLIM2 binds to and promotes polyubiquitination of p65 through its LIM domain. In addition, PDLIM2 targets p65 to discrete intranuclear compartments, called PML nuclear bodies. Polyubiquitinated p65 is ultimately degraded by proteasomes in these compartments. However, it remains unclear how the activity of PDLIM2 is regulated. We are therefore attempting to isolate proteins that bind to PDLIM2 and control its activity. We have recently found that heat shock protein 70 (HSP70) translocates into the nucleus in response to TLR stimulation, binds to PDLIM2, and augments the activity of PDLIM2 to degrade p65 and suppress NF-κB signaling. We have further demonstrated that HSP70 also interacts with BAG-1, a protein that can

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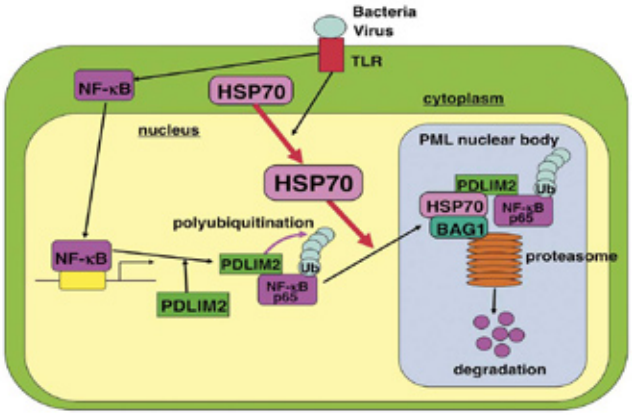

Figure1: PDLIM2 binds to the p65 subunit of NF-κB and promotes p65 polyubiquitination in the nucleus. PDLIM2 then targets p65 into discrete intranuclear compartments, called PML nuclear bodies. HSP70 binds to PDLIM2 and facilitates delivery of the NF-κB-PDLIM2 complex to the proteasome cooperatively with BAG1. Polyubiquitinated p65 is ultimately degraded by the proteasome in PML nuclear bodies.

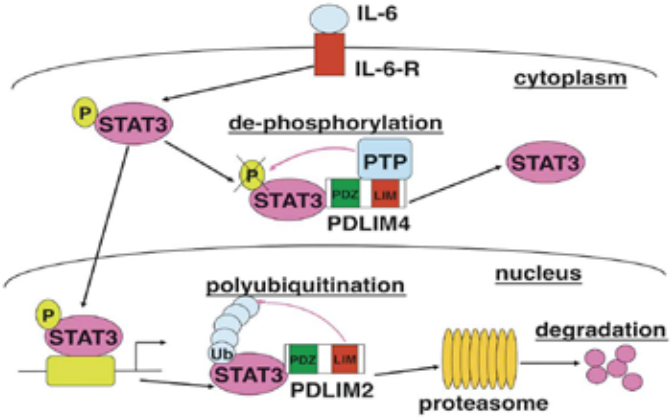

Figure 2: PDLIM2 and PDLIM4 regulate Th17 cell-mediated inflammatory responses by different mechanisms. PDLIM2 interacts with STAT3 and promotes its ubiquitin/proteasome-dependent degradation, whereas PDLIM4 binds to STAT3, but recruits PTP and enhances its dephosphorylation.

bind to both HSP70 and the proteasome, and facilitates the delivery of the NF-κB-PDLIM2 complex to the proteasome. Consistently, knockdown of either HSP70 or BAG-1 by small interfering RNA resulted in larger amounts of nuclear p65 and augmented production of proinflammatory cytokines. These data delineate an essential role of HSP70 and BAG-1 in PDLIM2-mediated termination of NF-κB activation.

The role of LIM proteins in the *in vivo* inflammatory response

The LIM protein family is classified into three groups and PDLIM2 belongs to Group 3 LIM proteins, which contain one or multiple LIM domains at their C-terminus. About 10 proteins have been identified in Group 3, but their functions in the immune system remain unclear. We have been using *in vivo* models to clarify how these LIM proteins differentially regulate inflammatory responses. Granuloma formation is an important host defense mechanism against intracellular bacteria. However, uncontrolled granulomatous responses cause tissue damage and impair normal organ function in several human autoimmune and inflammatory diseases. Recent studies have suggested that exaggerated Th17 cell-mediated responses may contribute to the development of granulomatous inflammation. We showed that PDLIM2 negatively regulated Th17 development and granulomatous responses by acting as a nuclear ubiquitin E3 ligase targeting STAT3, the transcription factor critical for commitment to the Th17 lineage. PDLIM2 promoted polyubiquitination and proteasome-dependent degradation of STAT3, thereby disrupting STAT3-mediated gene activation. PDLIM2 deficiency resulted in increased amounts of nuclear STAT3, enhanced Th17 cell differentiation, and exacerbated *Propionibacterium* acnes-induced liver granuloma formation. Moreover, we found that PDLIM4, another LIM protein family member, also negatively regulated Th17 differentiation by suppressing STAT3 activation. Interestingly, however, PDLIM4 did not promote STTA3 degradation but instead inhibited tyrosine phosphorylation of STAT3. We further demonstrated that PDLIM4 bound to and recruited a protein tyrosine phosphatase, which facilitated dephosphorylation of STAT3. Thus, PDLIM2 and PDLIM4 suppress STAT3 signaling through distinct mechanisms. Our findings delineate an essential role of these LIM proteins in negatively regulating Th17-mediated inflammatory responses and provide a potential therapeutic target for inflammatory diseases.

Research Unit for Therapeutic Model

Unit Leader **Kanako Shimizu**

Technical staff : **Miki Asakura**

The goal of our laboratory is to develop immunotherapeutic models for cancer. We have been focusing on the biological role of dendritic cells (DCs) *in vivo* as a link between innate and adaptive immunity. NKT cells have unique immunoregulatory features that include the ability to rapidly produce large quantities of cytokines. We have attempted to 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. We have recently established an immunotherapeutic strategy using 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 elucidated the mechanism of DCs in detail in the AAVC-treated mice. Also, to lead to the launch of clinical studies, we have been performing preclinical studies in collaboration with Dr. Fujii, Cellular Immunotherapy Unit. Second, we have studied the biological interactions between NK cells and DCs following DC therapy. The NK antitumor response in DC-vaccinated mice depended on $CD4^+$ T cells, but not on $CD8^+$ T cells or NKT cells.

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

We previously demonstrated that the administration of tumor/Gal cells could generate innate and adaptive immunity through the maturation of DCs in a tumor-specific manner. In the current study, instead of tumor cells, we used allogeneic fibroblast cells loaded with α-GalCer and transfected with antigen-encoding mRNA, thus combining the adjuvant effects of iNKT cell activation with delivery of an antigen of choice to DCs *in vivo*. We found that these cells produce antigen protein and activate NK and iNKT cells. When injected into MHC mismatched mice, they elicited antigen-specific T cell responses and provided tumor protection, suggesting that these immune responses depend on host DCs. Also, antigen-expressing fibroblasts loaded with α-GalCer lead to a more potent T cell response than those expressing NK cell ligands. Thus, glycolipid-loaded,

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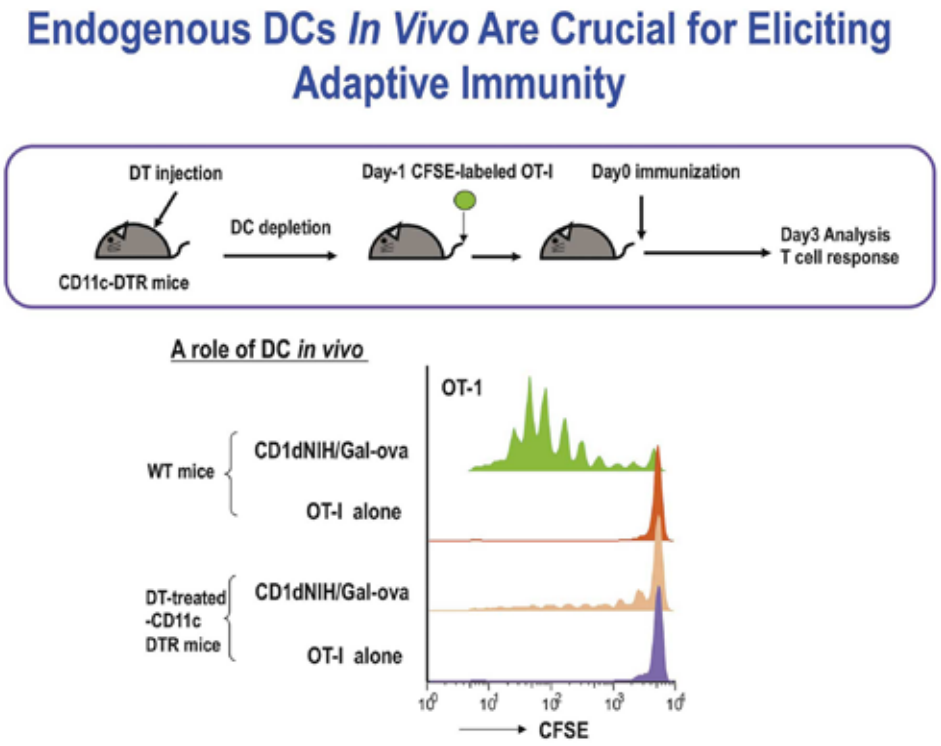

Figure : **Endogenous dendritic cells *in vivo* play a key role for induction of adaptive immunity in the AAVC therapy model.**

OVA-specific CD8 T cells (OT-1) were transferred into WT mice, which were then injected with OVA mRNA-trasnfected AAVCs, and robust OT-1 cell proliferation was observed. However, if we used CD11c^{-}DTR mice as recipients and depleted host CD11c^{+}DC by DT injection, the OT-1 cells did not proliferate, indicating that host dendritic cells, but not AAVC, play a key role in cross-priming in the AAVC therapy system.

mRNA-transfected allogeneic fibroblasts act as adjuvant vector cells (AVCs) to promote iNKT cell activation, leading to DC maturation and T cell immunity. By harnessing the innate immune system and generating an adaptive immune response to a variety of antigens, this unique tool could prove clinically beneficial in the development of immunotherapies for malignant and infectious diseases.

Anti-tumor NK cell response by dendritic cell (DC) therapy

We have examined the relationship between NK cells and endogenous DCs following administration of DCs. We vaccinated mice with DCs and evaluated long term resistance to tumor challenge. When DC-treated mice were challenged with tumor cells even several months later, NK cells were quickly activated to express CD69 and produce IFN-γ. The NK cells could resist a challenge with several different tumors *in vivo*. Even if the NK cells were depleted with anti-NK1.1 Ab treatment, the activated cells recovered, indicating that tumor-responsive NK cells were being generated continuously as a result of vaccination with DCs and were not true memory cells. The NK antitumor response in DC-vaccinated mice depended on CD4^{+} T cells, but not on CD8^{+} T cells or NKT cells. However, both vaccine DCs and endogenous DCs were required for the immune response. These results indicate that DC therapy in mice induces long lasting innate NK cell activation through a pathway that requires host DCs and CD4^{+} T cells.

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

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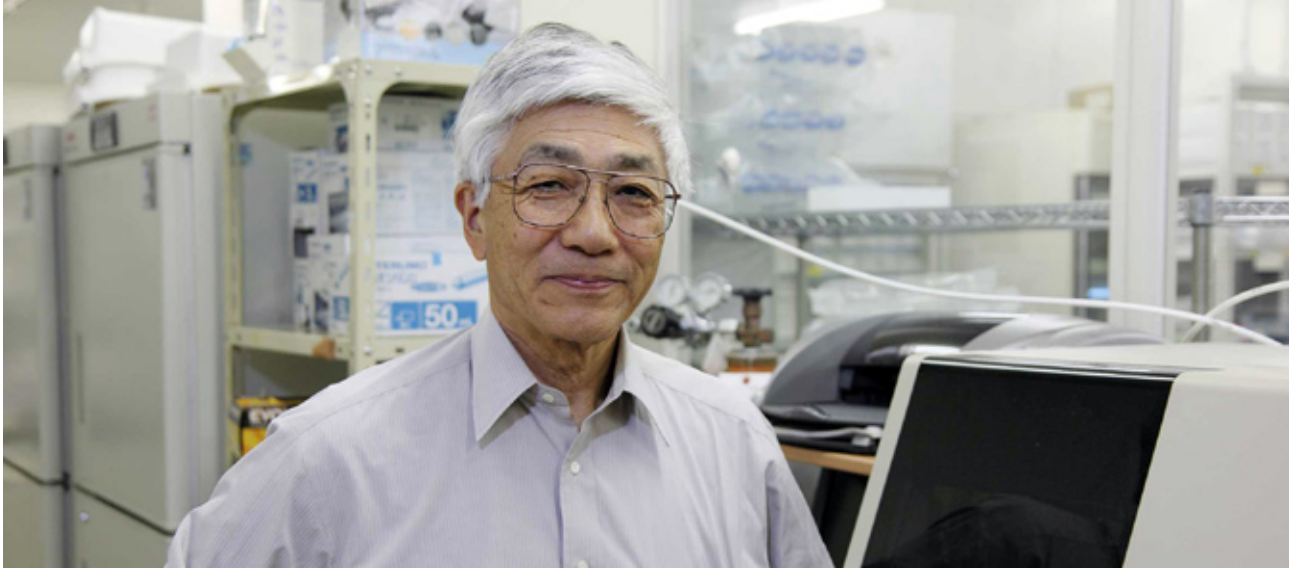

Laboratory for Immune Regulation

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Senior Research Scientist : **Hiroshi Watarai**
Research Scientists : **Takuya Tashiro**, **Masao Shiozaki**, **Michishige Harada**
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Technical Staff : **Sakura Sakata**, **Etsuko Sekine**, **Yuko Nagata**, **Tomokuni Shigeura**, **Yuko Sasaki**, **Chihiro Sasaki**, **Diana Eissens**

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

Identification of $CD4^-CD8^-$ double-negative natural killer T cell precursors in the thymus

It is well known that CD1d-restricted Vα14 invariant natural killer T (NKT) cells are derived from cells in the $CD4^+CD8^+$ double-positive (DP) population in the thymus. However, the developmental progression of NKT cells at earlier stages remains unclear, and the possible existence of NKT cell precursors in stages earlier than the DP stage remains to be tested. We have demonstrated that NKT cell precursors that express invariant Vα14-Jα18 transcripts, but are devoid of surface expression of the invariant Vα14 receptor, are present in the late $CD4^-CD8^-$ double-negative (DN)4 stage and have the potential to generate mature NKT cells both *in vivo* and *in vitro*. Moreover, this DN4 population in CD1dKO mice is similar to that with NKT cell potential in wild-type C57BL/6 mice, but failed to develop into NKT cells *in vitro*. However, these CD1dKO precursors could develop into NKT cells when co-cultured with normal thymocytes or in an *in vivo* experimental setting, indicating that functional NKT cell precursors are present in CD1dKO mice. Together, these results demonstrate that the thymic DN4 fraction contains NKT cell precursors. Our findings have provided new insights into the early development of NKT cells prior to surface expression of the invariant Vα14 antigen receptor and suggest a possible alternative developmental pathway of NKT cells.

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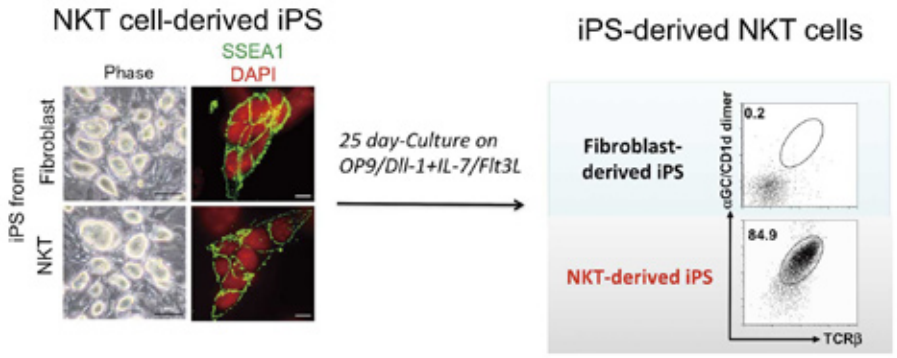

Figure :
Methods for generating clonal NKT cells from iPS cells. iPS cells derived from somatic cells give rise to diversified lymphocytes due to the gene rearrangement during their differentiation (*upper*). On the other hand, (i.e. NKT cell) iPS cells derived from a mature lymphocyte (e.g., an NKT cell in this example) generate clonal lymphocytes with desired functions (*lower*).

Generation of functional NKT cells *in vitro* from embryonic stem cells (ESCs) bearing a rearranged invariant Vα14-Jα18 TCRα gene

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

High-mobility group box 1 (HMGB1) is involved in the initial events of the early loss of transplanted islets in mice

Islet transplantation for the treatment of type 1 diabetes mellitus is limited in its clinical application mainly due to early loss of the transplanted islets, resulting in low transplantation efficiency. NKT cell–dependent IFN-γ production by Gr-1^{+}CD11b^{+} cells is essential for this loss, but the upstream events in the process remain undetermined. We demonstrated that HMGB1 plays a crucial role in the initial events of early loss of transplanted islets in a mouse model of diabetes. Pancreatic islets contain abundant HMGB1, which was released into the circulation soon after transplantation of islets into the liver. Treatment with an HMGB1-specific antibody prevented the early islet graft loss and inhibited IFN-γ production by NKT cells and Gr-1^{+}CD11b^{+} cells. Moreover, there was no early islet graft loss in mice lacking either of the known HMGB1 receptors, TLR2 or receptor for advanced glycation end products (RAGE), but such loss did occur in mice lacking the known HMGB1 receptor TLR4. Mechanistically, HMGB1 stimulated hepatic mononuclear cells (MNCs) *in vivo* and *in vitro*; in particular, it upregulated CD40 expression and enhanced IL-12 production by DCs, leading to NKT cell activation and subsequent NKT cell–dependent augmented IFN-γ production by Gr-1^{+}CD11b^{+} cells. Thus, treatment with either IL-12– or CD40L-specific antibody prevented the early islet graft loss. These findings indicate that the HMGB1-mediated pathway eliciting early islet loss is a potential target for intervention to improve the efficiency of islet transplantation.

Murine induced pluripotent stem cells (iPSCs) can be derived from and differentiate into NKT cells

NKT cells demonstrate antitumor activity when activated to produce Th1 cytokines by DCs loaded with α-galactosylceramide, the prototypic NKT cell-activating glycolipid antigen. However, most cancer patients do not have sufficient numbers of NKT cells to induce an effective immune response in this context, indicating the need for a source of NKT cells that could be used to supplement the endogenous cell population. iPSCs hold tremendous potential for cell-replacement therapy, but whether it is possible to generate functionally competent NKT cells from iPSCs had not been rigorously assessed. We successfully derived iPSCs both from embryonic fibroblasts from mice harboring functional NKT cell-specific rearranged T cell receptor loci in the germline and from splenic NKT cells from WT adult mice. These iPSCs could be differentiated into NKT cells *in vitro* and secreted large amounts of the Th1 cytokine IFN-γ. Importantly, iPSC-derived NKT cells recapitulated the known adjuvant effects of natural NKT cells and suppressed tumor growth *in vivo*. These studies demonstrate the feasibility of expanding functionally competent NKT cells via an iPSC phase, an approach that may be adapted for NKT cell-targeted therapy in humans.

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Laboratory for Dendritic Cell Immunobiology

Team Leader **Katsuaki Sato**

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Dendritic cells (DCs) are essential antigen-presenting cells (APCs) that initiate primary immune responses. DCs consist of heterogeneous subsets, mainly classified into conventional DCs (cDCs) and plasmacytoid DCs (pDCs), which are distinguishable by surface and intracellular phenotypic markers, immunologic function, and anatomic distribution. Immature DCs (iDCs) serve as sentinels, recognizing invading pathogens or virus-infected cells through various pattern-recognition receptors. Subsequently, they become mature DCs (mDCs) with up-regulated expression of MHC and costimulatory molecules under inflammatory conditions. The mDCs then 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. The tolerogenic mechanisms include recessive tolerance mediated by clonal deletion and anergy as well as dominant tolerance involving active immune suppression by $CD4^+Foxp3^+$regulatory T (T_{reg}) cells in the periphery, a function of likely importance in self-tolerance as well as 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.

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

Oral tolerance is a key feature of intestinal immunity, generating systemic tolerance to food antigens. However, the molecular mechanism mediating oral tolerance remains unclear. In this study, we examined the role of the B7 family members of costimulatory molecules in the establishment of oral tolerance. Deficiencies of B7-H1 and B7-DC abrogated oral tolerance; instead there was an immune response accompanied by enhanced antigen-specific $CD4^+$T cell-

Recent publications

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

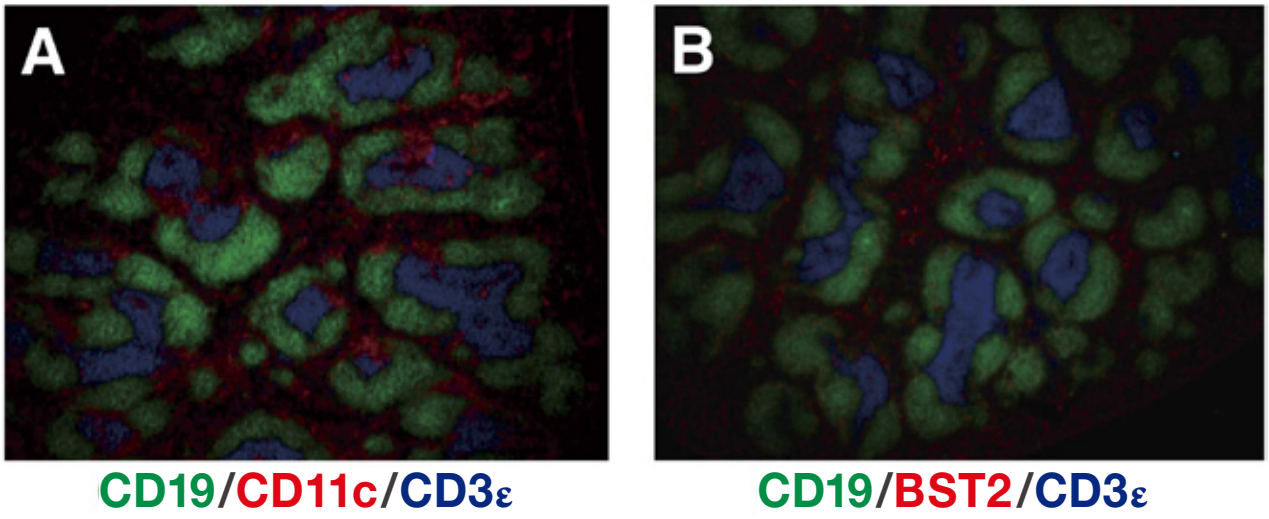

Figure : **Splenic localization of CD11c^{high}cDCs and BST2^{+}pDCs.** Immunofluorescent microscopic analysis was performed on frozen sections that were stained for CD19 (*green*), CD3ε (*blue*) and CD11c (*red*; A) or BST2 (*red*; B). Original magnification; 4x.

responses and IgG_1 production. Mesenteric lymph node (MLN) dendritic cells (DCs) displayed higher levels of B7-H1 and B7-DC than systemic DCs, whereas they showed similar levels of CD80, CD86, and B7-H2. Relative to systemic DCs, the MLN DCs enhanced the antigen-specific generation of $CD4^+Foxp3^+$inducible regulatory T (iT_{reg}) cells from $CD4^+Foxp3^-$T cells rather than $CD4^+$effector T (T_{eff}) cells, owing to their dominant expression of B7-H1 and B7-DC. Furthermore, the antigen-specific conversion of $CD4^+Foxp3^-$T cells into $CD4^+Foxp3^+iT_{reg}$ cells occurred in MLNs to a greater extent than in peripheral organs during oral tolerance under steady-state conditions. Moreover, this conversion required B7-H1 and B7-DC more than other B7 family members, whereas it was severely impaired under inflammatory conditions. In conclusion, our findings suggest that B7-H1 and B7-DC expressed on MLN DCs are essential for establishing oral tolerance through the *de novo* generation of antigen-specific $CD4^+Foxp3^+iT_{reg}$ cells.

Naturally occurring CD49b$^+$CD200R3$^+$regulatory DCs induce regulatory T cell-mediated peripheral tolerance

Despite their key functions in multiple aspects of the immune system, there is much to be learned about the DCs, including which subset is specialized for the induction of immunological tolerance. We have identified a naturally occurring DC subset with a regulatory function, termed regulatory DCs (DC_{regs}). DC_{regs} generated from bone marrow *in vitro* (BM-DC_{regs}) exclusively expressed CD200 receptor 3 (CD200R3), which exerted a suppressive function in the antigen-specific $CD4^+$T-cell response. $CD49^+CD200R3^+$cells showed similarities in phenotype and function to BM-DC_{regs}, which formally distinguishes them from other leukocytes, suggesting that they are the natural counterpart of BM-DC_{regs}. *In vivo*, $CD49^+CD200R3^+$cells not only generated antigen-specific anergic $CD4^+$T cells but also induced the peripheral conversion of $CD4^+CD25^+Foxp3^+$regulatory T (T_{reg}) cells from $CD4^+CD25^-Foxp3^-$T cells. In addition, $CD49^+CD200R3^+$cells as well as BM-DC_{regs} protected against experimental autoimmune encephalomyelitis (EAE), an effect mediated through the suppression of the development of pathogenic T helper type (T_H)1 cells and T_H17 cells, and the depletion of these subsets enhanced EAE pathogenesis. In conclusion, $CD49^+CD200R3^+$cells act as naturally occurring DC_{regs} to maintain immunological tolerance and to regulate immune pathogenesis.

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Laboratory for Immunochaperones

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Student Trainee : **Shizuha Uda**

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

The role of hsp90 in antigen cross-presentation

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

Recent publications

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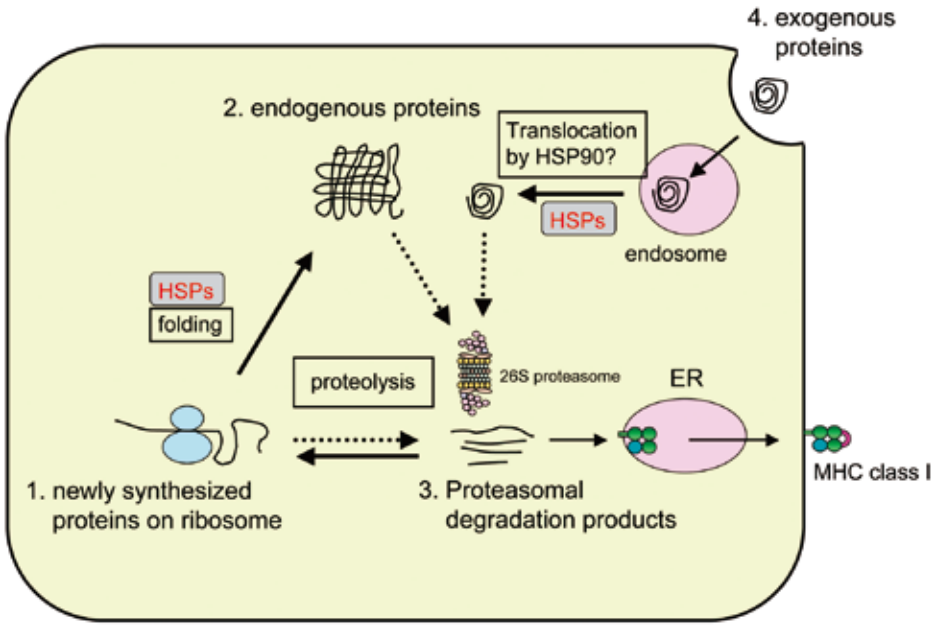

Figure :
HSPs mediate degradation of a few percent of newly synthesized proteins as well as mature proteins. In dendritic cells, HSP90 seems to facilitate the degradation of even internalized exogenous proteins following their translocation to the cytoplasm. The proteolysis step depends on the ubiquitin-proteasome system to produce peptides presentable by MHC class I molecules and amino acids that are reused for protein synthesis.

in the intracellular antigenic milieu. Each step towards proteolysis by the proteasome is regulated, at least in part, by HSPs. In considering how HSPs are involved in cross-presentation (in terms of translocation of endosomal antigen to the cytosol), there might be two distinct mechanisms. First, HSPs direct carboxyl terminus of hsc70-interacting protein (CHIP)-mediated polyubiquitinylation of retro-translocated unfolded proteins for proteasomal degradation. Second, HSPs direct retro-translocation of proteins from the endosome to cytosol, a mechanism that is supported by evidence that Bip and cytosolic hsp70/hsp90 drive polypeptides into the ER and the mitochondria, respectively. Our recent observations clearly indicate that HSPs play a pivotal role in antigen trafficking, which links to antigen cross-presentation as well as endogenous antigen processing.

The role of cell surface hsp90 on dendritic cells

HSP90 is an abundant cytosolic molecular chaperone associated with protein folding, transport and degradation. With a novel mAb, 6H8, we found that HSP90 is also expressed on the surface of bone marrow derived dendritic cells (BMDCs) and macrophages. The HSP90 expression level is enhanced by type I and II interferons but not by other cytokines. The 6H8 mAb bound to DCs was rapidly internalized, and the immobilized form stimulates BMDCs to produce IL-12. Incubation of 6H8 coupled with OVA (6H8-OVA) with DCs resulted in efficient cross-presentation of the dominant epitope, $OVA_{257-264}$, a process which was blocked by free 6H8 mAb, indicating that the cross presentation truly depends on cell surface HSP90. Alexa-labeled 6H8 mAb accumulated on $CD8^+$ but not on $CD8^-$ DCs *in vivo* one hour after intravenous injection. Administration of less than a microgram of a 6H8-OVA conjugate induced vigorous proliferation of adoptively transferred OT-I $CD8^+$ T cells, a response that did not occur in DC-deficient mice or TAP (transporter associated antigen processing) 1-deficient mice. Furthermore, 6H8-OVA induced CTL in naïve mice and protected them from lung metastasis of melanoma MO5 cells expressing OVA.

These results indicate that targeting cell surface HSP90 by 6H8 bearing antigens of interest enables cross-priming but not cross-tolerance of antigen-specific $CD8^+$ T cells, raising the possibility of a novel antibody-based vaccine strategy for induction of cytotoxic T cell immunity.

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

5, Yamano T, Sugahara H, Mizukami S, Murata S, Chiba T, Tanaka K, Yui K and Udono H. Allele-selective effect of PA28 in MHC class I antigen processing. ***J.Immunol.*** 181, 1655-1664 (2008)

Research Unit for Immune Homeostasis

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Technical Staff : **Takahisa Miyao**, **Guanying Wang**, **Kuniko Kamiya**

A small subpopulation of T lymphocytes known as regulatory T (Treg) cells plays a central role in preventing pathological immune responses including autoimmunity, inflammation and allergy, thereby ensuring self-tolerance and immune homeostasis. This has been well illustrated by the findings that the development and function of Treg cells is controlled by the transcription factor Foxp3, and that defective generation of functional Treg cells underlies the catastrophic autoimmune pathology that develops in Foxp3-mutant *scurfy* mice and in human patients with the IPEX syndrome.

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

Resolving the controversies over Treg cell plasticity

Early studies of organ-specific autoimmunity provoked by neonatal thymectomy, and of transplantation tolerance induced by thymic epithelium grafts have suggested that Foxp3$^+$ Treg cells represent a thymus-committed cell lineage, which is distinct from CD4$^+$ helper and CD8$^+$ cytotoxic T cell lineages. The discovery of Foxp3 as the "master regulator" of Treg cells has further strengthened this notion of a committed lineage by suggesting that it is Foxp3 that programs and maintains their regulatory functions. The irreversibility of the Treg cell fate has been believed to be necessary to ensure the robustness of self-tolerance and immune homeostasis in a changing environment.

Recent findings have challenged this notion of a committed Treg cell lineage by suggesting that these cells retain developmental plasticity- the capacity to be "reprogrammed" to various Foxp3- helper T (Th) cell subsets in response to environmental perturbations such as lymphopenia and inflammation. However, this issue of Treg cell lineage plasticity remains controversial because unequivocal evidence for lineage reprogramming is lacking and because there are other recent studies that argue against the plasticity phenomena. We have previously proposed that Foxp3$^+$ T cells are heterogeneous and consist of the majority population committed to the Treg lineage and a minority uncommitted (or un-programmed) population that retains plasticity. We have addressed the nature of the "uncommitted" Foxp3$^+$ T cells converting to Foxp3$^-$ Th cells under physiological, lym-

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3. Tsuji M., Komatsu N., Kawamoto S., Kanagawa O., Honjo T., Hori S.*, Fagarasan S.* (*corresponding/ senior authors) Preferential generation of follicular B helper T cells from Foxp3$^+$ T cells in gut Peyer's patches. ***Science*** 323, 1488-1492 (2009)
4. Komatsu N., Mariotti-Ferrandiz M.E., Wang Y., Malissen B., Waldmann H., Hori S. Heterogeneity of natural Foxp3$^+$ T cells: a committed regulatory T cell lineage and an uncommitted minor population retaining plasticity. ***Proc. Natl. Acad. Sci. U.S.A.*** 106, 1903-1908 (2009)

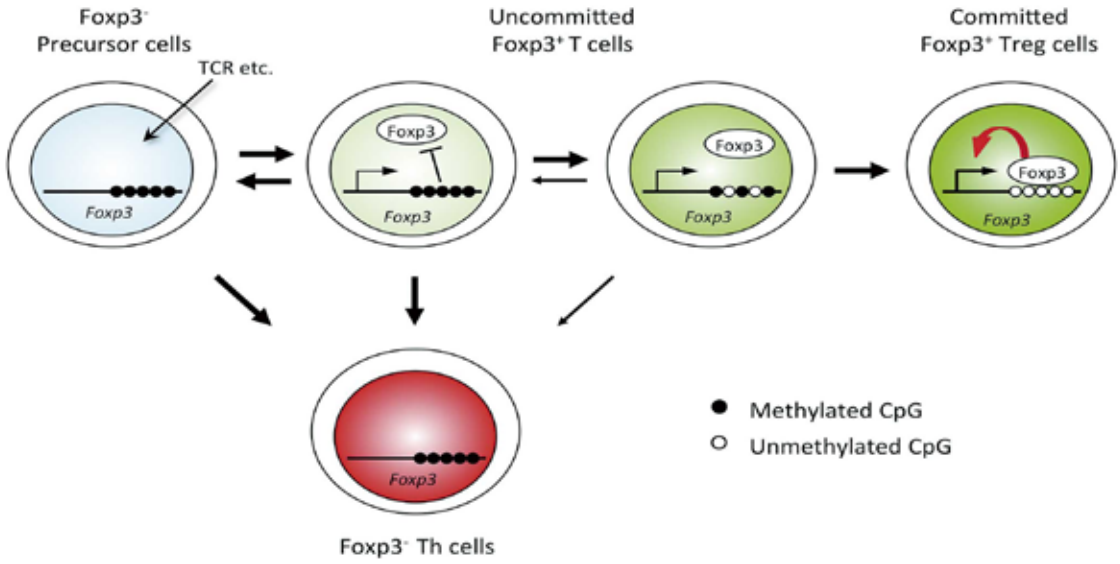

Figure : A model of Treg lineage commitment and plasticity. During thymic or peripheral Treg cell differentiation, TCR and other signals specify precursor cells to the Treg cell differentiation pathway and induce Foxp3 expression. At this stage, the *Foxp3* locus remains methylated and the induced Foxp3 expression can be easily extinguished by external perturbations such as inflammatory signals. The locus gradually becomes demethylated but such partially demethylated states may still be susceptible to external perturbations. Once the locus becomes fully demethylated, Foxp3 binds to it in cooperation with other transcription factors and self-enforces its own transcription.

phopenic, and inflammatory conditions. By using serial adoptive transfer as well as fate mapping strategies, we showed that there is certain time window in thymic and peripheral Foxp3⁺ T cell differentiation during which Foxp3 expression is transient. After this time window, Foxp3 expression becomes fixed and Foxp3⁺ T cells become irreversibly committed to the Treg cell fate. These findings resolve the current controversies over Treg cell plasticity and indicate that Treg lineage commitment is a multilayered process, determined not by Foxp3 expression *per se* but by higher-order regulation, which involves "epigenetic" modifications of the *Foxp3* locus and Foxp3-dependent self-enforcement of *Foxp3* transcription (Figure).

Impact of naturally occurring Foxp3 gene mutations on Treg cell differentiation and function

In order to understand the molecular mechanisms by which Foxp3 controls Treg cell differentiation and function, we have been addressing whether and how Foxp3 mutations that had been identified in human IPEX impair Treg cell development and function. We previously showed that all the missense mutations examined were amorphic or hypomorphic in that, upon retroviral transduction, these mutants fail to convert conventional T cells into fully functional Treg cells. To determine the impact of these mutations *in vivo*, we have established three independent knock-in mouse strains harboring *foxp3* mutations that mirror those in human IPEX. We found that hemizygous mutant males developed a *scurfy*-like lymphoproliferative autoimmune disease, although mice harboring hypomorphic mutations developed a milder disease than mice with *scurfy* or amorphic mutations. Thymic differentiation, peripheral maintenance, and suppressive functions of Foxp3⁺ Treg cells were impaired in two of the three mutant mice. By contrast, Foxp3⁺ Treg cells from the other mutant strain showed compromised peripheral homeostasis of Treg cells, while displaying normal thymic differentiation, *in vitro* suppressor function, and overall gene expression profiles. Strikingly, this homeostasis defect was accounted for by the absence of a particular Treg cell subset. These results suggest that the defective differentiation and/or maintenance of this Treg cell subset underlie the development of autoimmune pathology in this mutant strain. We are currently testing this hypothesis and addressing how this particular Foxp3 gene mutation results in this remarkable phenotype.

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

Laboratory for Cytokine Signaling

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Technical Staff : **Ayumi Ito**, **Masami Kawamura**, **Mayumi Hara**
Student Trainees : **Byn Beum-Ho (JRA)**, **Aiko Hasegawa**

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

Mast cell biology and molecular mechanisms of its functions

We are investigating cellular and molecular mechanisms of the involvement of mast cells in allergy, inflammation, and autoimmune diseases. Many researchers have believed that mast cells are major players in allergic responses, and IgE-dependent activation of mast cells leads to degranulation and cytokine production. However, how these distinct mast cell responses are differentially controlled is still unclear. We dissected Fc epsilon RI-mediated signal transduction in mast cell degranulation and cytokine production. First, we found that the Zinc transporter, ZnT5/Slc30a5 is required for contact hypersensitivity, a mast cell-mediated delayed-type allergic response, but not for passive cutaneous anaphylaxis, an immediate response. In mast cells from *Znt5*-KO mice, Fc epsilon RI-induced cytokine production was diminished, but degranulation was intact. ZnT5 was essential for Fc epsilon RI-induced translocation of PKC to the plasma membrane and the nuclear translocation of NF-kappa B. In addition, we found that the zinc finger–like motif of PKC was required for its plasma membrane translocation and binding to diacylglycerol. Thus, ZnT5 is selectively required for the mast cell-mediated delayed-type allergic response and is a novel player in PKC signaling (Nishida et al., J Exp. Med, 2009). Second, recently we found that Gab2 knock-in mice that express Gab2 mutated in either the PI-3K or SHP-2 binding sites have a defect in mast cell-mediated immediate-type allergic responses, but not in delayed-type responses. We also showed that the PI-3K-binding site, but not the SHP-2-binding site, was involved in Fc epsilon RI-dependent ARF1 activation and granule translocation to the plasma membrane during the degranulation process, further dissecting the signals required for degranulation. Finally, we established the molecular framework of the Fyn/Gab2/PI-3K-dependent

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2, Hojyo, S*., T. Fukada*, S. Shimoda, W. Ohashi, B-H. Bin, H. Koseki, T. Hirano. (*equal contribution) The zinc transporter SLC39A14/ZIP14 controls G-protein coupled receptor-mediated signaling required for systemic growth. ***PLoS ONE*** 6(3): e18059. (2011)

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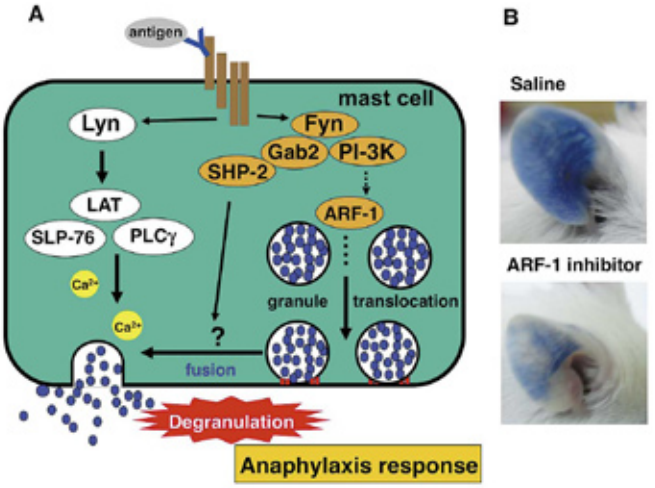

Figure1: A: Model of the Fc epsilon RI-mediated Fyn/Gab2/PI-3K/ARF1 signaling pathway involved in granule translocation and mast cell degranulation and the anaphylaxis response. B: Inhibition of ARF1 inhibits the IgE-mediated anaphylaxis response. Both ears were injected with antigen-specific IgE antibodies. After 16-18 h, the antigen was administered intravenously together with a blue dye and the amount of dye extravasating into the ear tissue was measured. One ear was pretreated with saline (*Upper panel*) and the other with an ARF-1 inhibitor (*Lower panel*) before antigen injection.

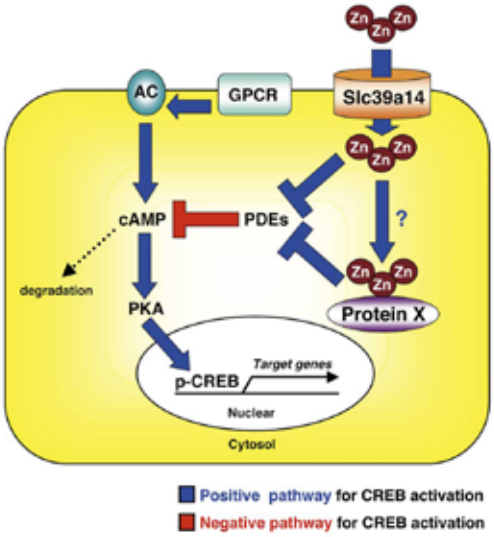

Figure2 : Schematic model of the regulation of GPCR-mediated signaling by Slc39a14. Slc39a14 regulates basal cAMP levels by suppressing PDE activity, which enhances the GPCR-cAMP-CREB pathway to control systemic growth.

ARF1-mediated granule translocation and mast cell degranulation (Fig.1) (Nishida et al., J. Immunol., 2011). Thus, this is the first demonstration of a molecular mechanism to differentiate the immediate and delayed allergic responses.

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 have shown that it has a role in cell migration during early zebrafish development. (Yamashita et al., Nature. 2004). We also found that LPS-induced maturation of DCs is partly mediated through lowering the intracellular concentration of free Zn by down regulating Zn transporters, including Slc39a6 (Kitamura et al., Nature Immunology., 2006), suggesting involvement of Zn in MHC class II cell surface expression through regulating endocytosis and membrane trafficking. In order to understand the role and function of Zn and Zn transporters *in vivo*, we have generated mice deficient in the Slc39/Zip family of zinc transporters. We found that Slc39a13/Zip13 knockout mice (*Slc39a13*-KO) show changes in connective tissues reminiscent of the human disease Ehlers-Danlos syndrome (EDS), in which there are defects in the maturation of osteoblasts, chondrocytes, odontoblasts, and fibroblasts. Impairment of bone morphogenic protein (BMP) and TGF-β signaling was observed in the corresponding tissues and cells of the knockout mice. Homozygosity for an *SLC39A13* loss of function mutation was identified in human sibs affected with a unique variant of EDS that recapitulates the phenotype observed in *Slc39a13*-KO mice. Hence, our results have revealed a crucial role of SLC39A13 in connective tissue development at least in part due to its involvement in BMP/TGF-β signaling pathways (Fukada et al, PLoS ONE, 2008). 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 G-protein -coupled receptors (GPCR) was observed in the growth plate, pituitary gland, and liver. We found that Slc39a14 -mediated Zn transport plays a role in control of GPCR-cAMP response element-binding protein (CREB) signaling via regulating cAMP levels by suppressing phosphodiesterase (PDE) activity (Figure 2, Hojyo et al, PLoS ONE, 2011), suggesting a crucial role for Slc39a14 in control of mammalian systemic growth.

Recently, we showed that Zn suppresses T_h17-mediated autoimmune diseases by inhibiting the development of T_h17 cells via attenuating STAT3 activation. In mice injected with type II collagen to induce arthritis, Zn treatment inhibited Th17 cell development. IL-6-mediated activation of STAT3 and *in vitro* T_h17 cell development were all suppressed by Zn. Mechanistically, we found that Zn binding changed the α-helical secondary structure of STAT3, disrupting its association with JAK2 kinase and with a phospho-peptide that included a STAT3-binding motif from the IL-6 signal transducer gp130. Thus, we conclude that Zn suppresses STAT3 activation, which is a critical step for T_h17 development (Kitabayashi et al, Int. Immunol., 2010).

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

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

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

Laboratory for Signal Network

Team Leader **Masato Kubo**

Research Scientist	: **Yuriko Nakatani**
Visiting Scientist	: **Yasutaka Motormura**
Technical Staff	: **Yoshie Suzuki**
Student Trainee	: **Minoru Sawaguchi**
Student Trainee/Part Timer	: **Keisuke Suzuki**

T cells play a central role in the effector and regulatory functions in immunological surveillance, and aberrations in these functions can lead to various immunological disorders. T helper 1 (T_H1) cells secrete interleukin-2 (IL-2), IFN-γ and TNF-α during the cellular immune response to intracellular pathogens and viruses. T_H2 cells, which are mainly protective against extracellular pathogens, but also account for allergic immune responses, produce IL-4, IL-5, IL-6, IL-10 and IL-13. T_H17 cells, which are important for defense against certain bacteria and fungal pathogens, especially at mucosal and epithelial surfaces, express IL-17 and IL-22 and are important effector cells in autoimmune tissue inflammation. These cytokines secreted by the different effector helper T cells play a critical role in controlling the outcome of immunological surveillance. The helper T cell subsets are differentiated from common precursor cells, naïve T cells, and these cells accomplish their differentiation by acquiring the capacity to produce certain types of cytokines. The overriding goal of our laboratory is to understand the molecular mechanisms of cytokine gene regulation during helper T cell differentiation.

Regulation of *Il4* gene expression in type 2 helper T cells by an *Il4* intronic enhancer HS2

Transcription of the T_H2-associated cytokine genes, IL-4, IL-5 and IL-13 is controlled by the T_H2 cell master regulator GATA3. However, the molecular basis of GATA3-mediated gene regulation during TH2 cell development is an inconclusive and controversial area of research. We recently showed that binding of GATA3 to the DNase-I hypersensitivity site 2 (HS2) in the second intron of the *Il4* locus is specifically required for chromosomal modifications at the *Il4* locus that allow transcription of *Il4*.

Several regulatory elements in the T_H2 locus have been identified; however whether T_H2-associated cytokine expression is controlled by a single element or by the coordinated activity of multiple elements is not known. To address this issue, we generated a series of mutant mice that lack each HS element in the *Il4–Il13* locus and assessed the affect of these mutations on cytokine production. T_H2 cells from mice that lack HS2 produced only low levels of IL-4 whereas the expression of IL-13 and IL-5 remained normal. By contrast, naive T cells that lack the conserved GATA3-response

Recent publications

1. Tanaka, S., Motomura, Y., Suzuki, Y., Yagi, R., Inoue, H., Miyatake, S., Kubo, M.: The enhancer HS2 critically regulates GATA-3-mediated *Il4* transcription in T_H2 cells. ***Nat. Immunol.*** 12, 1, 77-85, 2011

2. Park, JH., Adoro, S., Guinter, T., Erman, B., Alag, A., Catalfamo, M., Kimura, M.Y., Cui Y., Lucas, P.J., Gress, R.E., Kubo, M., Hennighausen, L., Feigenbaum, L., Singer, A.: Signaling by intrathymic cytokines, not T cell receptors, specifies CD8-lineage choice and promotes differentiation of cytotoxic-lineage T cells ***Nat. Immunol.*** 11, 3, 257-265, 2010

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

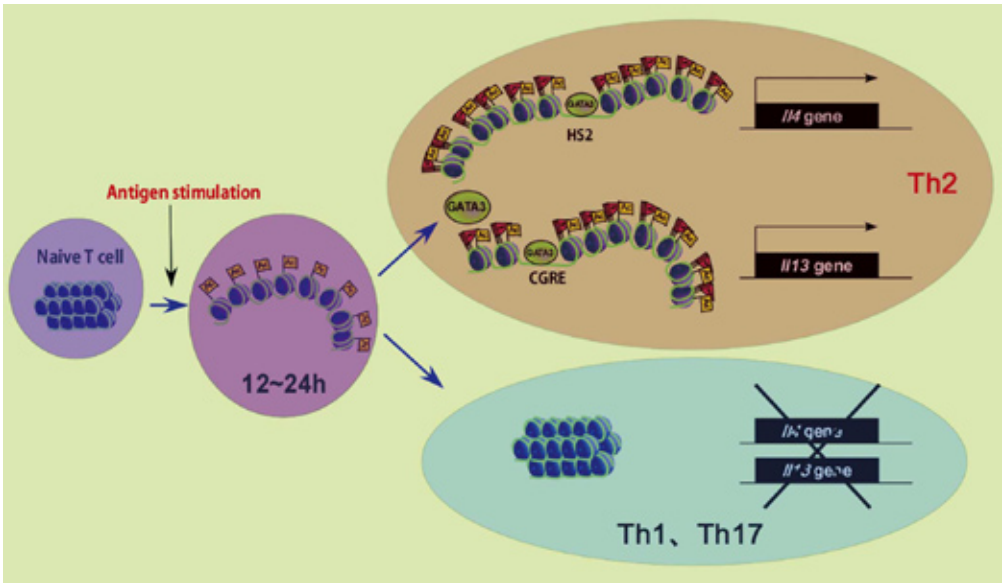

Figure :
Modification of chromatin structure at Th2 cytokine loci during helper T cell differentiation. The chromatin structure at the Th2 cytokine loci is compacted in naïve T cells, and antigen stimulation via the T cell receptor randomly promotes a permissive situation though acetylation (Ac) of core histones (H3). GATA-3 binds to tits cognate binding site localized in each Th2 cytokine gene, and this binding gives rise to further acetylation and methylation (Me) of particular lysine residues on histones. By contrast, GATA-3 is not expressed in Th1 and Th17 cells, therefore, the chromatin structure in the Th2 cytokine loci remains condensed.

element (GCRE) in the *Il13* locus gave rise to T_H2 cells producing normal levels of IL-4 but not IL-13, indicating that this element regulates *Il13* transcription.

These gene expression defects were reflected by abnormalities in transcription-permissive epigenetic changes normally observed at the *Il4* and *Il13* loci. Indeed, the permissive histone code; acetylation of histone H3 at Lys9 and Lys14 and trimethylation of histone H3 at Lys4 was impaired in HS2-deficient T_H2 cells, but only at the *Il4* locus. By contrast, deletion of GCRE resulted in impaired methylation of histone H3 at Lys4 at the *Il13* locus but not the *Il4* locus. In the T_H2 differentiation process, GATA3 functions as a master regulator, so it is possible that binding of GATA3 to HS2 and CGRE is required for T_H2 differentiation. HS2 and CGRE are crucial GATA3-binding sites in the *Il4* and *Il13* locus, respectively, and GATA3-binding to these regions independently regulates IL-4 and IL-13 expression.

Role of basophils and mast cells in inflammatory responses

We established a diphtheria toxin (DT)-based conditional deletion system using *Il4* enhancers specific for either mast cell (MC)s or basophils to drive the expression of the DT receptor (DTR) (Mas-DTR and Bas-DTR mice). While diphtheria toxin treatment of Bas-DTR mice resulted in specific deletion of basophils, treatment of Mas-DTR mice resulted in deletion of both MCs and basophils. Using these mice, we sought to tease out the role of MCs and basophils in several IgE-mediated disease models. We found that MCs and basophils played distinctive roles in the early and chronic phases of the IgE-mediated allergic response. In an asthma model, MCs appeared to play a critical role in the effector phase but not in the induction phase. Although basophil deletion partially impaired cysteine protease-mediated IL-4 production by antigen-specific T cells, basophils were dispensable for asthmatic responses, and for IgE production induced by systemic antigen immunization and by *Trichinella spiralis* infection. By contrast, MC deletion resulted in increased serum IgE levels, which were stably sustained for more than 15 days. Thus, these newly established Mas-DTR and Bas-DTR mice have allowed us to demonstrate distinct roles of MCs and basophils in IgE-mediated allergic responses and in regulation of serum IgE levels.

Using the Mas-DTR mouse model, we also examined the role of mast cells in contact hypersensitivity (CHS). CHS was attenuated when MCs were depleted during the sensitization phase. In addition, both maturation and migration of skin dendritic cells (DCs) were abrogated by MC depletion. Consistent with these findings, co-culture with bone marrow-derived MCs (BMMCs) enhanced maturation and chemotaxis of BMDCs *in vitro*. These results suggest that MCs interact with DCs in the skin and enhance DC functions. This interaction might be essential for establishing the sensitization phase of CHS.

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

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

Laboratory for Immunogenetics

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

ENU mutant panel study

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

During the first 4 years of this project we have screened 7,600 recessive inheritances from 80 mouse pedigrees. For basic phenotypic screening, we examined the levels of immunoglobulins, cytokines, and autoantibodies, as well as expression of a panel of cell surface markers on peripheral blood cells. 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

Recent publications

1. Matsushima Y, Kikkawa Y., Takada T., Matsuoka K., Seki Y., Yoshida H., Minegishi Y., Karasuyama H., and Yonekawa H. An Atopic Dermatitis-Like Skin Disease with Hyper-IgE–emia Develops in Mice Carrying a Spontaneous Recessive Point Mutation in the *Traf3ip2 (Act1/CIKS)* Gene. ***J Immunol***. 185(4),2340-9 (2010)
2. Wada H., Yasuda T., Miura I., Watabe K., Sawa C., Kamijuku H., Kojo S., Taniguchi M., Nishino I., Wakana S., Yoshida H., Seino K. Establishment of an improved mouse model for infantile neuroaxonal dystrophy that shows early disease onset and bears a point mutation in Pla2g6. ***Am J Pathol***. 175(6), 2257-63 (2009)
3. Kakugawa K., Yasuda T., Miura I., Kobayashi A., Fukiage H., Satoh R., Matsuda M., Koseki H., Wakana S., Kawamoto H., Yoshida H. A novel gene essential for the development of single positive thymocytes. ***Mol Cell Biol***. 29(18), 5128-35. (2009)
4. Aoki H. ,Yoshida H. ,Hara A. ,Suzuki T. ,and Kunisada T.: Transplantation of melanocytes into iris: method for iris repigmentation.. ***Transplantation*** 85, 492--494. (2008)

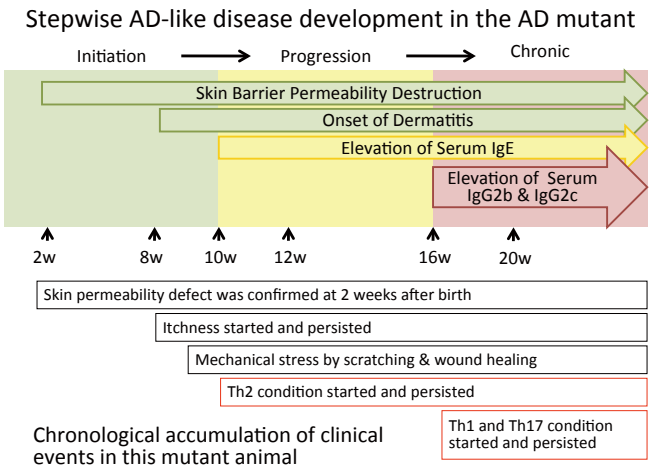

Figure 1: An atopic dermatitis-like mutant mouse was established from the ENU screen. The above scheme illustrates the stepwise progression of AD disease in the mutant animals. The responsible point mutation in a single gene and the developmental mechanism(s) of this dermatitis have been clarified (manuscript under submission).

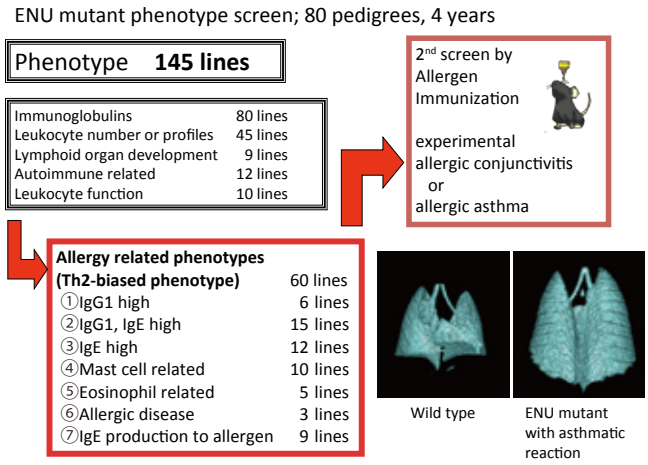

Figure 2: Summary of four years of ENU mutant screening. In total, approximately 8,000 mutant mice from 80 genomes have been screened and 145 phenotypes related to immunity or allergy have been identified. The images in the bottom right show the 3 dimensional renderings of the lungs of WT and mutant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

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 typical human allergic disease. The ear skin became thicker and red and, as the mice aged, they started to scratch the ear skin or face. The serum IgG1 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. Finally, they developed 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 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 the wild type animal. 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 (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 2010, 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., 2009), and 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 model for a primary immunodeficiency disease (Kakugawa et al., 2009).

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

Laboratory for Vaccine Design

Team Leader **Yasuyuki Ishii**

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Visiting Scientists : **Kenichi Masuda**, **Omar Duramad**
Technical Staff : **Haruka Katagiri, Risa Nozawa**

Allergen-specific immunotherapy (ASIT) is used globally for treatment of various forms of pollinosis. However, in Japan, the only ASIT is subcutaneous immunotherapy (SCIT) for Japanese cedar pollinosis and this therapy is not popular due to the long treatment time and unclear mechanism of action. We have been studying two types of vaccine technologies whose aim is the clinical application of ASIT as therapy for Japanese cedar pollinosis. The first vaccine candidate is a PEGylated recombinant fusion protein of Cry j 1 with Cry j 2, which has much less risk of anaphylaxis than natural allergen extracts, and can be utilized for not only for SCIT but also for sublingual immunotherapy (SLIT). The fusion protein project was licensed to Torii Pharmaceutical Co. The second candidate vaccine is liposomal α-GalCer, a ligand for invariant natural killer T (iNKT) cells, encapsulating the Cry j 1/2 fusion protein. The liposomes not only reduced ongoing IgE antibody formation but also suppressed tertiary antibody responses. Experiments are ongoing to elucidate the detailed mechanisms of the liposome vaccine. Preclinical and phase I/IIa studies to confirm the proof of concept are being done in collaboration with a biotech company, REGiMMUNE Co, and supported by the Japan Science and Technology Agency (JST).

Mode of action of liposomal α-GalCer-antigen

To elucidate the detailed mechanism of liposome vaccine-mediated suppression of IgE antibody responses, target cells of the liposome vaccine after intravenous injection were identified by using rhodamine-labeled liposomal α-GalCer -OVA protein. Splenic $B220^{+}CD1d^{high}$ cells, but not $B220^{+}CD1d^{low}$ cells, preferentially incorporated liposomes. Immunofluorescence analysis of spleen sections showed that the $B220^{+}CD1d^{high}$ cells were localized in the marginal zone area and interacted there with iNKT cells. We found that CXCR6, which is a major chemokine receptor for iNKT cells, and its ligand, CXCL16 were expressed on iNKT cells and marginal zone $B220^{+}CD1d^{high}$ cells, respectively. To test whether CXCR6/CXCL16 was involved in the interaction of the iNKT cells with the $B220^{+}CD1d^{high}$ cells, co-administration of neutralizing antibody against CXCL16 and rhodamine-labeled liposomes into NKT-Venus cloned mice was carried out. As shown in Figure, iNKT cells failed to traffic to the marginal zone area. The significance of interaction of iNKT cells with marginal zone $B220^{+}CD1d^{high}$ cells was supported by the result that co-administration of neutralizing antibody against CXCL16 with liposomal α-GalCer-OVA in OVA-primed mice diminished the immunosuppressive effects of the liposome vaccine. Taken together, these results suggest

Recent publications

1. Okayama T, Matsuno Y, Yasuda N, Tsukui T, Suzuta Y, Koyanagi M, Sakaguchi M, Ishii Y, Olivry T, Masuda K. Establishment of a quantitative ELISA for the measurement of allergen-specific IgE in dogs using anti-IgE antibody cross-reactive to mouse and dog IgE. ***Vet. Immunol. Immunopathol.*** 2011, 139(2-4):99-106.
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*** 2011, 17, 1154-68

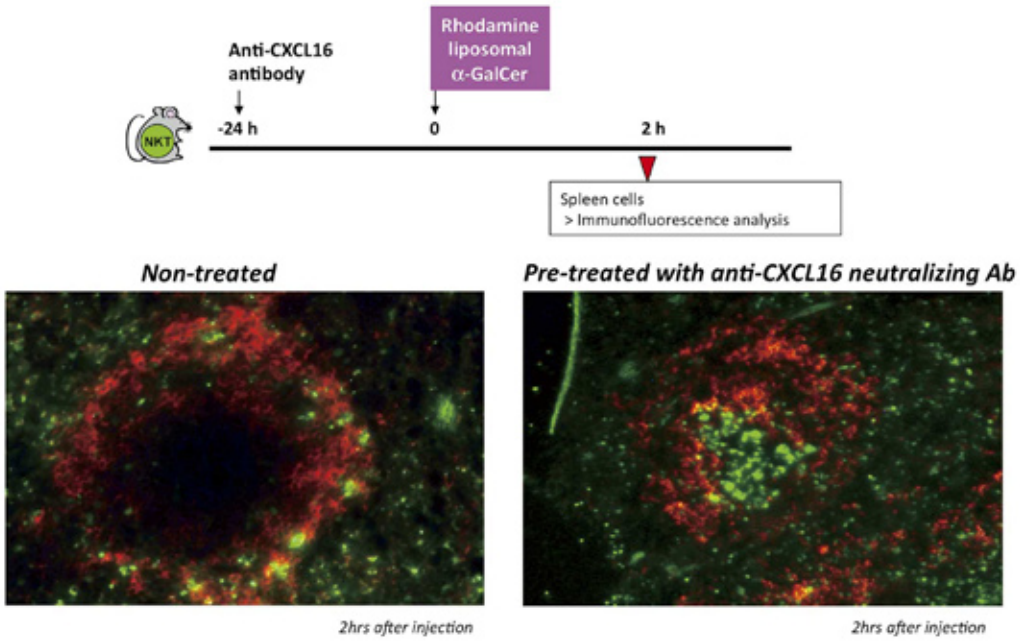

Figure : **Venus-NKT cells (*green*) failed to traffic to the MZ B220^{+} cells, which have taken up the liposomal antigen (*red*) after anti-CXCL16 antibody treatment**

that the interaction of iNKT cells with marginal zone B220^{+}CD1d^{high} cells is essential for the immunosuppressive activity of the liposomal α-GalCer -Cry j 1/2 fusion protein.

The next question was why IgE antibody response are preferentially suppressed by the liposome treatment. One possibility is the production of IL-21 which could induce apoptosis of Bε cells. To test this possibility, *in vitro* culture of liposome-treated B220^{+}CD1d^{high} cells, OVA-primed CD4^{+}T cells with or without iNKT cells was performed. The result of quantitative PCR analysis indicated that IL-21 mRNA expression was only enhanced in the presence of iNKT cells.

Development of liposomal α-GalCer -Cry j 1/2 fusion protein (Code: RGI-1001)

Supported by a JST grant, development of RGI-1001 was started at the end of 2008. Preclinical studies including manufacturing, pharmacology, toxicology, pharmacokinetics and pharmacodynamics are ongoing.

In the manufacturing process, each component of RGI-1001 was independently produced. The first GMP lot of α-GalCer, KRN7000 was released three years ago. Feasibility studies of liposome formulations had also been done. Optimized liposome compositions for protein encapsulation were determined. High level of production of recombinant Cry j 1/2 fusion protein was successful in a fed-batch culture system. A scalable purification process was developed, which made it possible to provide GLP grade material. GMP lot release is in preparation.

In pharmacology studies using non-GLP liposomal α-GalCer -Cry j 1/2 fusion protein, the Cry j-sensitized mouse asthma model showed that treatment with liposomes could suppress not only the Cry j-specific IgE antibody response after Cry j antigen challenge but also airway hyperreactivity. Cry j-sensitized monkey studies were just started to confirm the efficacy of RGI-1001 in a primate model.

In toxicology studies, several safety concerns have already been alleviated. One is that RGI-1001 might interfere with host defense against viral and bacterial infection. Studies of murine CMV and *Listeria monocytogenes* showed that the treatment with RGI-1001 did not suppress host defense. Anaphylaxis risk of recombinant Cry j 1/2 fusion protein was examined by using Cry j-sensitized mice. The results indicated that the anaphylaxis score and body temperature were not affected, even when a large amount of the recombinant protein was intravenously injected into the sensitized mice with high IgE antibody titers.

Next year, GLP toxicology and other studies required for IND filing should be started.

3. Ishii Y, Motohashi S, Shimizu K, Nakayama T, Taniguchi M, and Fujii S, Application of NKT cells in immunotherapy, ***Curr. Immunol. Rev***, 2010, 6, 109-115.

Research Unit for Human Disease Model

Unit Leader **Fumihiko Ishikawa**

Senior Research Scientist : **Yoriko Saito**
Technical Staff : **Mariko Tomizawa-Murasawa, Akiko Sone, Nahoko Suzuki, Hiroshi Kajita**
Graduate Students : **Yuki Aoki, Shinsuke Takagi, Yuho Najima**

The ultimate goal of our lab is to understand normal human hematopoiesis and immunity and to conquer human diseases that impact these systems. To do so, we have created a humanized mouse model through intravenous injection of human stem cells into newborn NOD/SCID/IL2rgKO mice. Transplantation of normal or diseased human stem cells results in reconstitution of human immunity or recapitulation of human diseases in the recipient mice. This year, in the area of normal human immunity, we have analyzed the development of human innate immunity and innate immune response *in vivo*. Among various human diseases, we have continued to focus our investigations on human myeloid and lymphoid leukemias for better understanding of these disease entities. In addition to these projects, we have begun to study human iPS cells along with somatic stem cells. Specifically, we are currently seeking a strategy for inducing hematopoietic stem/progenitor cells from human iPS cells and to understand the role of transcription factors in hematopoietic development.

Reconstitution of human adaptive and innate immunity in mice

Lack of an HLA-restricted human immune response has long been one of the major obstacles in humanized mouse research. To overcome this limitation, in collaboration with Dr. Leonard D. Shultz at The Jackson Laboratory, we have created HLA class A02 transgenic NOD/SCID/IL2rγKO mice. Human $CD8^+$ T cells developing in the HLA class I positive NOD/SCID/IL2rγKO recipients expressed functional molecules such as granzyme, in accordance with their phenotypic maturation from naïve, central memory, to effector memory cells. Furthermore, we demonstrated that these human $CD8^+$ T cells are functional in cytokine production and cytotoxicity, recognizing EBV-infected cells and virus-associated peptides. These functions are inhibited by the addition of anti-HLA class I antibody. Since we have detected a human CTL response against a species-specific virus, we are currently analyzing the HLA-restricted human T cell response to peptides.

Regarding human innate immunity, human myeloid cell development in humanized mice has yet to be determined. We therefore set out to analyze differentiation and function of human myeloid cells in the recipients transplanted with purified human cord blood $CD34^+CD38^-$ HSCs. In the recipient bone marrow and spleen, we detected HLA-DR^- granulocytes and HLA-DR^+ antigen presenting cells within the human $CD45^+$ cell fraction. $CD15^+CD33^{low}$ neutrophils, c-$Kit^+CD203c^+$ mast

Recent publications

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2. 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. Cell cycle entry potentiates elimination of quiescent chemotherapy-resistant human AML stem cells. ***Nat. Biotechnol.*** 28-3,275-280, 2010
3. Shultz LD, Saito Y, Najima Y, Tanaka S, Ochi T, Tomizawqa 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. Nat. Acad. Sci. U. S. A.***107,13022-13027, 2010

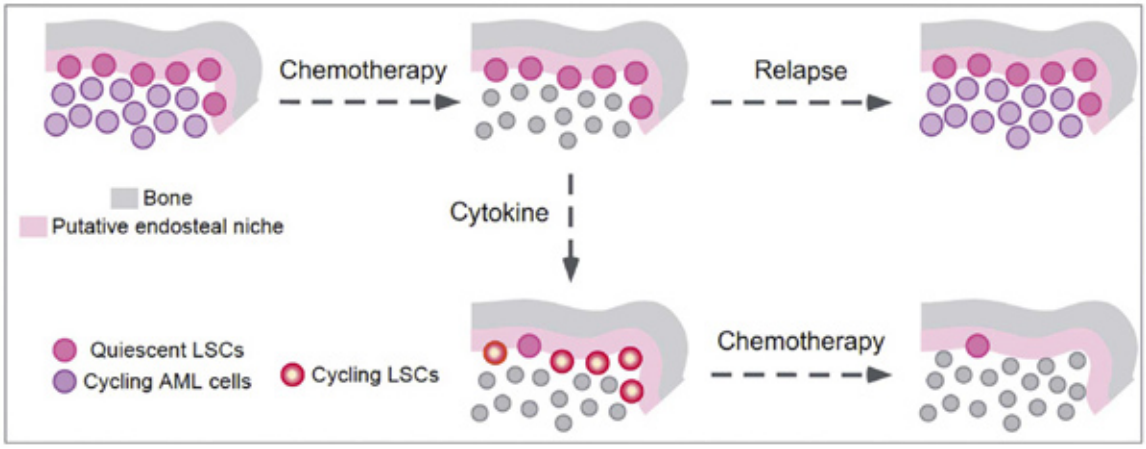

Figure : **Chemotherapy can efficiently eliminate non-stem leukemia cells in the bone marrow cavity, but not AML stem cells, which reside in the endosteal region of the bone marrow.** Cytokines or antibodies disrupt the interaction between AML stem cells and their niche, thereby inducing the stem cells to enter cell cycle and become susceptible to chemotherapy.

cells, and c-Kit$^-$CD203c$^+$ basophils were observed among human granulocytes developing in vivo. Among HLA-DR$^+$ human APCs, CD14$^+$ monocytes, BDCA1/3$^+$ conventional DCs and plasmacytoid DCs were present in the bone marrow and spleen of the recipient mice. These human myeloid cells express cytokine receptors and TLRs in a similar manner as seen in primary human myeloid cells. We are continuing to evaluate the function of these human myeloid cells in terms of cytokine production and inflammatory responses.

Targeting human acute myeloid leukemia (AML) stem cells

AML is one of the most intractable hematologic malignancies in adults. This year, we have continued our efforts to better understand the mechanism for AML relapse and to create effective therapies enabling AML patients to survive long-term through collaboration with Dr. Shuichi Taniguchi at Toranomon Hospital and Dr. Osamu Ohara at RIKEN.

To date, we have determined that CD34$^+$CD38$^-$ AML cells satisfy the functional criteria for leukemia stem cells (LSCs) and that LSCs exhibit significant chemotherapy-resistance. Through *in vivo* analysis, we found that human LSCs preferentially reside in the endosteal region of the bone marrow and are cell cycle quiescent. The relative chemotherapy-resistance of LSCs may be accounted for by their cell cycle status and specific location.

Therefore, to achieve long-term relapse-free survival of AML patients, we need to eliminate the chemotherapy-resistant human LSCs residing within the endosteal region of the bone marrow. As a potential novel therapeutic strategy, first, we modified the cell cycle status of LSCs by inhibiting the stem-niche interaction at the endosteal region. To accomplish this, we have treated AML engrafted mice with G-CSF for five days. Though we observed significant case-dependent variability among patients, G-CSF induced LSCs to enter cell cycle in all the cases examined. Consistent with the successful cell cycle induction, conventional chemotherapy following G-CSF treatment resulted in significantly enhanced eradication of AML stem cells at the niche. By analyzing other cytokines and antibodies for their effect on cell cycle status in AML stem cells, we aim to construct cytokine-enhanced chemotherapy as a post-remission, curative anti-LSC therapy.

Global gene expression profiling has given us much information on potential therapeutic targets expressed by human AML stem cells. In 2010, we identified *FCGR2A* and *IL2RA* as genes encoding plasma membrane molecules that could be promising targets for antibody drug development. We are continuing to pursue the identification of anti-LSC markers through global expression profiling followed by functional validation. To cope with the significant biological heterogeneity in AML, we are performing analyses of more homogeneous patient groups based on clinically-verified AML classifications including those using morphology (FAB classification) and cytogenetic abnormalities. In addition to plasma membrane molecules, we are focusing on WT1, a zinc-finger transcription factor and well known tumor-associated antigen, as a potential target for effective immune therapy. By using HLA class I Tg NOD/SCID/IL2rγKO mice, we are currently developing methods that allow efficient induction of WT1-targeting human CD8$^+$ T cells *in vivo*.

4. Ishikawa F, Yoshida S, Saito Y, Hijikata A, Kitamura H, Tanaka S, Nakamura R, Tanaka T, Tomiyama H, Saito N, Fukata M, Miyamoto T, Lyons B, Ohshima K, Uchida N, Taniguchi S, Ohara O, Akashi K, Harada M, Shultz L D. Chemotherapy-resistant human AML stem cells home to and engraft within the bone marrow endosteal region. ***Nat. Biotechnol.*** 25,1315-1321, 2007.

5. Shultz LD, Ishikawa F, Greiner DL. Humanized mice in translational biomedical research. ***Nat. Rev. Immunol.*** 7,118-130, 2007

Research Unit for Cellular Immunotherapy

Unit Leader **Shin-ichiro Fujii**

Technical staff : **Hidetoshi Sugahara**
Jun Shinga

Student Trainee : **Yusuke Sato**

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

We have also studied the role of DCs *in situ* for tumor immunity by focusing on the link between innate and adaptive immunity. Recently, we successfully developed a strategy for the induction of antigen-specific T cell responses using tumor-associated antigen-expressing, α-GalCer-loaded allogeneic fibroblasts. Based on these findings in mice, we will attempt to launch clinical studies, in collaboration with Dr. Shimizu, Therapeutic Model Research Unit.

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

We have been developing a Phase I/IIa clinical study of the application of NKT cell therapy for advanced non-small cell lung cancer (NSCLC) patients refractory to standard treatment as a post second line therapy (stage IIIB, IV or recurrence). α-GalCer-pulsed APCs ($1x10^9/m^2$ PBMC-derived DCs) were intravenously administered four times. In this study, when we compared the responder group, which had an increased number of IFN-γ producing NKT cells, and the non-responder group, the MST (median survival time) was longer in the responder group (31.9 months versus 9.7 months). Thus, the increased IFN-γproduction by NKT cells upon α-GalCer stimulation was significantly associated with clinical outcome. Also, we detected a larger number of NKT cells capable of producing IFN-γ in the lung tumor. These phase I/IIa trial results are encouraging and warrant further

Recent publications

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2. Watarai H, Fujii S, Yamada D, Rybouchkin A, Sakata S, Nagata Y, Iida-Kobayashi M, Sekine-Kondo E, Shimizu K, Shozaki Y, Sharif J, Matsuda M, Mochiduki S, Hasegawa T, Kitahara G, Endo TA, Toyoda T, Ohara O, Harigaya K, Koseki H, Taniguchi M. Murine induced pluripotent stem cells can be derived from and differentiate into natural killer T cells. ***J Clin Invest*** 120, 2610-2618 (2010)
3. Fujii S, Motohashi S, Shimizu K, Nakayama T, Yoshiga Y, Taniguchi M. Adjuvant activity mediated by iNKT cells. ***Semin Immunol*** 22, 97-102 (2010)

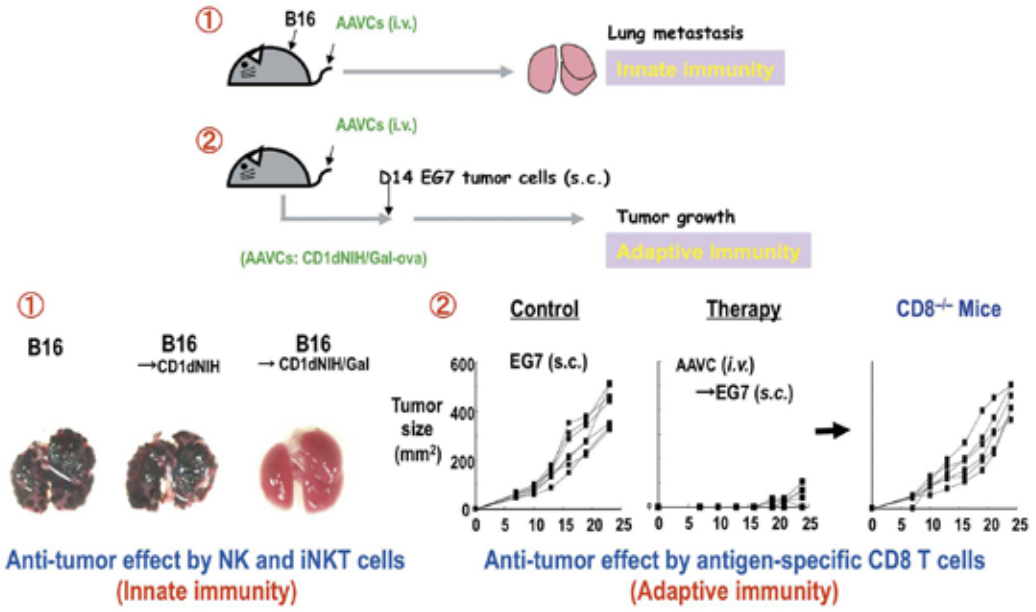


Figure : **Efficacy of AAVCs for induction of Innate and Adaptive Immunity**

In the murine models, we evaluated the efficacy of artificial adjuvant vector cells (AAVCs) that had been loaded with α-GalCer, and were transfected with ova Ag mRNA. ①When we used the AAVCs in the B16 lung metastasis model, we observed the eradication of tumor cells. ②Also, when we treated EG7 tumor (ova-expressing tumor) bearing mice with AAVC, we observed resistance to tumor cells, but not in CD8-deficient mice.

evaluation of the survival benefit of this immunotherapy. To understand the mechanism of the DC therapy response and identify predictive biological markers, we have been analyzing NKT cells in the lung tumors in murine models and cancer patients.

Antigen mRNA-transfected allogeneic fibroblasts loaded with NKT cell ligand confer antitumor immunity (A collaboration with the University of Tokyo, Yamaguchi University and RCAI Drs. Ishii and Shimizu)

We previously demonstrated that administration of tumor/Gal cells can generate both innate and adaptive immunity through the maturation of DCs in a tumor specific manner. In the current study, instead of tumor cells, we used allogeneic fibroblast cells loaded with α-GalCer and transfected with antigen-encoding mRNA (CD1dNIH/Gal, Fig.), thus combining the adjuvant effects of iNKT cell activation with delivery of antigen to DCs *in vivo*. We found that these cells produce antigen protein and activate NK and iNKT cells. When injected into MHC mismatched mice, they elicited antigen-specific T cell responses and provided tumor protection, suggesting that these immune responses depend on host DCs. Also, antigen-expressing fibroblasts loaded with α-GalCer generated a more potent T cell response than those expressing NK cell ligands. Thus, glycolipid-loaded, mRNA-transfected allogeneic fibroblasts act as artificial adjuvant vector cells (AAVCs) to promote iNKT cell activation, leading to DC maturation and T cell immunity. By harnessing the innate immune system and generating an adaptive immune response, this unique tool could prove clinically beneficial in the development of immunotherapies for malignant and infectious diseases. Presently, we have been setting up preclinical studies to establish AAVCs in humans.

4. Fujii S, Goto A, Shimizu K. Antigen mRNA-transfected, allogeneic fibroblasts loaded with NKT-cell ligand confer antitumor immunity. ***Blood*** 113, 4262-72 (2009)
5. Shimizu K, Fujii S. DC therapy induces long-term NK reactivity to tumors via host DC. ***Eur J Immunol***. 39, 457-68 (2009)
6. Fujii S. Exploiting dendritic cells and naturatl killer T cells in immunotherapy against malignancies. ***Trends Immunol***. 29, 242-9 (2008)

Laboratory for Cellular Systems Modeling

Team Leader

Mariko Okada-Hatakeyama

Research Scientists : **Takeshi Nagashima**
Yuichi Shiraishi
Yuko Saeki

Technical Staff : **Noriko Yumoto**
Kaoru Takahashi

Student Trainees : **Felix Jonas (IPA)**, **Hanna Iribe**

Trainee : **Karin Mitosch**

The Laboratory for Cellular Systems Modeling has been carrying out research to identify and reconstruct biological networks and basic regulatory principles from quantitative experimental data using computational, theoretical and experimental biology approaches. The main topic of our current research is mechanistic understanding of membrane receptor signaling and its transcriptional control in the cell fate decision process.

For this purpose, we take both bottom-up and top-down approaches. For bottom-up studies, kinetic mathematical modeling of a pathway using computational and experimental approaches is carried out. This tactic is useful when the components and their interconnections in the reaction network are already known. Based on our in-house data and the literature, we constructed ordinary differential equation (ODE)-based kinetic models that allow us to understand intracellular signaling dynamics. For the past few years, we have worked on the model to explain digital (all-or-none) outputs of c-Fos transcription factor activation (Cell, 2010). The model can explain that a structure where there is co-existence of ERK signal and c-Fos transcription (an AND-gate structure) can generate the digital output of c-Fos. The model predicted and our experimental results verified that DUSP, an ERK phosphatase, together with as yet unknown negative regulators determine c-Fos dynamics. This model is thought to be valid for production of transcription factors during B-cell development and neurite elongation of PC12 cells; therefore it is most likely that the model captures general network principles of the signal network during a variety of cellular differentiation process.

In addition, for the modeling of signaling pathways, we try to obtain information on molecular binding interactions and kinetics in the pathway. For this purpose, we closely collaborate with Dr. Yasushi Sako in RIKEN ASI to determine association and diffusion constants of protein-protein interactions in living cells. Particularly we are focusing on the dynamics of SH2 domain proteins and ligand-receptor interactions because critical intracellular signaling dynamics often originate based on the binding behavior of membrane receptors. Our analysis so far indicates that interactions

Recent publications

1. 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, 818-896 (2011).
2. Shiraishi Y, Kimura S, Okada M. Inferring Cluster-based Networks from Differently Stimulated Multiple Time-course Gene Expression Data. ***Bioinformatics*** 26, 1073-1081 (2010).
3. 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.
4. Suenaga A, Hatakeyama M, Kiyatkin AB, Radhakrishnan R, Taiji M, Kholodenko BN. Molecular Dynamics Simulations Reveal that Tyr-317 Phosphorylation Reduces Shc Binding Affinity for Phosphotyrosyl Residues of Epidermal Growth Factor Receptor. ***Biophys. J.*** 96, 2278-2288 (2009).

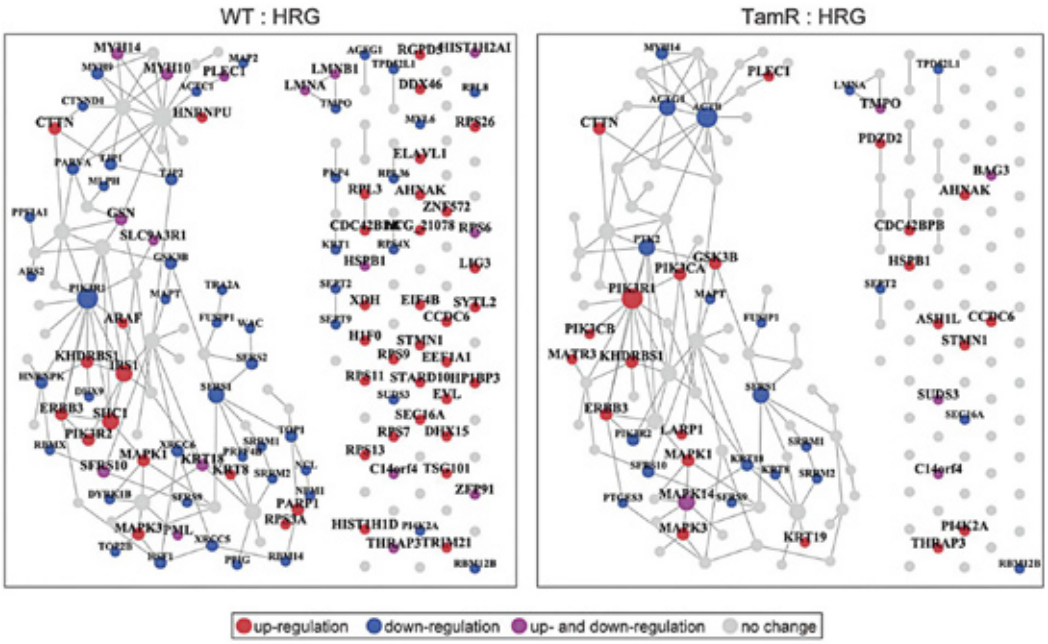


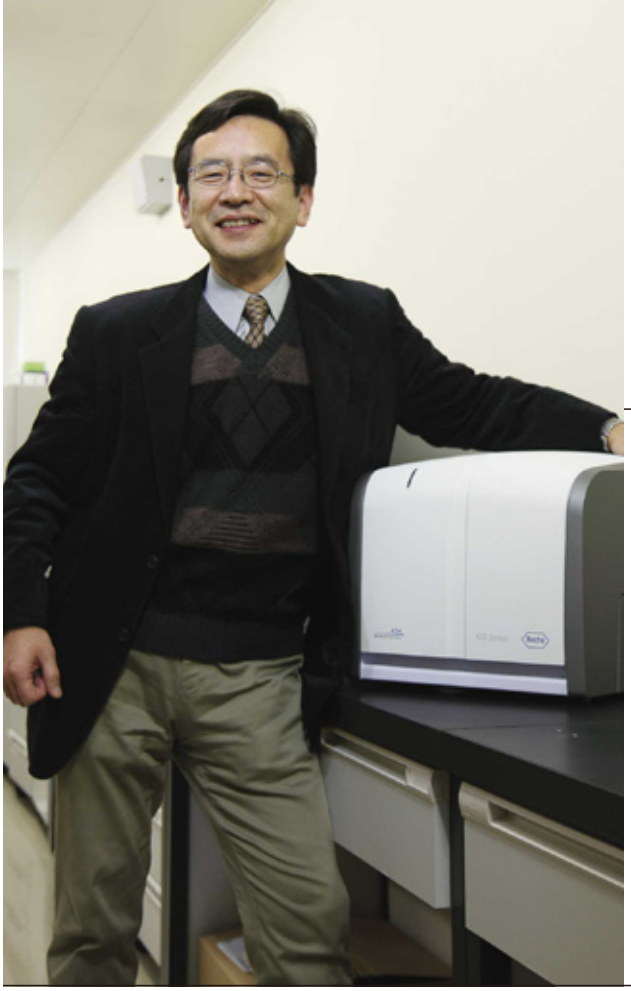
Figure:
Interaction network of phosphorylated proteins in the HRG-stimulated wild type (WT) and drug resistant (TamR) cells. The reconstructed network comprises one large subnetwork and small subnetworks with fewer than four nodes. Node size reflects the number of interaction partners. Node color indicates phosphorylation changes: *red*, up-regulation; *blue*, down-regulation; *purple*, up- and down-regulation; *gray*, no change.

between receptors and Grb2 or Shc, p85 and p110 of PI3K in living cells might be different from the schemes that had been suggested based on *in vitro* studies. Molecular dynamics (MD) simulation done in collaboration with Dr. Makoto Taiji in RIKEN also indicated an unexpected role of Shc as a potential molecular switch to turn on/off the signal coming from the receptor, in that binding affinity to the receptor might be reduced after the phosphorylation at Tyr317 (Biophys. J, 2009). Thus, by combining various experimental and computational approaches, we are able to construct more realistic mathematical models of signaling networks and identify key regulators that determine cell fate.

As for the top-down approach, we perform transcriptome and proteome analyses to determine overall systems properties. We performed phospho-proteome analysis using LC mass spectrometry in collaboration with Dr. Oyama (Tokyo Univ.) et al. to understand drug-resistance mechanisms in breast cancer cells (J. Biol. Chem., 2010). The analysis indicated an overall down-regulation of protein phosphorylation in drug-resistant cells whereas specific signaling pathways were up-regulated. Integrative statistical analysis with gene expression data indicated that the activation of AP-1 and CREB transcription factors was dysregulated in the drug-resistant cells. We thus proposed and then experimentally verified that GSK3beta phosphorylation status might be considered as a marker for drug-resistance. These analyses showed that the approach of using combinatorial analysis of signaling and transcription allows us to understand the drug-resistance properties at the network level, which should narrow down the potential therapeutic targets.

To realize our goal of understanding signal-transcription networks, we developed several computer algorithms and methods to analyze time-course data, infer networks and to estimate model parameters. The methods are useful to estimate positive and negative regulatory relationship among the molecules when their functions are experimentally unknown. Particularly, we developed a method to simultaneously cluster and infer regulatory relationship among genes from the time-course gene expression data (Bioinformatics, 2010). The method allowed us to predict functional regulatory relationship of genes in breast cancer cells under several conditions. The method is also useful for analysis of any time-course data as well to estimate molecular functions.

5. Seo J-H, Suenaga A, Hatakeyama M, Taiji M, Imamoto A. Structural and functional basis of an essential role for CRKL in FGF8-induced feedforward pathway. ***Mol. Cell. Biol***. 29, 3076-3087 (2009).



Laboratory for Immunogenomics

Group Director **Osamu Ohara**

Research Scientists : **Takashi Watanabe** , **Yoko Kuroki**, **Yoshitaka Shirasaki (SPDR)**

Research Associatess : **Atsushi Hijikata**, **Mai Yamagishi**

Technical Staff : **Masatoshi Ito**, **Nobutake Suzuki**, **Atsuo Kobayashi**, **Tomoko Hasegawa**, **Keiko Takahashi**, **Naomi Inagaki**, **Ryosuke Yashi** (temporary employee), **Fumie Yokoyama** (temporary employee), **Noriko Utsumi** (temporary employee), **Emi Abe** (temporary employee)

Student Traineess : **Nanako Shimura** (Junior Research Associate), **Asahi Nakahara**, **Masayuki Ishii**

It has been our important and basic mission to function as a "Gateway" to genomics for immunologists. Thus, our research activities have been three-pronged since our group was launched: (1) central support activities; (2) strategic and collaborative research activities; and (3) exploratory research activities aimed at new technology development. However, these research activities are never mutually exclusive, but should be synergistically stimulated. In 2010, we have placed a significant emphasis on development of new technology to eventually enhance strategic and collaborative research activities as well as the central support activities. Our current emphasis is on the development of technology for single-cell analyses at the mRNA and the protein levels. We believe that single-cell genomic data together with bioimaging data will serve as the foundation of systems immunology, where cell-to-cell interactions play a crucial role in determining the behavior of the whole immune system. Our recent proteomic method development, an assay system for secretion of cytokines from single cells, also enables us to retrieve the single cells on demand, as described below.

Characterization of multiple alternative forms of heterogeneous nuclear ribonucleoprotein K by phosphate-affinity electrophoresis

Post-translational modifications of proteins often play an important biological role and, among these modifications, phosphorylation is one of the most common and dynamic. For example, the phosphorylation of heterogeneous nuclear ribonucleoprotein K (hnRNP K) is thought to play an important role in cell cycle regulation and signal transduction. However, the relationship between hnRNP K phosphorylation and cellular events has only been indirectly examined, and the phosphorylated forms of endogenous hnRNP K have not been biochemically characterized in detail. Taking hnRNP K as a model protein for development of analysis method for protein phosphorylation, we extensively examined the phosphorylated isoforms of endogenous hnRNP K by direct protein-chemical characterization. The proteins were isolated by phosphate-affinity electrophoresis, which uses polyacrylamide-bound Mn^{2+}-Phos-Tag™ ligand as a copolymer in the separating gel matrix, followed by immunoblotting and mass spectrometry. Phosphate-affinity electrophoresis enabled us to detect and separate the

Recent publications

1. Kimura Y, Nagata K, Suzuki N, Yokoyama R, Yamanaka Y, Kitamura H, Hirano H, Ohara O. Characterization of multiple alternative forms of heterogeneous nuclear ribonucleoprotein K by phosphate-affinity electrophoresis. ***Proteomics*** 10, 3884-3895 (2010)
2. Suyama M, Harrington ED, Vinokourova S, von Knebel Doeberitz M, Ohara O, Bork P. A network of conserved co-occurring motifs for the regulation of alternative splicing. ***Nucleic Acids Res***. 38, 7916-7926 (2010)
3. 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 analysing mutations from human genetic diseases. ***DNA Res***. 17, 197-208 (2010)
4. Keerthikumar S, Raju R, Kandasamy K, Hijikata A, Ramabadran S, Balakrishnan L, Ahmed M, Rani S, Selvan LD, Somanathan DS, Ray S, Bhattacharjee M, Gollapudi S, Ramachandra YL, Bhadra S, Bhattacharyya C, Imai K, Nonoyama S, Kanegane H, Miyawaki T, Pandey A, Ohara O, Mohan S.

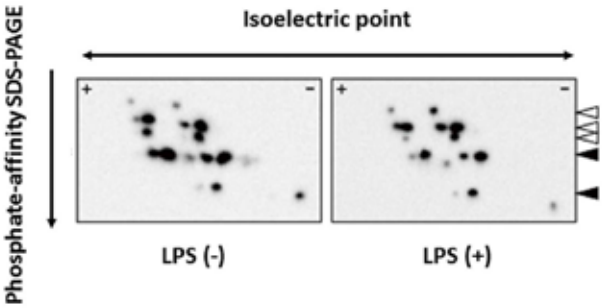


Figure1: hnRNP K phosphorylation profiles revealed by phosphate-affinity two-dimensional electrophoresis.
Nuclear extracts from J774.1 cells treated with and without lipopolysaccharide (LPS- and LPS+) were separated by phosphate-affinity two-dimensional electrophoresis. The *white* and the *black* arrows indicate the positions of phosphorylated and unphosphorylated protein spots, respectively, on phosphate-affinity SDS-PAGE gel images.

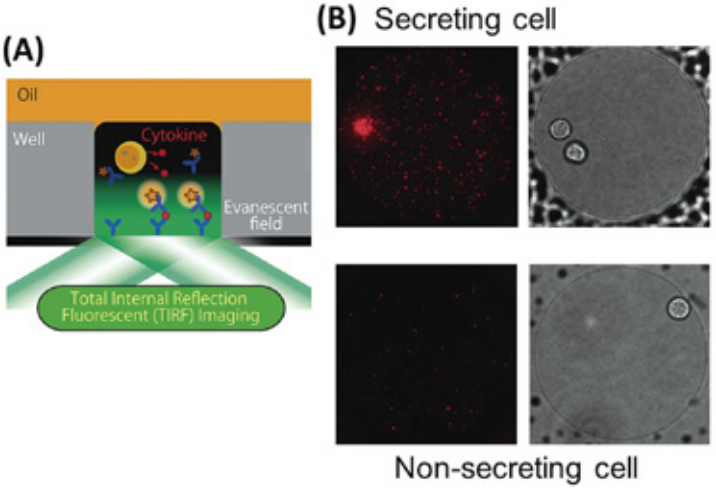


Figure2: Real-time monitoring of protein secretion from single cells.
(A) A schematic of real-time observation of cytokine secretion in micro fabricated wells by total internal reflection fluorescence imaging. Cytokines secreted from an immune cell are detected by a real-time sandwich immunosorbent assay.
(B) Secretion of IL-1β from a single MC/9 cell. *Red* spots at the bottom of the microwells correspond to single molecules of the IL-1β/sandwich antibody complex. The number of red fluorescent spots increases with time only in the case of the secreting cell *(upper panel)*.

phosphorylated forms of hnRNP K with high sensitivity. When we used two-dimensional gel electrophoresis with phosphate-affinity SDS-polyacrylamide gel electrophoresis in the second dimension, the nuclear fraction contained more than 20 spots of endogenous hnRNP K on the two-dimensional map. We found that the multiple forms of hnRNP K were produced by combinations of alternative splicing of the single *HNRNPK* gene and phosphorylation of Ser116 and/or Ser284. Furthermore, the subcellular localization of these proteins revealed by the two-dimensional map correlated with their phosphorylation states and alternative splicing patterns. The results also indicated that the multiple forms of hnRNP K were differentially modulated in response to external stimulation with bacterial lipopolysaccharide or serum. This example demonstrates very well the high resolving power of phosphate-affinity two-dimensional gel electrophoresis, which will enable us to analyze complex phosphorylation states of proteins in the immune system.

Real-time monitoring of protein secretion from single cells

While mRNA profiling at the single-cell level is now feasible by various established methods, we still lack the ability to obtain protein profiles of single cells, providing a snapshot for comprehensive characterization of their functional state. This is a significant challenge and thus, one of the focal points of our research is to develop a protein analysis method at the single cell level (Sasuga et al., *Anal Chem*. (2008) 80, 9141-9149). As an extension of the previous study, we have now succeeded in monitoring secretion of proteins from each single cell in real-time over 12 hours. This measurement could be performed in parallel for about 150 single cells with the current analysis platform. A combination of fully automated total internal reflection fluorescence microscopic technology (TIRFM) and microfluidics technology modified to be compatible with TIRFM has made this feasible. Taking advantage of these technologies, it becomes possible to assess the single cell response to stimulation by monitoring secretion activity with time. An important aspect of this technology is that monitoring of protein secretion is non-invasive as it sensitively reflects the state of the cell. This has interesting implications. For example, we could analyze mRNA profiles and/or epigenetic states of cells at a particular moment of interest, which could be identified by the protein secretion profile. In fact, our preliminary results showed that individual cells undergoing protein secretion analysis could be separately retrieved and then characterized in terms of mRNA profiles and/or genomic sequences. If we can successfully develop this system to make it more user-friendly and high-throughput, various applications of the technology will become possible. Most interestingly, because the immune system is composed of various types of immune cells, this technology may enable us to uncover unexpected complexity, which would be missed in the averaged bulk biochemical data. This concept is well-illustrated in the case of somatic mosaicism of chronic infantile neurologic, cutaneous, articular syndrome caused by NLRP3 mutation. Together with bioimaging data, single-cell genomic data of heterogeneous cell populations would greatly assist us in tackling multiscale problems in the immune system.

RAPID : Resource of Asian Primary Immunodeficiency Diseases. ***Nucleic Acids Res*** 37, D863-7 (2009)

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

Research Unit for Immunoinformatics

Unit Leader **S. Sujatha Mohan**

Research Associate : **Suresh Kumar Ramadoss**

The main mission and focus of our research unit is to develop and maintain an open access bioinformatics platform and data resource in order to gain insights into primary immunodeficiency diseases (PID) through genomic, transcriptomic and proteomic data. It has been our basic aim to provide relevant, up-to-date and validated information on PID in an easily decipherable and usable format as per global community standards. This would assist physicians in the early diagnosis and effective treatment of PID in order to minimize clinical deterioration, provide researchers with vital clues to analyze candidate PID genes, and create awareness among PID patients to lead a near normal quality life and also support affected family members with relevant PID information.

RAPID : Resource of Asian Primary Immunodeficiency Diseases – "An integrated informational platform"

We have recently developed an open access PID database designated "Resource of Asian Primary Immunodeficiency Diseases (RAPID)", a web-based compendium of molecular alterations and gene expression at the mRNA and protein levels of all PID genes reported from PID patients in the public literature. The database also includes other pertinent information about protein-protein interactions, mouse studies and microarray gene expression profiles in various organs and cells of the immune system. RAPID (published in Nucleic Acids Research, 2009) can be accessed at http://rapid.rcai.riken.jp.

PID gene mutations in RAPID were mapped to NCBI RefSeq genomic, cDNA and protein sequences as per the recommendations of the Human Genome Variations Society (HGVS). The frequent updates of RAPID have resulted in exponential data growth since its launch, as depicted in Fig. 1. Each mutation entry in the database is also linked to "Mutation@A glance", a web-based graphical user interface (GUI)-enabled tool that allows users to visualize the mutation position both at the level of DNA and protein sequences to obtain homology based three-dimensional structures with various types of information such as SNP, protein domains and functional sites, as well as to predict deleterious and novel mutations using the SIFT program.

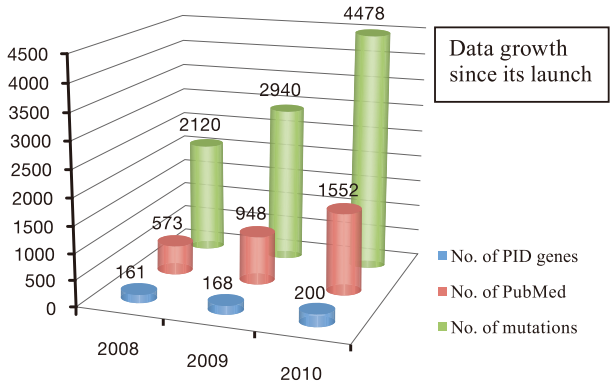


Figure 1: RAPID data growth as of December 2010. No. of PubMed refers to number of annotated reference papers with mutation data and No. of mutations indicates non-redundant entries of mutation data annotated in RAPID.

Recent publications

1. Hiroshi Masuya, Yuko Makita, Norio Kobayashi, *et al.* The RIKEN integrated database of mammals. ***Nucleic Acids Research***, 39 (Database issue) : D861-70 (2011)
2. Atsushi Hijikata, Rajesh Raju, Shivakumar Keerthikumar *et al.* Mutation@A Glance: an integrative web application for analyzing mutations from human genetic diseases. ***DNA Research***, 17 (3) : 197-208 (2010).
3. Kandasamy, K.*, Mohan, S.S*, Raju, R., *et al.* NetPath: A Public Resource of Curated Signal Transduction Pathways. ***Genome Biology***, 11 (1) : R3 (2010). *These authors contributed equally.
4. Kandasamy, K., Keerthikumar, S., Rajesh, R., *et al.* PathBuilder – Open Source Software for Annotating and Developing Pathway Resources. ***Bioinformatics***, 25 (21) : 2860-2 (2009).

PID candidate gene prediction

RAPID also hosts the candidate PID gene list obtained from Support Vector Machine (SVM) based parameter classification approach. We have predicted 1442 candidate PID genes using 69 binary features of 148 known PID genes and 3162 non-PID genes as a training data set (published in DNA Research, 2009). Currently, 19 SVM candidate genes have been confirmed as PID-causing genes, while others remain attractive candidates that are to be analyzed in patients where the disease etiology cannot be ascribed to any of the known PID genes.

RAPID mutation data analysis

With our enormous collection of PID mutation data, we have implemented an *in silico* approach to deduce interacting domains of PID genes which lose function due to observed mutations. The workflow of this approach is shown in Fig. 2. A submitted manuscript describing this approach has been accepted for oral presentation at the International Conference on Bioscience, Biochemistry and Bioinformatics (ICBBB 2011), Singapore which is to be published by IEEE and will be archived in the IEEE Xplore.

Development of a PID pathway resource

We have developed an internal pathway annotation tool (Fig. 3) to construct immune cell / tissue-specific molecular pathways of PID processes thereby highlighting various signaling and regulatory events that are curated from the published literature. These pathway reactions are depicted using 3-D visualization and high-throughput expression data analysis software tools such as Protein Lounge, ePath (www.proteinlounge.com) and PathVisio (www.pathvisio.org). This tool should aid users in evaluating gene expression patterns by overlaying expression data on the pre-constructed pathways as well as in analyzing defined pathophysiology of PID along with specific identification of potential candidate genes involved in PID for further experimental validation.

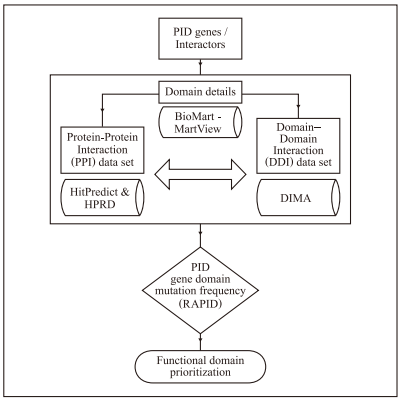


Figure2: Overview of our *in silico* approach to prioritize interacting domains associated with occurrence of high frequency PID gene-specific mutations. In this process, domain spanning regions for a PID gene and its interactors are retrieved from BioMart. Protein-protein interaction (PPI) and domain-domain interaction (DDI) data sets are collected from HitPredict & HPRD and DIMA web resources respectively. Functional domain annotation and prioritization are performed using UniProt and RAPID, respectively, to gather PID gene-specific mutations reported with equal to or more than 80% of observed mutation frequency for further integrated analysis.

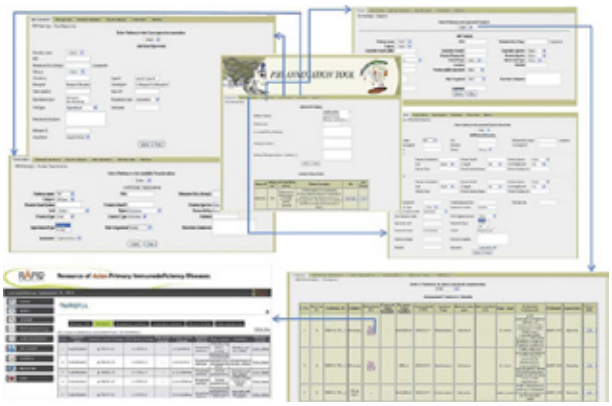


Figure3: Internal pathway annotation tool showing various features to curate PID pathway-specific data from the published literature.

PID phenotype standardization

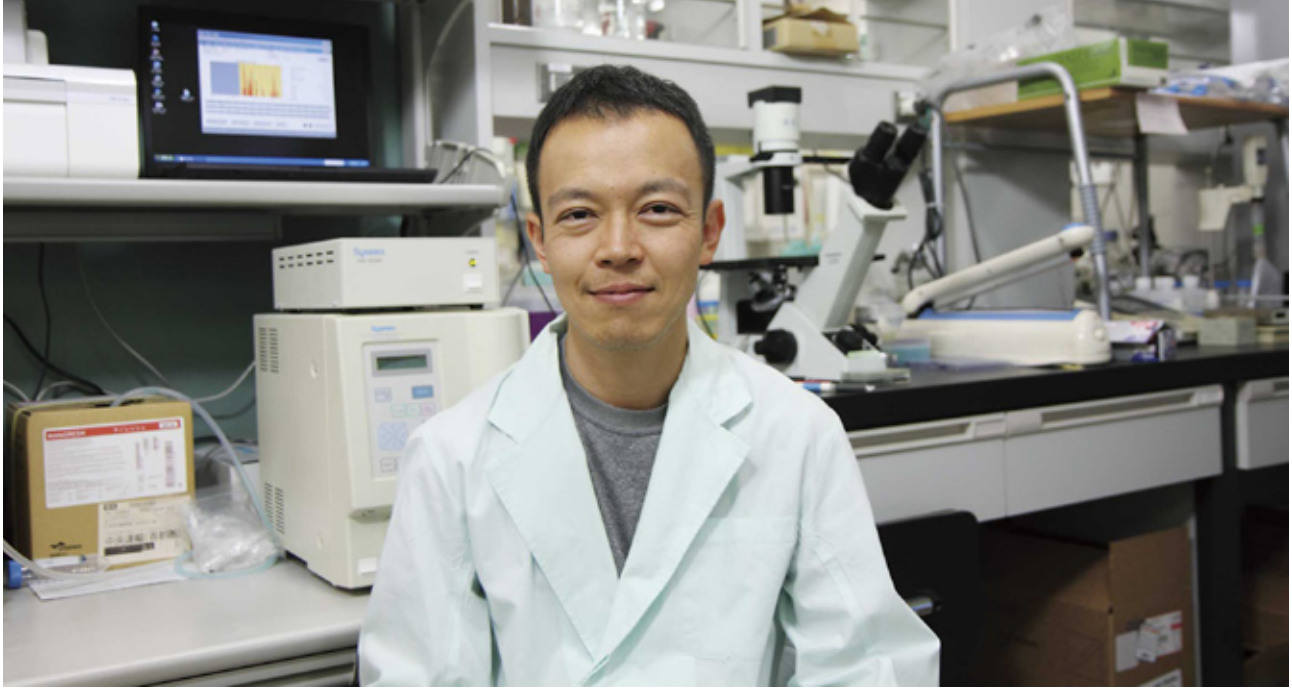
The main challenge for *in silico* genotype-phenotype correlation for any genetic diseases is to standardize phenotype ontology terms. We are in the process of collecting PID mutation specific phenotypes from the literature and Immunodeficiency resources (IDR). The generated phenotype list is then mapped to standard phenotype ontologies obtained from Human Proteome Ontology (HPO), human diseases (DOID) and Symptom ontology browser (SYMP) as provided by the ontology lookup service (http://www.ebi.ac.uk/ontology-lookup/). We also recommend the list of unmapped PID phenotypes to the HPO team to build a standard ontology. This kind of analysis should bridge a gap between genotype and phenotype correlation, thereby improving phenotype-based genetic analysis of PID genes. Also, it should aid clinicians in confirming early PID diagnosis and in implementing proper therapeutic interventions.

Collaboration and funding

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

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

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



Research Unit for Immunodynamics

Unit Leader **Takaharu Okada**

Research Scientists : **Masahiro Kitano (SPDR), Yoshikazau Ando**
Technical Staff : **Noriko Takahashi**

The goal of our research is to understand the mechanisms regulating cell migration and interactions in the tissues that shape immune responses. For this purpose we use real time imaging, in particular multi-photon microscopy, to analyze *in vivo* cellular migration and interactions. This microscopy method, which was introduced only 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. This year, we have been focusing on understanding how cellular dynamics are regulated by lymphocyte differentiation during the formation of germinal centers (GCs), the primary site for long-term, high-affinity antibody responses. GC B cells and follicular helper T (Tfh) cells play pivotal roles in GC reactions, and the transcription factor BCL6 is attracting a lot of attention in the field because it is now recognized as the master regulator for both of these cell types. However, *in vivo* dynamics of BCL6 protein expression in B cells and T cells have not been examined, despite the knowledge that *Bcl6* mRNA levels are sometimes a poor indicator of BCL6 protein expression. In particular, it is currently uncertain where in the tissue B cells begin to upregulate BCL6 protein, and how BCL6 influences B cell dynamics, leading to their clustering in GCs. Moreover, little is known about the stability of BCL6 expression in Tfh cells despite its potential importance in determining their fate.

Roles for BCL6 upregulation in controlling pre-GC B cell dynamics

In order to understand BCL6 protein expression dynamics during the antibody response, we generated a novel fluorescent reporter mouse strain by gene targeting. Utilizing the reporter animals we found that antigen-engaged B cells upregulated BCL6 while they were in the outer part of B cell follicles before clustering in GCs. Two-photon microscopic analysis indicated that this early upregulation of BCL6 in pre-GC B cells contributed to sustaining their interactions with Tfh cells in the outer follicle. The BCL6-dependent sustainment of cellular conjugate formation may play an impor-

Recent publications

1. Tanizaki H, Egawa G, Inaba K, Honda T, Nakajima S, Moniaga CS, Otsuka A, Ishizaki T, Tomura M, Watanabe T, Miyachi Y, Narumiya S, Okada T, Kabashima K. Rho-mDia1 pathway is required for adhesion, migration, and T-cell stimulation in dedritic cells. ***Blood*** 116, 5875-5884 (2010)
2. Okada T. Two-photon microscopy analysis of leukocyte trafficking and motility. ***Semin. Immunopathol.*** 32, 215-225 (2010)
3. Katagiri K., Katakai T., Ebisuno Y., Ueda Y., Okada T., Kinashi T. Mst1 controls lymphocyte trafficking and interstitial motility within lymph nodes. ***EMBO J.*** 28, 1319-1331 (2009)
4. Grigorova I.L., Schwab S.R., Phan T.G., Pham T.H., Okada T., Cyster J.G. Cortical sinus probing, S1P1-dependent entry and flow-based capture of egressing T cells. ***Nat. Immunol.*** 10, 58-65 (2009)

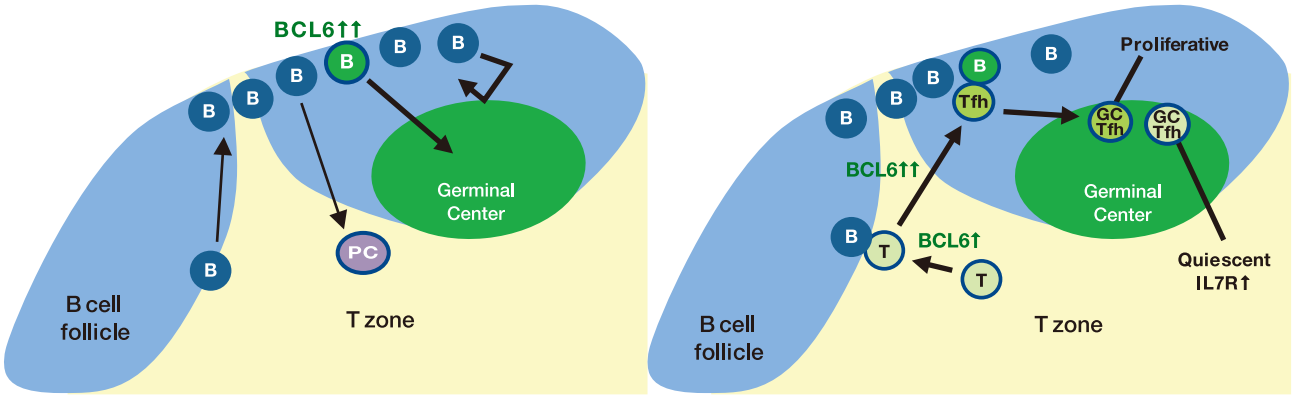


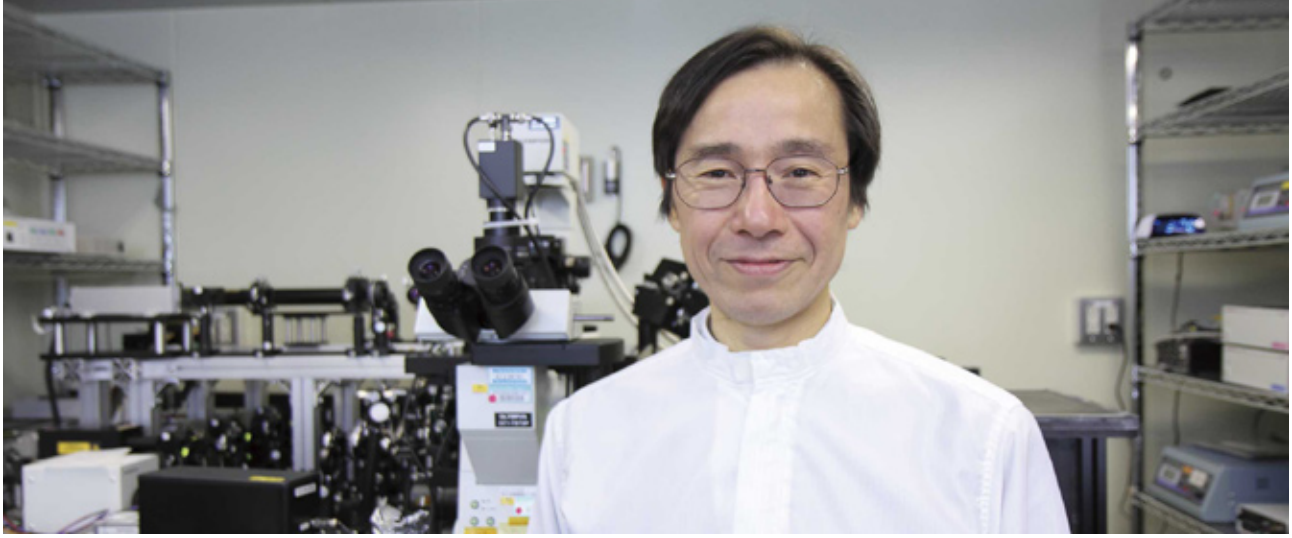
Figure : **BCL6 expression and migration of antigen-engaged B cells and helper T cells in the lymph node.**
After initial interaction with helper T cells at the B-T boundary region, antigen-engaged B cells are localized in the outer follicle region. In the outer follicle, some of these cells are committed into the plasma cell (PC) pathway, whereas others upregulate BCL6, which enables them to enter the GC region in the inner part of the follicle (*left*). Helper T cells partially upregulate BCL6 without interacting with B cells, presumably through interactions with dendritic cells. Helper T cells further upregulate BCL6 through cognate interactions with B cells to become Tfh cells. In the outer follicle, Tfh cells preferentially form conjugates with BCL6-upregulating pre-GC B cells. After their development, some of Tfh cells downmodulate BCL6. These BCL6-low Tfh cells, which are found even in GCs, terminate proliferation and upregulate the IL-7 receptor (*right*).

tant role in the GC formation, as our preliminary results suggest that blockade of integrin molecules at the time when antigen-engaged B cells and Tfh cells are in the outer follicle partially impaired their conjugation and almost completely abrogated subsequent GC formation. Moreover, the dynamic imaging analysis revealed that if antigen-engaged B cells were impaired in upregulation and function of BCL6 they failed to enter the GC region in the inner part of the B cell follicles, indicating that BCL6 upregulation in the outer follicle directs pre-GC B cell migration into the GC. These results clarify the role of BCL6 upregulation in dynamics of B cells committed to the GC pathway.

Heterogeneity of BCL6 expression in Tfh cells

BCL6 expression dynamics in helper T cells during the antibody response was also investigated. We observed that a small fraction of antigen-engaged helper T cells in the T cell zone expressed BCL6. Furthermore, antigen-engaged $CD4^+$ T cells partially upregulated BCL6 protein even in B cell-deficient animals, suggesting that helper T cells begin to express BCL6 protein in the T cell zone and further increase its expression as they became Tfh cells. When BCL6 function was genetically compromised, helper T cells were defective in the formation of the cells with the highest expression levels of CXCR5 and PD-1. Two-photon microscopy of LNs and subsequent flow cytometric analysis of the same LNs showed that the Bcl6 mutant helper T cells were most severely reduced in the GCs. These results support a model in which Tfh cells with the highest expression of CXCR5 and PD-1 are the Tfh cell population inhabiting GCs. Interestingly, after their development, Tfh cells gradually downmodulated BCL6 protein. BCL6-low Tfh cells were less proliferative than BCL6-high cells, and upregulated the IL-7 receptor. These results highlight the heterogeneity of BCL6 protein expression in Tfh cells, and suggest functional consequences. We propose that BCL6-low Tfh cells are precursors of memory helper T cells derived from Tfh cells.

5. Yamamoto S., Shimizu S., Kiyonaka S., Takahashi N., Wajima T., Hara Y., Negoro T., Hiroi T., Kiuchi Y., Okada T., Kaneko S., Lange I., Fleig A., Penner R., Nishi M., Takeshima H. and Mori Y. TRPM2-mediated $Ca2^+$ influx induces chemokine production in monocytes that aggravates inflammatory neutrophil infiltration. ***Nat. Med***. 14, 738-747. 2008.



Research Unit for
Molecular Systems Immunology

Unit Leader **Makio Tokunaga**

Student Trainees : **Akira Okada, Yuma Itou, Jun Takimoto, Katsuo Ichinomiya, Masahiro Shimozawa**

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 reconstructed three-dimensional images of the nuclear pore complex (NPC) in cells from z-scanned serial images (Fig. 1b). Clear point-like images of the Sp1 transcriptional factor were obtained without the need for deconvolution to remove out-of-focus haze. The fluorescence intensity of the NPCSp1 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.

We examined the thickness of the illumination beam by measuring illumination intensity profiles in the z-direction. We decreased the illumination thickness with the reduction of the illumination diameter. Notably, the full width at half maximum of the profile, i.e., the illumination thickness, was

Recent publications

1. Shiina N., Tokunaga M.: RNA Granule Protein 140 (RNG140): A Paralog of RNG105 Localized to Distinct RNA Granules in Neuronal Dendrites in the Adult Vertebrate Brain. ***J. Biol. Chem.***, 285, 24260-24269 (2010).
2. 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).
3. Fukagawa, A., Hiroshima, M., Sakane, I., Tokunaga M.: Stochastic emergence of multiple intermediates detected by single-molecule quasi-static mechanical unfolding of protein. ***BIOPHYSICS***, 5, 25-35 (2009).
4. Tokunaga, M., Imamoto, N., Sakata-Sogawa, K.: Highly inclined thin illumination enables clear single-molecule imaging in cells. ***Nat. Methods*** 5 (2), 159-161 (2008).

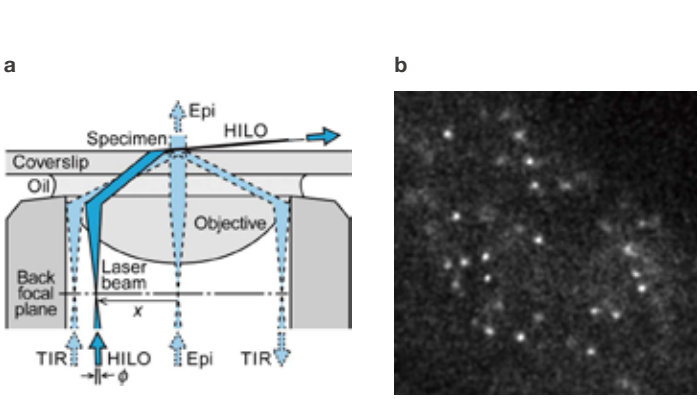


Figure 1: Molecular imaging enables one to visualize and quantify molecular dynamics, interactions, and kinetics in cells for molecular systems biology. (a) HILO microscopy for molecular imaging in cells. Illumination by a highly-inclined and thin beam increases image intensity and decreases background 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.

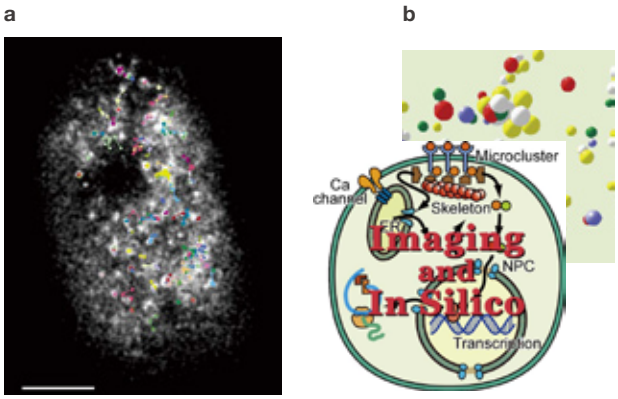


Figure 2 : The combination of single molecule quantification and "in silico" modeling opens new approaches for developing molecular systems biology. (a) Automatic tracking of single molecules of the Sp1 transcription factor visualized in a living cell. Molecular dynamics and interactions can be quantified 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.

less than 7 μm at the diameter below 20 μm.

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

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

"In silico" Modeling and Simulation

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

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

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

5. Yokosuka, T., Kobayashi, W., Sakata-Sogawa, K., Takamatsu, M., Hashimoto-Tane, A., Dustin, M. L., Tokunaga, M., Saito, T.: Spatiotemporal regulation of T cell co-stimulation by TCR-CD28 microclusters through PKCq translocation. *Immunity* 29 (4), 589-601 (2008).

Research Unit for
Thymic Environments

Unit Leader **Willem van Ewijk**

Research Scientist : **Katrin Ishii-Schrade**

In the thymic environment, lympho-stromal interaction forms the basis for both lymphoid and stromal cell development. On the one hand, thymic epithelial cells (TECs) promote maturation of T lymphocytes. On the other hand, developing thymocytes induce maturation and functional stability of the thymic stroma, a phenomenon earlier designated as "thymic crosstalk" (Ewijk W van, Shores EW, Singer A. *Immunol Today.* 1994, 15:214-217).

The artificial thymus *in vitro*

Notch signaling in developing hematopoietic cells induces restriction of these cells along the T-cell lineage (*Radtke F., et al. Immunity 1999, 10:547-558*).

Conversely, Notch signaling in TECs promotes the establishment of appropriate thymic microenvironments (Masuda et al. *Mol. Immunol.,* 2009). Based on these findings, we have set up an *in vitro* culture system to generate T lymphocytes. We have infected the mesenchymal cell line TSt4 with a retrovirus containing the Notch ligand DLL4. Previously, we found that on TSt4/DLL4 monolayers, hematopoietic progenitor cells become committed to the T cell lineage and develop into $CD4^+CD8^+$ (DP) thymocytes, but do not differentiate further. Apparently, these pre-selection DP cells are awaiting signals to be positively selected. TSt4/DLL4 cells cannot provide such key signals, although they express MHC class I molecules. Thus, although the TCR-MHC interaction is the cornerstone of positive selection, there must be other candidate molecules that contribute to the process by as yet undiscovered mechanisms. In 2010, we began focusing on one such candidate molecule, the recently discovered proteasome subunit β5t. β5t, which is only expressed in thymic cortical epithelial cells (cTECs), is crucial for the development of CD8SP cells (Murata S, Sasaki K, Kishimoto T, Niwa S, Hayashi H, Takahama Y, Tanaka K. *Science* 2007, 316:1291-1292).

Is β5t the missing molecule that works together with TCR and MHC to initiate positive selection of CD8SP cells from pre-selection DP cells?

To answer this question, we have examined whether retrovirally transduced β5t expressed in TSt4/DLL4 cells, bone marrow derived dendritic cells (BMDC), DP cells, or B cells can confer on these cells the ability to positively select CD8SP cells from pre-selection DP cells *in vitro* (Fig.1A,C).

None of the β5t expressing cells induced CD8SP cell development using the monolayer culture system. Because a 2-D organized monolayer culture itself may not provide an optimal microenvironment for positive selection, we have redesigned our initial system into a (3-D) re-aggregate culture

Recent publications

1. Vroegindeweij, E, Itoi M, Satoh R, Zuklys S, Crobach S, Germeraad WTV. Cornelissen JJ, Cupedo T, Holländer G, Kawamoto H, van Ewijk W. Thymic cysts originate from Foxn1 positive thymic medullary epithelium. ***Mol. Immunol.*** 2010 47(5):1106-13

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

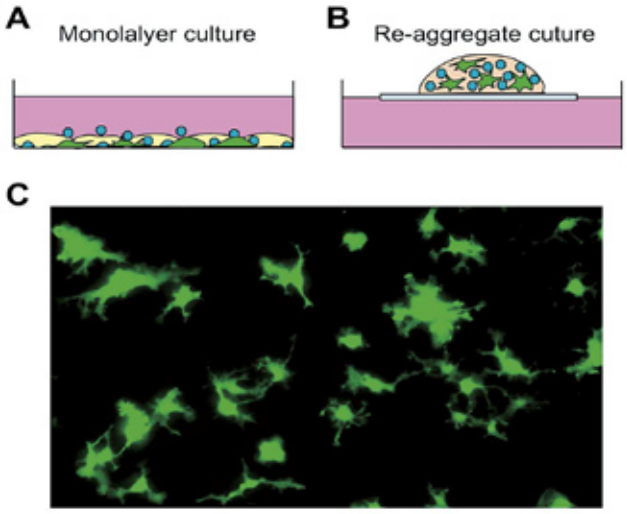


Figure 1:
A) Developing T cells (*blue*) and β5t expressing antigen presenting cells (*green*) growing on mono-layered TSt4/DLL4 stromal cells (*yellow*).
B) The same cells as in A) growing as a tight re-aggregate on a filter that floats on medium.
C) We have labeled BMDCs (*green*) to study how well they grow in monolayer culture together with DP cells and Tst4/DLL4 cells (both not visible)

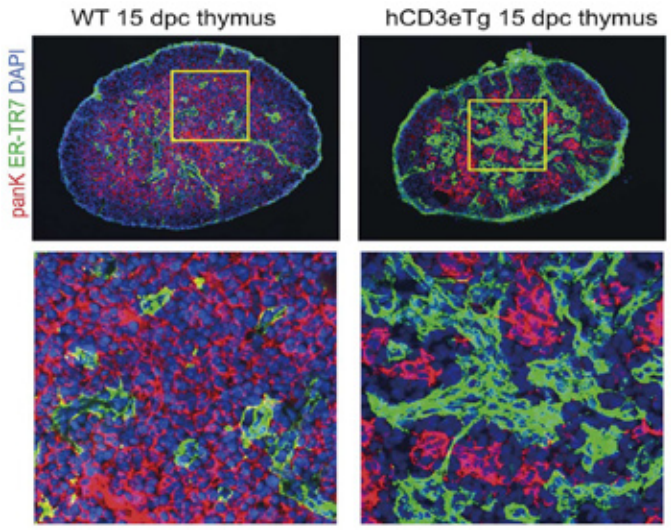


Figure2: Thymic lobes of normal and hCD3Tg fetal mice

system (RTOC) where pre-selection DP cells can be mixed with β5t over-expressing cells in known numbers to form discrete, small volume aggregates, ensuring maximal cell-to-cell interaction (Fig.1B). We found that, again, none of the β5t expressing antigen presenting cells promoted CD8SP development. However, cTECs isolated from deoxyguanosine (dGuo) treated fetal thymic lobes were able to generate CD8 SP cells.

We conclude that simple overexpression of β5t in these antigen presenting cells is not sufficient to confer the capacity for positive selection. Thus, additional unknown molecules, unique to cTECs associated in a network configuration, must act in concert with β5t, TCR, and MHC in the process of CD8SP development.

In vivo microenvironments

The normal thymic environment is largely composed of epithelial cells. Thymic fibroblasts form a minority population and are only found in the thymic capsule, in septae, around blood vessels, and as free fibroblasts in the medulla.

In following up our crosstalk experiments, we noticed that an early block in T cell development induces major changes in the thymic environment, leading both to the generation of thymic cysts, as well as to changes in the development of mesenchymal cells. Regarding the development of thymic cysts, we have now provided formal proof that cysts differentiate from the 3-D organized thymic epithelium (2).

Regarding the development of mesenchymal cells in the thymus, we noticed that loss of thymic crosstalk signaling leads to expansion of these cells in the thymic environment. Thus, in the thymus of fetal hCD3tg mice, which have a block in T cell development at the TN-1 stage, the organization of TEC's is disturbed, going hand in hand with a marked increase in mesenchymal cells, detected by ERTR7 expression (Fig 2). Similarly, fetal thymi cultured with dGuo show increased numbers of fibroblasts.

BrdU labeling experiments showed that fibroblasts proliferate under these experimental conditions. We are presently focusing on the role of epithelial derived cytokines in stimulating the growth of thymic mesenchymal cells.

In sum, our data indicate:

(1) Further research is required to enable the production of fully differentiated T cells *in vitro*.
(2) In the *in vivo* thymus, TECs dominate the thymic environment in the presence of crosstalk signaling. Absence of crosstalk signaling impairs TECs, but stimulates growth of fibroblasts. Such increased activity of mesenchymal cells might prepare a scaffold for repair of the affected thymic epithelial environment.

Research Unit for Immunoepigenetics

Unit Leader **Miguel Vidal**

Research Scientist : **Kaori Hisada**

Technical staff : **Asako Shibano**

Polycomb group (PcG) complexes function as transcriptional repressors in cells undergoing cell fate transitions. Thus, maintenance of stem cells and utilization of their full developmental potential are some of the essential functions regulated by PcG activity. PcG complexes are chromatin modifiers endowed with histone modifying activities (trimethylation and monoubiquitylation of histones H3 and H2A). How these modifications relate to transcriptional repression and how PcG complexes are recruited to their targets are a subject of active research.

We identified RYBP (Ring1 and YY1 binding protein) as a PcG histone H2A monoubiquitin ligase interactor that also interacts with YY1, features that made it appear as a candidate for PcG recruiting. RYBP function, however, is poorly characterized, in part due to the embryonic lethal phenotype of constitutive RYBP mutant alleles.

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

RYBP it thought to be part of the pluripotency network of transcriptional regulators of ES cells. To study RYBP function we have generated ES cells that carry both a floxed RYBP allele and a ubiquitously expressed allele encoding a Cre-ER recombinase that can be activated by hydroxytamoxifen. RYBP-deficient ES cells maintain their self-renewal properties and show a limited alteration in the mRNA expression pattern of protein-coding genes. We have addressed the possibility of redundancy by knocking down the only RYBP paralog, Yaf2, using an shRNA approach, but preliminary results do not support such a function. Interestingly, a subset of upregulated mRNAs in RYBP-deficient ES cells encode germline specific products and are also derepressed in ES cells lacking components of the DNA methylation machinery. To gain insight in RYBP mechanisms we generated (in collaboration with Drs. M. Endoh and T. Endo at RCAI) maps of chromatin association for RYBP in wild type, Dnmt1-deficient and eed-deficient ES cells. Preliminary analysis of the results showed RYBP association to characteristic Polycomb targets whose repression is independent of RYBP. By contrast, RYBP silenced targets, including genes marked by partial DNA methylation, show poor association of PcG subunits. In both cases, depletion of

Recent publications

1. Román-Trufero, M., Méndez-Gómez, H., Pérez, C., Hijikata, A., Fujimura, Y., Endo, T., Koseki, H., Vicario-Abejón, C. and Vidal, M. (2009) Maintenance of undifferentiated state and self-renewal of embryonic neural stem cells by Polycomb protein Ring1B. ***Stem Cells*** 27:1559-1570
2. Vidal, M. (2009) Role of Polycomb proteins Ring1A and Ring1B in the epigenetic regulation of gene expression ***Int. J. Dev. Biol***. 53:355-370
3. Hirabayashi and, Y., Suzki, N., Tsuboi, M., Endo, T.A., Toyoda, T., Shinga, J., Koseki, H., Vidal, M. Gotoh, Y. (2009) Polycomb limits the neurogenic competence of neural precursor cells to promote astrogenic fate transition. ***Neuron*** 63: 600-613
4. Calés, C., Román-Trufero, M., Pavón, L., Serrano, I., Melgar, T., Endoh, M., Pérez, C., Koseki, H. and Vidal, M. (2008) Inactivation of the Polycomb group protein Ring1B unveils an antiproliferative role in hematopoietic cell expansion and cooperation with tumorigenesis

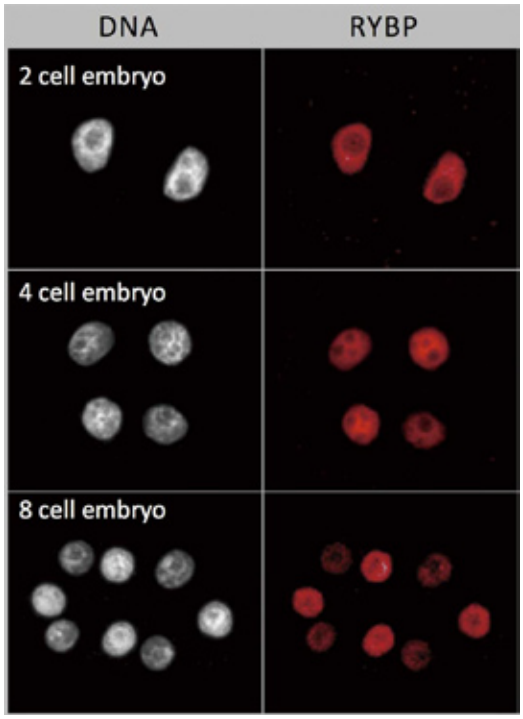


Figure 1: RYBP protein expression in preimplantation embryos.

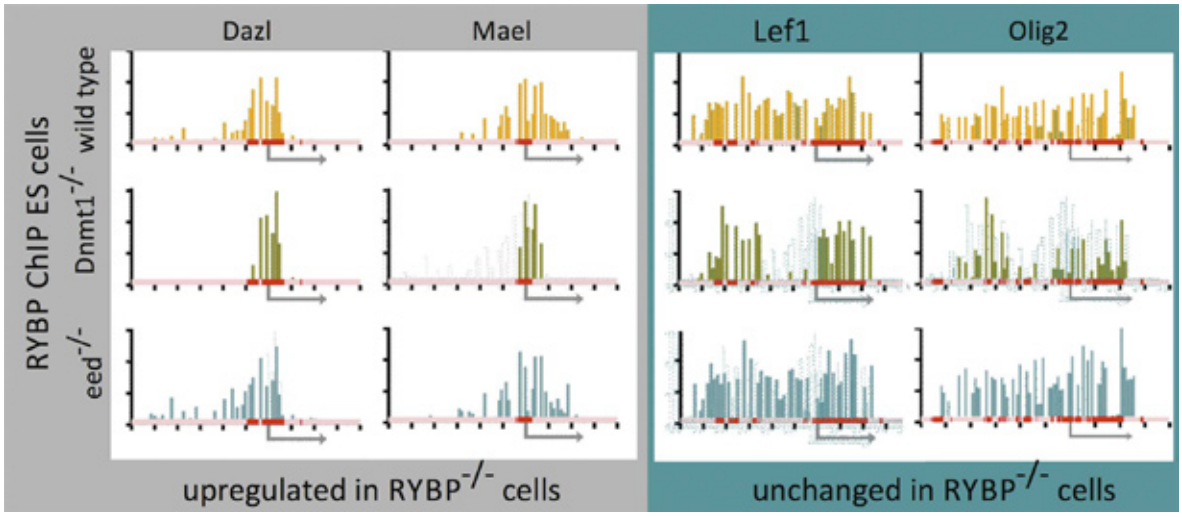


Figure 2: RYBP association to chromatin of ES cells of the indicated genotypes.
Representative loci of the groups of genes whose transcriptional output was upregulated or unchanged in RYBP-deficient ES cells. *Arrows* indicated transcription start sites and direction of elongation. CpG islands are indicated in *red*.

H3K27me3 marks or of CpG methylation (eed and Dnmt1-deficient ES cells) has little effect on RYBP chromatin association. Future work will involve validation of the hypothesis raised by these results suggesting that RYBP, which is expressed from the earliest developmental stages, may contribute to DNA methylation marking and the recruitment of Ring1A/Ring1B Polycomb complexes.

associated to *Ink4a* deletion. ***Mol. Cell. Biol***. 28:1018-1028

Central Facilities

Central Facilities in RCAI provides all researchers in the Center with access to the most advanced equipment and technologies. The Central Facilities consist of five sections: the FACS, Confocal, and Monoclonal Antibody Laboratories managed by Takashi Saito, the Genomics Laboratory managed by Osamu Ohara, and the Animal Facility managed by 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 2010, FACS Lab. added new FACS machines, FACS Aria II and Canto II. In addition, the lab installed ImageStream100 (Fig. 1) that combines the speed, sensitivity, and quantitation of flow cytometry with the visual detail of microscopy in a single platform. Ikuo Ishige, Engineer from BM Equipment Co. Ltd., provides training courses, consultations for experiments and operation and analysis services for using ImageStream.

Table 1: FACS instruments in the Central Facilities

Machine types	Machines	Number of machines
FACS cell analyzer	Calibur	5
	Canto II	1
FACS cell sorter	Aria I	2
	Aria II	1
	Vantage	2
	Diva (digital vantage)	1
Imaging flow cytometer	ImageStream100	1

Figure 1 : Ikuo Ishige with ImageStream100

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

1. **Technical support and training**
   In 2010, the facility offered 9 technical courses (4 for cell sorting and 5 for cell analysis) both in English and Japanese. Courses were held at 3 different levels, Calibur basic, Canto II and Aria basic. A total of 41 researchers in RCAI took the courses in 2010.

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 2010, the number of operation service provided by the lab was 266.

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.

Monoclonal Antibody Lab.

Technical Staff : Tomomi Aoyama, Mayuko Matsuda

The Monoclonal Antibody (mAb) Lab. aims to produce mAbs that meet the needs of RCAI researchers, and

also focus on more strategic development of mAb, such as those for allergy-related molecules, and several GPCRs that are difficult targets. The activity is partially supported by Becton Dickinson, and two technical staff members, Tomomi Aoyama and Mayuko Matsuda, are engaged to produce mAbs. In 2010, the Lab. established mAbs against 15 different antigens, which were requested from 7 laboratories. The lab also succeeded in the establishment of an antibody against the human mast cell IgE receptor that inhibits IgE-binding and would have potential therapeutic applications.

Confocal Lab.

Visiting Scientist : Yasutaka Wakabayashi (Leica Microsystems)

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

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

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

3. Leica SP5 was newly introduced in February, 2011. SP5 is the successor of SP2. Brighter and more beautiful image will be possible because of the improved optical system.

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

5. Newly installed inverted intravital system with visual laser, MP, OPO laser and high speed scanner that can be used for *in vivo* imaging of various tissues. Especially OPO laser can excite red fluorophore in deeper area because its tunable wave length is longer than MP.

6. 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 applications such as light stimulation (Fig 2).

During 2010, Yasutaka Wakabayashi from Leica provided training course for 18 people. The total running time of the microscopes was over 1860 hours.

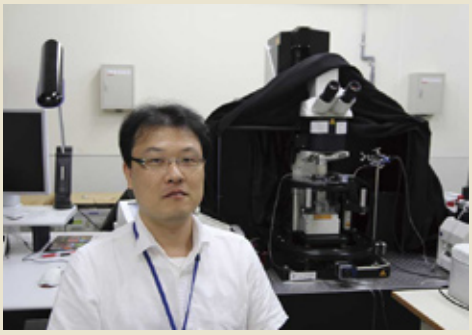


Figure 2 : Yasutaka Wakabayashi with intravital upright system

Genomics Lab.

Research Scientist : Takashi Watanabe
Research Associates : Mai Yamagishi, Atsushi Hijikata
Technical Staff : Tomoko Hasegawa, Nobutake Suzuki, Akio Kobayashi, Ryosuke Yashi, Fumie Yokoyama

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 2010

Proteomics	# of samples	# of teams
Two-dimentional electrophoresis	14	1
Mass Spectrometry Analysis	367	2
Multiplex suspension array	# of samples	# of teams
	3414	13
Affymetrix Genechip (Exon array, Gene array, miRNA array)	# of samples	# of teams
Human	148	6
Mouse	441	17
Total	589	23
DNA sequencing	# of samples	# of teams
36cm capillary	18,473	18
50cm capillary	13,425	16
Total	31,898	34
cDNA clone delivery	# of clones	# of teams
	121	6
Primer/labeled probe delivery	# of sets	# of teams
	551	4

Because progress in genome technology is very rapid, the Genomics Lab. is keen to provide the Center with the most updated technology on demand. As an example, a Fluidigm BioMark system is newly introduced to expedite single-cell quantitative PCR processes. The BioMark system enables us to obtain a high throughput qPCR data based on single cell using a 48.48 Dynamic Array chip and/or more high-throughput array chips (Fig. 3).

Figure 3 : BioMark™ system and BioMark™ 48.48 Dynamic Arrays

Animal Facility

Senior Research Scientist : Takanori Hasegawa
Technical Scientist : Shinobu Mochizuki
Technical Staff : Tomoyuki Ishikura, Isamu Hisanaga, Yusuke Iizuka, Hiromi Tatsumi, Yurie Kawamoto, Chie Yoshida
Animal care takers (outsourced) : 22 people

The animal facility at RCAI provides a high-standard facility for animal experiments for RIKEN Yokohama Institute. Also, the facility supports the generation of knock-out, transgenic and humanized mice.

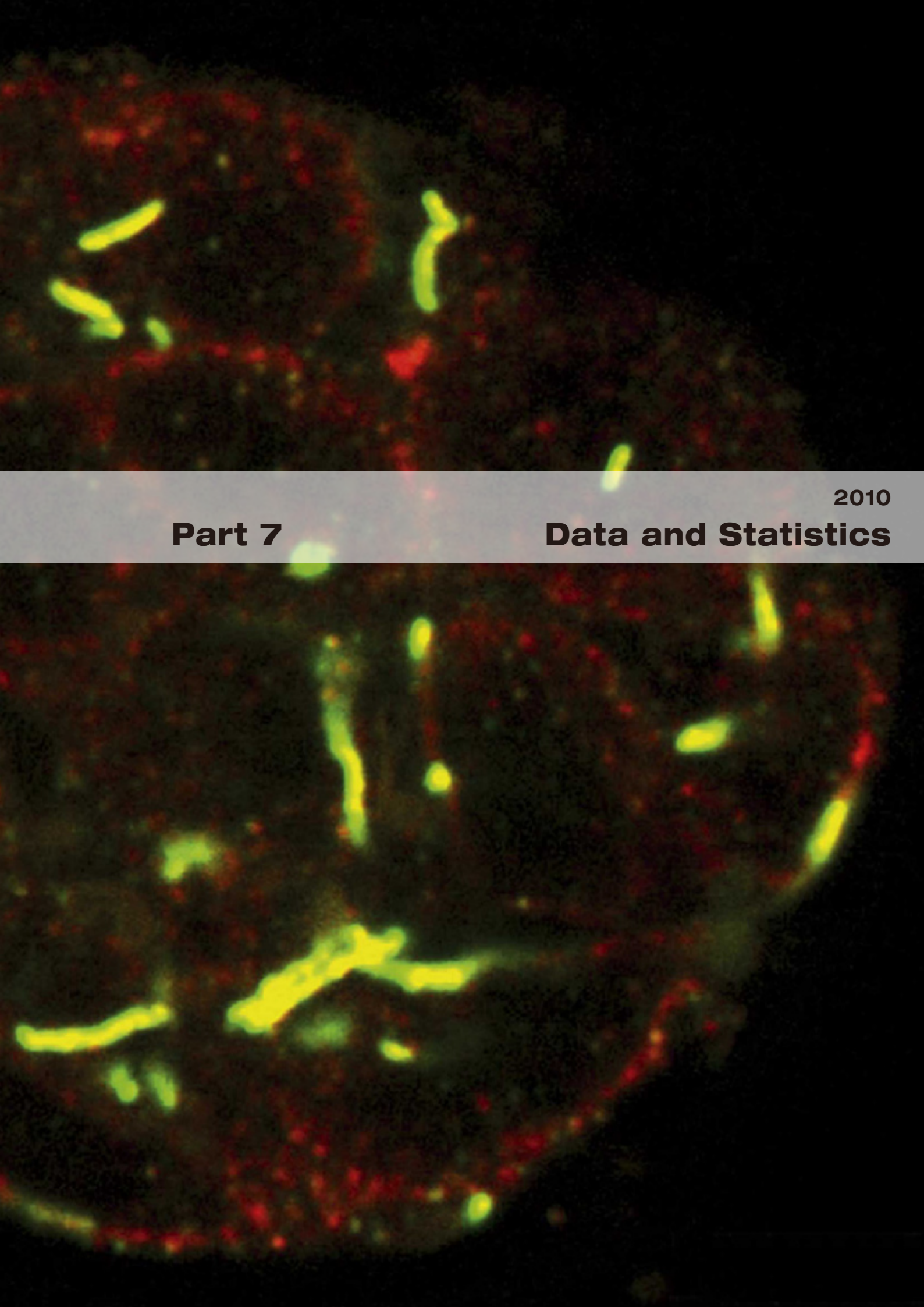
1. Maintenance, generation and cryostocks of genetic resources

In 2010, over 52,300 mice and 6 rats were maintained in the SPF area and 725 mice in an isolated area. They have newly introduced 641 mouse lines into the SPF area by a combination of *in vitro* fertilization (IVF) and embryo transfer and generated cryostocks of genetic resources for 502 lines. They also maintained relatively large colonies for several commonly used strains such as NOD/SCID/γCKO mice, Rag1KO and cre deleters and provided them to users on demand. The facility staff provided technical assistance to generate chimeras (188 lines) and transgenic mice (10 lines) and to establish and maintain ES cells. The facility has also generated an internally available database for genetic resources.

2. Introduction of human BAC clones into NOD/SCID/γCKO mice

In collaboration with Osamu Ohara (RCAI Lab. for Immunogenomics) and Fumihiko Ishikawa (RCAI Research Unit for Human Disease Model), they launched a new activity to improve the efficacy of transplantation of human hematopoietic stem cells into immunodeficient NOD/SCID/γCKO 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/γCKO mice. Up to now, they have established 3 BAC transgenics and confirmed the expression of human genes on a C57BL6 background and begun back-crossing onto the NOD/SCID/γCKO mice using the speed-congenic method.

Figure 4 : Bio-bubble for the maintenance of immunodeficient mice



2010

Part 7 Data and Statistics

Publications (Apr. 2010 - Mar. 2011)

Table : RCAI Publications (Apr. 2010-Mar. 2011)

Journals	IF (2009)	FY2010
Nature	34.5	1
Cell	31.2	1
Science	29.7	2
Nat Immunol	26.0	4
Cell Stem Cell	23.6	2
Immunity	20.6	4
J Clini Invest	15.4	3
J Exp Med	14.5	6
Dev Cell	13.4	1
Curr Opin Immunol	10.9	1
Blood	10.6	5
Immunol Rev	10.1	2
J Cell Biol	9.6	1
Proc Natl Acad Sci USA	9.4	5
Semin Immunol	9.2	1
EMBO J	9.0	1
Nucleic Acids Res	7.5	2
Arthritis Rheum	7.3	1
Lab Chip	6.3	1
Mol and Cell Biol	6.1	2
J Immunol	5.6	10
Semin Immunopathol	5.5	1
J Biol Chem	5.3	1
J Proteome Res	5.1	1
J Mol Cell Cardiol	5.0	1
Bioinformatics	4.9	1
DNA Res	4.9	1
Proteomics	4.4	1
PLoS One	4.4	1
Arthritis Res Ther	4.3	1
BMC Syst Biol	4.1	1
Prog Biophys Mol Biol	4.0	1
Int Immunol	2.5	7
Other Journals		39
Total		113

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Invited Presentations

Date	Title	Speaker	Meeting
Apr-10	M-Sec, a factor promoting the formation of tunneling nanotubes interconnecting the plasma membrane of two remote cells.	**Ohno, H.**	18th Neuroimmunology Forum (Fukushima, Japan)
Apr-10	Role of AP-1B in the formation of biological front-line barrier	**Hase, K.**	14th Meeting of Young Investigators for IBD (Niigata, Japan)
Apr-10	Detection de molecules uniques sur cellule vivante: presentation d'une nouvelle technique de microscopie a fluorescence	**Tokunaga, M.** and **Sakata-Sogawa, K.**	27eme Rencontre Scientifique Francophone de Tokyo (Tokyo, Japan)
Apr-10	Zinc signaling in immunity, allergy and inflammation	**Hirano, T.**	109th Annual Meeting of the Japanese Dermatological Association (Osaka, Japan)
Apr-10	IL-6 amplifier and autoimmune/chronic inflammatory diseases	**Hirano, T.**	54th Annual General Assembly and Scientific Meeting of the Japan College of Rheumatology (Kobe, Japan)
Apr-10	Elucidation of regulatory mechanisms for dendritic cell functions by gene targeting mice	**Kaisho, T.**	The Seminar of Osaka University Hospital Medical Center for Translational Research (Osaka, Japan)
May-10	Regulation of immune response by cytokine and zinc signaling	**Hirano, T.**	JSI symposium at IMMUNOLOGY 2010 (Bethesda, USA)
May-10	Single molecule quantification and systems biology	**Tokunaga, M.**	Symposium of Science Council of Japan (Tokyo, Japan)
May-10	New aspects of IgA synthesis in Peyer's patches	**Fagarasan, S.**	IMMUNOLOGY 2010 TFH symposium (Baltimore, Maryland, USA)
May-10	Immune Regulation by Apoptotic Cell Clearance	**Tanaka, M.**	A Research Workshop of Israel Science Foundation Clearance of Dying Cells in a Healthy and Diseased Immune System (Jerusalem, Israel)
May-10	Overview on bacteria-immune interactions in gut	**Fagarasan, S.**	External examiner, The School of Graduate Studies at University of Toronto (University of Toronto, Canada)
May-10	M-Sec promotes intercellular logistics via membrane nanotube formation	**Hase, K., Kimura, S., Ohno, H.**	62nd Annual Meeting of the Japan Society for Cell Biology (Osaka, Japan)
May-10	The future of allergen immunotherapy: how the new mechanisms lead to better immunotherapy?	**Ishii, Y.**	KACCI Spring Congress (Soul, South Korea)
May-10	Role of sinus macrophages in the induction of of anti-tumor immunity	**Tanaka, M.**	CIMT2010 Cancer Immunotherapy 8th Annual Meeting (Mainz, Germany)
Jun-10	Antigen transcytosis by M cells: from molecular entities to biological significance	**Hase, K.**	4th Symposium of Immunology Frontier Research Center (IFReC)(Osaka, Japan)
Jun-10	Immunosurveillance on the mucosal surface by specialized epithelial M cells	**Hase, K.**	16th Annual Meeting of Japanese Society for Helicobacter Research (Kyoto, Japan)
Jun-10	The Dynamic Mechansim of and Fundamental Technology for Biological System	**Hirano, T.**	CREST Symposium 2010 (Osaka, Japan)
Jun-10	Zinc and Immunity/inflammation/allegy: Zinc is signaling molecule	**Hirano, T.**	26th Annual Meeting of the Japan Society of Drug Delivery System (Osaka, Japan)
Jun-10	Zinc is an intracellular signaling molecule	**Hirano, T.**	20th Symposium on Role of Metals in Biological Reactions, Biology and Medicine (Tokushima, Japan)
Jun-10	The zinc transporter: its involvement in intracellular signaling and inheritated disorders	**Fukada, T.**	10th Annual Meeting of The Protein Science Society of Japan (Sapporo, Japan)
Jun-10	Zinc and Zinc transporter in cytokine/growth factor signaling and diseases	**Fukada, T.**	75th Japanese Society of Interferon and Cytokine Research (Kitakyushu, Japan)
Jun-10	Unique properties of memory B lymphocytes	**Kurosaki, T.**	Freiburg Institute for Advanced Studies Monday Seminar Series (Freiburg, Germany)
Jun-10	Glycoprotein 2: An Antigen-Uptake Receptor on Intestinal Epithelial M Cells for Mucosal Immunity	**Ohno, H.**	iCeMS International Symposium: "Traffic.dk: Future Directions in Cellular Trafficking" (Kyoto, Japan)
Jun-10	Antigen mRNA-transfected, allogeneic fibroblasts loaded with NKT cell ligand confer antitumor immunity.	**Shimizu, K.**	6th Kirin-Juku meeting, (Tokyo, Japan)
Jun-10	Molecular mechanisms for dendritic cell heterogeneity	**Kaisho, T.**	NZ-RIKEN-CHIBA Joint Workshop (Yokohama, Japan)
Jun-10	Crucial role of plasmacytoid dendritic cells for the regulation of inflammation and T cell immunity in vivo	**Sato, K.**	NZ-RIKEN-CHIBA Joint Workshop (Yokohama, Japan)
Jun-10	iNKT cell-triggered in vivo DC targeting immunotherapy	**Fujii, S.**	NZ-RIKEN-CHIBA Joint Workshop (Yokohama, Japan)
Jun-10	Immune regulation by apoptotic cell clearance	**Tanaka, M.**	NZ-RIKEN-CHIBA Joint Workshop (Yokohama, Japan)
Jun-10	Dynamic interactions between bacteria and mucosal immune system	**Fagarasan, S.**	14th Annual Scientific Meeting for Intestinal Microbiology (Kyoto, Japan)
Jun-10	Control of immune response by dendritic cells	**Sato, K.**	20th Annual Meeting of the Japanese Dendritic Cell Society (Niigata, Japan)
Jun-10	Discovery of NKT cells with adjuvant activity and their clinical application in patients with advanced lung cancer	**Taniguchi, M.**	2nd International Conference-Current Advances in Microbiology, Immunology and Allergology (Ulaanbaatar, Mongol)
Jun-10	Translational research on human leukemia stem cells using flow cytometric analysis	**Ishikawa, F.**	20th Annual Meeting of the Japan Cytometry Society (Tokyo, Japan)
Jun-10	Immunotherapy utilizing iNKT cells to target in vivo dendritic cells (DCs)	**Fujii, S.**	Global COE symposium on current and future directions of dendritic cell biology and immunotherapy. The Institute of Medical Science, The University of Tokyo (IMSUT) (Tokyo, Japan)
Jun-10	Mathematical analysis of signal transcriptional network for cell fate decisions	**Okada, M.**	RIKEN ASI colloquium series: Cellular Systems I "Theoretical Biology 3: Signal Network" (Wako, Japan)
Jun-10	M-Sec sec-ures membrane nanotubes with RalA and exocyst	**Hase, K.**	International Symposium on Organelle Network: Microbiology, Immunology & Cell Biology (Osaka, Japan)
Jun-10	Comparative genome analysis of the mammalian Y chromosomes for understanding of the male-specific genome evolution	**Kuroki, Y.**	IDBS2010, International Symposium on Biodiversity Sciences. ''Genome, Evolution and Environment (Nagoya, Japan)
Jun-10	Dendritic cell functions	**Kaisho, T.**	The Seminar of Hayashibara Biochemical Laboratories (Okayama, Japan)
Jun-10	The Future of Osaka University's School of Medicine	**Hirano, T.**	In Commemoration of the 200th Anniversary of OGATA Koan's Birth, the School of Medicine is offering a Course open to the Public (Osaka, Japan)
Jun-10	Single molecule imaging in living cells and stochastic feature of molecular interactions	**Tokunaga, M.**	Universität Heidelberg – Tokyo Institute of Technology JOINT WORKSHOP (Heidelberg, Germany)
Aug-10	Preferential localization of IgG memory B cells adjacent to contracted germinal centers	**Kurosaki, T.**	4th International Symposium on B cells and Autoimmunity (Nara, Japan)
Aug-10	Store-operated Calcium Entry in B cells	**Kurosaki, T.**	14th International Congress of Immunology (Kobe, Japan)
Aug-10	Localization of IgM and IgG memory B cells	**Kurosaki, T.**	INSERM/Pasteur-RIKEN Joint Immunology Workshop 2010 (Yokohama, Japan)
Aug-10	IL-6-STAT3-SOCS3 form a homeostatic circuit; STAT3 and SOCS3 independently regulate allergic and psoriatic skin inflammation	**Kubo, M.**	INSERM/Pasteur-RIKEN Joint Immunology Workshop 2010 (Yokohama, Japan)
Aug-10	MARCH-I: a new regulator of dendritic cell function	**Ishido, S.**	INSERM/Pasteur-RIKEN Joint Immunology Workshop 2010 (Yokohama, Japan)
Aug-10	What is "Systems Immunology" for?	**Ohara, O.**	14th International Congress of Immunology (Kobe, Japan)
Aug-10	Sequencing of the targeted chromosome by using next-generation sequencer	**Kuroki, Y.**	12th Annual Meeting of Society of Evolutionary Studies, Japan (Tokyo, Japan)
Aug-10	Anti-tumor immunity by dead cell clearance	**Tanaka, M.**	19th Annual Meeting of Japanese Society for Cell Death Research (Nagoya, Japan)
Aug-10	Transcriptional Regulation of CD4/CD8 Lineage Choice	**Taniuchi, I.**	14th International Congress of Immunology 2010 (Kobe, Japan)
Aug-10	How dendritic cells respond to nucleic acid adjuvants	**Kaisho, T.**	9th International Veterinary Immunology Symposium (Tokyo, Japan)

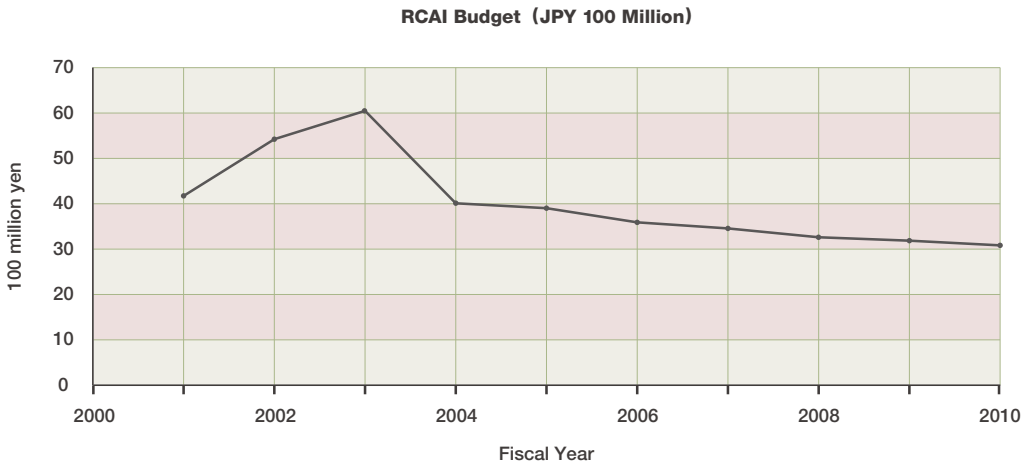
Aug-10	Investigating normal & diseased human immune system using humanized mouse	**Ishikawa, F.**	RCAI International Summer Program 2009 (Yokohama, Japan)
Aug-10	How dendritic cells sense and respond to nucleic acid adjuvants	**Kaisho, T.**	RCAI International Summer Program 2010 (Yokohama, Japan)
Aug-10	Transcriptional Regulation of CD4/CD8 Lineage Choice	**Taniuchi, I.**	Chiba GCOE Sympozium (Chiba, Japan)
Aug-10	Zinc signaling in bone and immunity	**Hirano, T.**	7th meeting of Bone Biology Forum (Shizuoka, Japan)
Aug-10	Role of interleukin 6 amplifier in autoimmune disease and inflammation	**Hirano, T.**	14th International Congress of Immunology (ICI 2010) (Kobe, Japan)
Aug-10	Adaptive immune regulation in the gut-T dependent and independent IgA responses	**Fagarasan, S.**	14th International Congress of Immunology (ICI 2010) (Kobe, Japan)
Aug-10	NKT cell-targeted adjuvant cell therapy-from basic to clinic	**Taniguchi, M.**	14th International Congress of Immunology (ICI 2010) (Kobe, Japan)
Aug-10	Reconciling stability and plasticity of Foxp3$^+$ regulatory T cell differentiation	**Hori, S.**	14th International Congress of Immunology (ICI 2010) (Kobe, Japan)
Aug-10	Spatio-temporal regulation of T cell activation	**Saito, T.**	14th International Congress of Immunology (ICI 2010) (Kobe, Japan)
Aug-10	Regulation of Thymocytes Development by Silencers	**Taniuchi, I.**	INSERM/Pasteur-RIKEN Joint Immunology Workshop 2010 (Yokohama, Japan)
Aug-10	Termination of myeloid potential in T progenitors: an essential developmental checkpoint for production of the T cell lineage	**Kawamoto, H.**	INSERM/Pasteur-RIKEN Joint Immunology Workshop 2010 (Yokohama, Japan)
Aug-10	Creating therapeutic strategies targeting human leukemia stem cells	**Ishikawa, F.**	INSERM/Pasteur-RIKEN Joint Immunology Workshop 2010 (Yokohama, Japan)
Aug-10	PDLIM2, a nuclear ubiquitin E3 ligase, negatively regulates inflammatory responses.	**Tanaka, T.**	INSERM/Pasteur-RIKEN Joint Immunology Workshop 2010 (Yokohama, Japan)
Sep-10	Elucidation of the onset mechanisms of allergy based on an evaluation platform of gut environment	**Uetake, C., Jukuda, S., Shima, H., Date, Y., Nakanishi, Y., Kato, T., Kikuchi, J., Ohno, H.**	5th Metabolome Symposium (Tsuruoka, Japan)
Sep-10	Analysis of CIN85 function in B cell signaling	**Kometani, K.** and **Kurosaki, T.**	National Institute for Physiological Sciences Conference (Okazaki, Japan)
Sep-10	Control of skin inflammation by Suppressor of cytokine signaling	**Kubo, M.**	Japanese Society of Psoriasis (Ube, Japan)
Sep-10	Technology development for the next generation humanized mouse model	**Ohara, O.**	Kazusa Chiba Collaboration Meeting for Academia, Government and Industries (Kisarazu,Japan)
Sep-10	From transcriptome analysis to systems immunology	**Ohara, O.**	5th Indo-Japan International Symposium on "Innovative Molecular Approaches in Global Health Research" (IJS-2010) (Jaipur, India)
Sep-10	Comparative analysis of the mammalian Y chromosomes	**Kuroki, Y.**	82nd Annual Meeting of the Genetics Society of Japan (Sapporo, Japan)
Sep-10	Comparative analysis of the genome constitution for understanding the conformation of the repetitive sequence blocks in primate evolution	**Kuroki, Y.**	International Primatological Society XXIII Congress Kyoto 2010 (Kyoto, Japan)
Sep-10	Visualization of a developmental checkpoint by inducing arrest and selfrenewal of progenitors	**Kawamoto, H.**	Life Science Seminar, Institute for Frontier Medical Sciences, Kyoto University (Kyoto, Japan)
Sep-10	Direct regulation of T cell activation by TLR ligands	**Saito, T.**	10th Awaji International Forum on Infection and Immunity (Awaji, Japan)
Sep-10	Molecular mechanisms for dendritic cell activation by nucleic acid adjuvants	**Kaisho, T.**	Vaccine Forum 2010 (Tokyo, Japan)
Sep-10	Immune regulation mechanism and clinical application of alpha-galactosylceramide	**Ishii, Y.**	Vaccine Forum 2011 (Tokyo, Japan)
Sep-10	Now, what are the elements expected to cancer research? – Specific proposals for cancer research –	**Hirano, T.**	69th Annual Meeting of the Japanese Cancer Association (Osaka, Japan)
Sep-10	iPS-derived NKT cells	**Taniguchi, M.**	40th Annual Meeting of the German Society for Immunology (Leipzig, Germany)
Sep-10	Chemotherapy resistance of quiescent AML stem cells	**Ishikawa, F.**	72nd Annual Meeting of the Japan Society of Hematology (Yokohama, Japan)
Sep-10	Control of immune response by regulatory dendritic cells	**Sato, K.**	72nd Annual Meeting of Japanese Society of Hematology (Yokohama, Japan)
Sep-10	Lineage stability and plasticity of Foxp3+ regulatory T cells	**Hori, S.**	72nd Annual Meeting of Japanese Society of Hematology (Yokohama, Japan)
Sep-10	Revision of the relationship of blood cell lineages: insight into the generation of LGL upon dasatinib administration	**Kawamoto, H.**	Corporate Seminar, Annul Meeting of Japanese Society of Hematology (Yokohama, Japan)
Oct-10	Modeling cellular signaling functions in breast cancer cells	**Okada, M.**	11th International Conference on Systems Biology (ICSB 2010) (Edinburgh, UK)
Oct-10	The myeloid-based model: revised scheme for hematopoiesis	**Kawamoto, H.**	Network Medicine Seminar, Tohoku University (Sendai, Japan)
Oct-10	Regulation CD4/CD8 lineage choice by transcriptional silencer in the thymus and the gut	**Taniuchi, I.**	Seminar at CSI(CSI Research Meeting) (Singapore)
Oct-10	Locus specific and non-specific regulation of silencers function	**Taniuchi, I.**	Aegean Sympo(5th International Conference on Gene Regulation in Lymphocyte Development) (Creta, Greece)
Oct-10	RAPID: Resource of Asian Primary Immunodeficiency Diseases	**Mohan, S.**	Center for the Study of Immunodeficiencies at the Hospital Necker-Enfants Malades (Paris, France)
Oct-10	Negative regulation of lymphocyte activation by LAPTM5-mediated TCR and BCR downmodulation	**Wang, J. Y.**	China Agricultural University (Beijing, China)
Oct-10	Zinc signaling in immunity, allergy and inflammation	**Hirano, T.**	52nd Annual Meeting of the Japanese Society of Gastroenterology (Yokohama, Japan)
Oct-10	From tolerance to symbiosis and back	**Fagarasan, S.**	Resistance and Tolerance to Infection (Mosteiro do Convento da Arrábida, Portugal)
Oct-10	Zinc is an intracellular signaling molecule: early and late signal	**Hirano, T.**	60th Fujihara Seminar Zinc Signaling and Cellular Functions (Osaka, Japan)
Oct-10	The zinc transporter Zip13: its involvement in connective tissue development and pathogenesis of mouse and human	**Fukada, T.**	61st Fujihara Seminar Zinc Signaling and Cellular Functions (Osaka, Japan)
Oct-10	Role of Zinc transporter, Znt5/Slc30a5 in mast cell mediated-allergic response	**Nishida, K.**	62nd Fujihara Seminar Zinc Signaling and Cellular Functions (Osaka, Japan)
Oct-10	T cell lineage commitment regulated by the transcription factor Bcl11b	**Ikawa, T.**	114th Pediatric Hematologist-oncologist Workshop (Tokyo, Japan)
Nov-10	BCL6 Protein Expression Shapes Pre-Germinal Center B Cell Dynamics and Follicular Helper T Cell Heterogeneity	**Okada T.**	LIAI-RCAI Joint Workshop 2010 (Yokohama, Japan)
Nov-10	The adaptor molecule Gab2, via PI-3K, regulates ARF1 in Fc epsilon RI-mediated granule translocation and mast cell degranulation	**Nishida, K.**	LIAI-RCAI Joint Workshop 2010 (Yokohama, Japan)
Nov-10	CIN85 regulates canonical NF-κB pathway and subsequent B cell responses	**Kurosaki, T.**	The CSI(Chinese Society of Immunology)-iFReC Joint Symposium on Immunology (Hangzhou, China)
Nov-10	Dynamic regulation of positive/negative T cell co-stimulation	**Saito, T.**	The CSI(Chinese Society of Immunology)-iFReC Joint Symposium on Immunology (Hangzhou, China)
Nov-10	On IgA, vitamin A and gut homeostasis	**Fagarasan, S.**	Frontiers of Immunology in Health and Diseases (Cold Spring Harbor Asia, Suzhou, China)
Nov-10	Molecular mechanisms for dendritic cell subset activation by nucleic acids -From mice to human-	**Kaisho, T.**	The Seminar of Hokkaido University Graduate School of Pharmaceutical Sciences (Sapporo. Japan)
Nov-10	Transcriptional Control of CD4/CD8 Lineage Choice	**Taniuchi, I.**	LIAI-RCAI Joint Workshop (Yokohama, Japan)

Nov-10	Retention and termination of myeloid potential in T progenitors: an essential developmental checkpoint for production of the T cell lineage	**Kawamoto, H.**	LIAI-RCAI Joint Workshop (Yokohama, Japan)
Nov-10	Molecular mechanisms for dendritic cell responses against nucleic acid adjuvants	**Kaisho, T.**	LIAI-RCAI Joint Workshop (Yokohama, Japan)
Nov-10	Involvement of zinc transporters in physiology and pathogenesis	**Fukada, T.**	2nd Metallomics Research Forum (Kyoto, Japan)
Nov-10	Regulation of T cell activation: a study using molecular imaging	**Yokosuka, T.**	2nd Bio-frontier Seminar "Imaging analysis for the regulation of the immune systems and diseases" (Tokyo, Japan)
Nov-10	Creating novel and curative therapies for adult leukemia	**Ishikawa, F.**	Science Forum by AAAS (The American Association for the Advancement of Science) (Yokohama, Japan)
Nov-10	Molecular mechanisms for dendritic cell subset functions	**Kaisho, T.**	60th Annual Meeting of Japanese Society of Allergology (Tokyo, Japan)
Nov-10	Development of allergy vaccines for IgE antibody suppression	**Ishii, Y.**	13th Workshop of Japan Health Sciences Foundation (Tokyo, Japan)
Nov-10	Regulation of immune responses by Foxp3$^+$ regulatory T cells	**Hori, S.**	60th Annual Meeting of Japanese Society of Allergology (Tokyo, Japan)
Nov-10	Crucial roles of B7-H1 and B7-DC expressed on mesenteric lymph node dendritic cells in the generation of antigen-specific CD4$^+$Foxp3$^+$regulatory T cells in the establishment of oral tolerance	**Sato, K.**	60th Annual Meeting of Japanese Society of Allergology (Tokyo, Japan)
Nov-10	Food Allergy, Intestinal Immunity and Oral Tolerance	**Ohno, H.**	60th Annual Meeting of Japanese Society of Allergology (Tokyo, Japan)
Dec-10	Specialized epithelial M cells contribute to immunosurveillance on the mucosal surface	**Hase, K.**	2010 Symposium by Japan Soceity of Immunology (Tokyo, Japan)
Dec-10	Regulation of lymphocyte dynamics during the immune response by the transcription factor BCL6	**Okada, T.**	Biochemistry and Molecular Biology 2010 (Kobe, Japan)
Dec-10	IL-6 amplifier and four step model	**Hirano, T.**	3rd Saitama Research Meeting of Therapeutic Approch of Anti-interleukin 6 (Saitama, Japan)
Dec-10	Zinc signaling in immunity, allergy and inflammation	**Hirano, T.**	26th Annual Meeting of the Japanese Society of Pediatric Oncology (Osaka, Japan)
Dec-10	Plasma cells differentiation from memory B cells	**Kometani, K.**	5th Chiba University Global COE Symposium (Tokyo, Japan)
Dec-10	Collaborative activity by academia, government and industries based on human DNA clones	**Ohara, O.**	Biochemistry and Molecular Biology 2010 Biotechnology Seminar (Kobe, Japan)
Dec-10	Molecular dynamics in living cells using single molecule imaging and quantification	**Tokunaga, M.** and **Sakata-Sogawa, K.**	Biochemistry and Molecular Biology 2010 Biotechnology Seminar (Kobe, Japan)
Dec-10	Understanding normal and diseased human immunity using humanized mouse	**Ishikawa, F.**	2010 Symposium by Japan Soceity of Immunology (Tokyo, Japan)
Dec-10	Elucidation of the effectirvness of probiotics against lethal infection of enterohemorrhagic *E. coli* O157:H7	**Ohno, H.**	2nd Danon Asian Probiotics Confention (Tokyo, Japan)
Dec-10	How vitamin A impact on innate and adaptive immunity	**Fagarasan, S.**	Annual Meeting of the Japanese Society for Immunology (Tokyo, Japan)
Dec-10	Lineage restriction process from hematopoietic stem cells to T cell progenitors	**Kawamoto, H.**	Award Lecture for 13th JSI Award (Tokyo, Japan)
Dec-10	Biological roles of essential trace elements and the prospect for their clinical applications	**Fukada, T.**	2nd SPring-8 Next Plan 2019 Symposium (Tokyo, Japan)
Dec-10	Memory B cell generation and functional maturation; distinct cellular pathways establish B cell memory	**Takemori, T.**	Development and Maintenance of Immune Memory (Tokyo, Japan)
Dec-10	Essential Role of Membrane Trafficking Factor AP-1B in Gut Immune Homeostasis	**Ohno, H.**	Biochemistry and Molecular Biology 2010 (Kobe, Japan)
Dec-10	M-Sec: a promoter of tunneling nanotube formation	**Ohno, H.**	Biochemistry and Molecular Biology 2010 (Kobe, Japan)
Dec-10	Molecular dynamics in living cells using single molecule imaging and quantification	**Tokunaga, M.** and **Sakata-Sogawa, K.**	Biochemistry and Molecular Biology 2010 (Kobe, Japan)
Dec-10	Construction of an Informational Platform for Primary Immunodeficiency Diseases in Asia	**Mohan, S.**	1st Asia-Pacific Symposium on Primary Immunodeficiency Diseases (Yokohama, Japan)
Dec-10	Immune regulation by dead cell clearance	**Tanaka, M.**	Biochemistry and Molecular Biology 2010 (Kobe, Japan)
Jan-11	iPS-Derived NKT Cells and their Adjuvant Effects	**Taniguchi, M.**	2011 Keystone Symposia on NK and NKT Cell Biology (Breckenridge, USA)
Jan-11	Lineage restriction process from hematopoietic stem cells to T cell progenitors: developmental checkpoints visualized by inducing arrest and self renewal of progenitors	**Kawamoto, H.**	Stem Cell Therapy Forum, The Institute of Medical Science, Tokyo University (Tokyo, Japan)
Jan-11	Visualization of a developmental checkpoint by inducing arrest and selfrenewal of progenitors	**Kawamoto, H.**	Nihon University Stem Cell Biology Forum (Tokyo, Japan)
Jan-11	Dynamics of proteins in the nucleus by single molecule analysis	**Tokunaga, M.** and **Sakata-Sogawa, K.**	International Symposium on the Physicochemical Field for Genetic Activities (Awaji, Japan)
Jan-11	Dual specificity receptor for damaged cells and pathogens	**Saito, T.**	US-Japan Cooperative Medical Science Program (USJCMSP), Adaptive and Innate Immune Responses to Neglected Tropical Diseases (San Diego, USA)
Jan-11	Molecular mechanisms for dendritic cell responses against nucleic acids	**Kaisho, T.**	1st symposium of Scientific Research on Innovative Areas, "Homeostatic inflammation" (Tokyo, Japan)
Jan-11	Dynamic regulation of T-cell costimulation by TCR microclusters and the c-SMAC	**Yokosuka, T.**	13th International Membrane Research Forum/ The 6th iCeMS International Symposium, Featuring Membrane Meso Mechanisms (Kyoto, Japan)
Feb-11	Dendritic cell subset responses against nucleic acid adjuvants	**Kaisho, T.**	29th Annual Meeting of Japan Society of Immunology and Allergology in Otolaryngology (Oita, Japan)
Feb-11	RAPID: Resource of Asian Primary Immunodeficiency Diseases A road ahead	**Mohan, S.**	2nd Asia-Pacific symposium on Primary Immunodeficiency Diseases (Chiba, Japan)
Feb-11	Visualization of a developmental checkpoint by inducing arrest and selfrenewal of progenitors	**Kawamoto, H.**	Special Seminar, Yamaguchi University (Yamaguchi, Japan)
Feb-11	Genetic factor determining allergic trait	**Kubo, M.**	20th Molecular Immunology Forum Tokyo (Tokyo, Japan)
Feb-11	Molecular Mechanisms that regulate early T cell lineage commitment	**Ikawa, T.**	20th Molecular Immunology Forum Tokyo (Tokyo, Japan)
Mar-11	Transcriptional control of thymocytes development	**Taniuchi, I.**	TCUID (Towards Comprehensive Understanding of Immune Dynamism) 2011 (Osaka, Japan)
Mar-11	Integrated omics approach identified acetate produced by probiotic bifidobacteria to protect host from enteropathogenic infection	**Fukuda, S** and **Ohno, H.**	4th Stage Surface Barrier Immunology Study Group (SBARIS) 3rd Meeting (Tokyo, Japan)
Mar-11	Analysis on the influence of natural medicine on mucosal immune system	**Sato, K.**	Seminar on Collaborative Reserch in Institute of Natural Medicine, University of Toyama (Toyama, Japan)
Mar-11	NKT cell-mediated adjuvant activity	**Taniguchi, M.**	World Immune regulation Meeting -V (Davos, Switzerland)
Mar-11	Interaction dynamics of B cells and T cells in the lymph node	**Okada T.**	115th Annual Meeting of Japanese Association of Anatomists (Symposium 1S1-PM1) (Morioka, Japan)
Mar-11	Molecular dynamics for T cell activation and costimulation	**Saito, T.**	Keystone Symposia, Lymphocyte Activation and Gene Expression (Breckenridge, CO, USA)
Mar-11	Control of immune response by dendritic cells	**Sato, K.**	Seminar on Institute of Natural Medicine, University of Toyama (Toyama, Japan)

Date	Speaker	Title
05-Apr-10	**Ken Shirasu** (RIKEN Plant Science Center, JAPAN)	**Common immunity systems in plants and animals**
11-Apr-10	**Koichi Kobayashi** (Dana-Farber Cancer Institute, Harvard Medical School, USA)	**Recognition of microbial components by Nod2:The role in the innate immunity and Crohn's disease**
14-Apr-10	**Shingo Iwami** (PRESTO, JST, JAPAN)	**Computational Virology and Immunology Based on in vivo / in vitro Experiments**
08-Jun-10	**Kenji Nakanishi** (Hyogo College of Medicine, JAPAN)	**Induction of innate and acquired type allergy by basophils**
30-Jun-10	**Hajime Karasuyama** (Tokyo Medical and Dental University, JAPAN)	**Novel roles for basophils in allergy and protective immunity**
15-Jul-10	**Yosuke Takahama** (Institute for Genome Research, The University of Tokushima, JAPAN)	**Role of thymic cortex-specific self-peptides in positive selection of T cells**
28-Jul-10	**Kazuhiro Aoki** (Complex Carbohydrate Research Center, University of Georgia, USA)	**Regulation of protein N- and O-glycosylation in the Drosophila melanogaster embryo by a Toll-like receptor**
15-Aug-10	**Jeffrey Browning** (Biogen Idec, USA)	**Exploration of the BAFF and Lymphotox in Pathways in Mice and Man**
31-Aug-10	**Dorina Avram** (Center for Cell Biology and Cancer Research, Albany Medical College, USA)	**BCL11B controls glycolipid presentation by DP thymocytes independent of the control of early iNKT precursors**
05-Sep-10	**Hidehiro Yamane** (National Institute of Allergy & Infectious Diseases, NIH, USA)	**Early Signaling Events Underlying the Fate Determination of Naïve $CD4^+$ T Cells towards Effector T Helper Subsets**
05-Sep-10	**Klaus Fruh** (Vaccine and Gene Therapy Institute, Oregon Health and Science University, USA)	**Viral Immune evasion**
27-Sep-10	**James Douglas Engel** (University of Michigan Medical School, USA)	**GATA3 reguktaion of early T cell and hematopoietic progenitor development**

27-Oct-10	**Richard Kroczek** (Robert Koch Institute, GERMANY)	**Understanding the $CD8^+$ T cells – DC communication axis: a starting point for a new type of vaccine?**
31-Oct-10	**Boris N. Kholodenko** (University College Dublin, IRELAND)	**Spatio-temporal coding of signal specificity and network reconstruction**
03-Nov-10	**Ichiro Hirao** (RIKEN Systems and Structural Biology Center, JAPAN)	**Expansion of the genetic alphabet by unnatural base pair systems**
15-Nov-10	**Reiko Onishi** (University of Pittsburgh, USA)	**A unique role for IL-17RA in IL-17 signal transduction**
05-Dec-10	**Jennifer Stow** (Institute for Molecular Bioscience, The University of Queensland, AUSTRALIA)	**Recycling endosomes-compartments for secretion and cell polarty**
09-Dec-10	**Randy L. Johnson** (University of Texas, MD Anderson Cancer Center, USA)	**Genetic analysis of the mammalian Hippo signaling pathway in organ size control and tumor suppression**
12-Dec-10	**Esteban C.Dell'Angelica** (University of California,Los Angeles, USA)	**Protein Complexes Involved in the Biogenesis of Lysosome-related Organelles**
20-Dec-10	**Florent Ginhoux** (Singapore Immunology Network (SIgN), Agency for Science, Technology and Research, SINGAPORE)	**Revisiting the ontogeny of the mononuclear phagocyte system:Embryonic versus adult hematopoiesis contribution**
23-Dec-10	**Michael Reth** (University of Freiburg, Max-Planck Institute of Immunobiology, BIOSS Excellence Cluster, GERMANY)	**The silence of the B cells**
26-Dec-10	**Michael Reth** (University of Freiburg, Max-Planck Institute of Immunobiology, BIOSS Excellence Cluster, Germany)	**Synthetic biology programme in Freiburg**
30-Jan-11	**David Grunwald** (Kavli Institute of Nano Science, Technical University Delft, The NETHERLANDS)	**Crossing the Gate: Export of Single mRNAs in vivo**
27-Feb-11	**Frederic Geissmann** (Center for Molecular and Cellular Biology of inflammation (CMCBI), King's College, UK)	**In vivo functions of monocytes**
02-Mar-11	**Lawrence E. Samelson** (Center for Cancer Research, National Cancer Institute, NIH, USA)	**Signaling at the T cell antigen receptor**

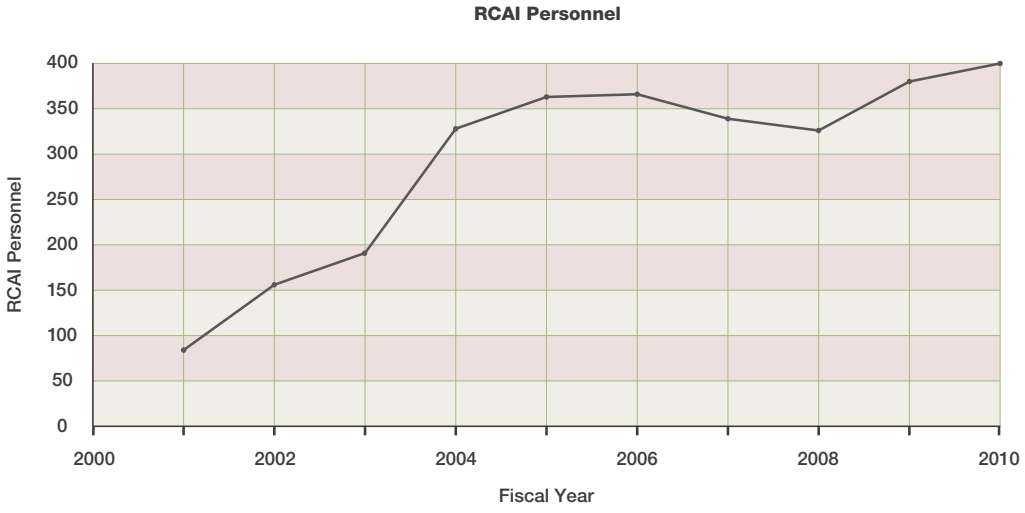
Budget, Personnel and Patents



RCAI Budget FY2001-2010 (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



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

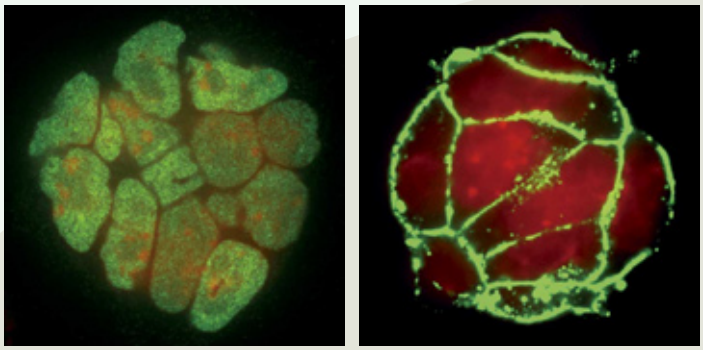
RCAI staff composition (as of Mar. 2011)

Category	Number
Director	1
Senior Advisor	1
Group Director	7
Team Leader	14
Unit Leader	10
Coordinator	1
Senior Scientist	12
Research Scientist	34
Special Postdoctoral Researcher	8
Foreign Postdoctoral Researcher	1
Research Associate	7
Junior Research Associate	17
International Program Associate	5
Student Trainee	58
Research Fellow	2
Research Consultant	2
Visiting Scientist	79
Senior Technical Scientist	1
Technical Scientist	5
Technical Staff I	35
Technical Staff II	65
Assistant	21
Temporary Employment	8
others	6
Total	400

RCAI Patents FY 2010 (as of Mar. 2011)

There were 28 patents filed from April 2010-March 2011.

Original Photos of the Cover and Front pages



Cover

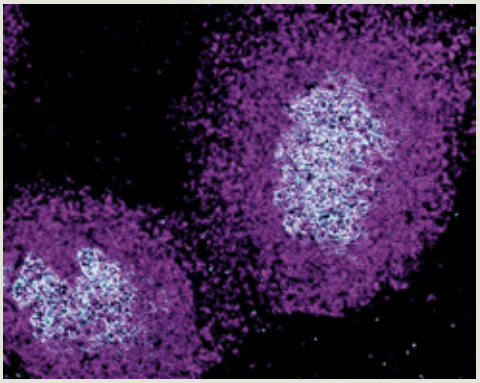
An internal RCAI collaboration among the Laboratories for Immune Regulation and Developmental Genetics and the Research Unit for Cellular Immunotherapy demonstrated the feasibility of expanding functionally competent NKT cells via an induced pluripotent stem cell (iPSC) phase, an approach that may be adapted for NKT cell-targeted therapy in humans (Watarai et al., *J Clin Invest*, 2010). (See Part 1 Creation of New Paradigms, p. 10).

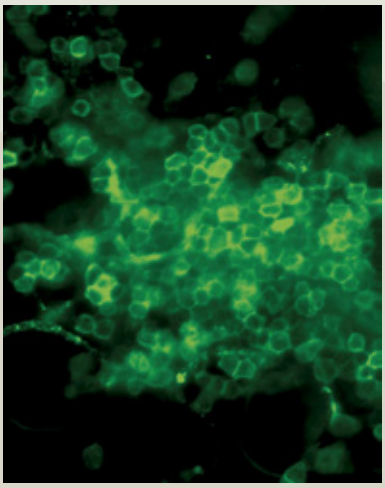
A cluster of undifferentiated iPSCs derived from reprogrammed NKTs highly express pluripotency markers Oct3/4 (*left, green*) and SSEA13 (*right, green*). DAPI (*red*) labels the nuclei. (Image courtesy of the collaborative groups.)

Front page of Part 1 Creation of New Paradigms

The follicular dendritic cell (FDC) is a specialized stromal cell in the germinal center. The Laboratory for Mucosal Immunity discovered that FDCs in the gut sense bacterial and food components and secrete the B-cell chemoattractant CXCL13 (Suzuki et al. *Immunity*, 2010). (See Part 1 Creation of New Paradigms, p. 3)

A cross-section of Peyer's patches showing B cell follicles (B220 stained in *magenta*) built upon FDCs (CXCL13 stained in *blue*). Image courtesy of the Laboratory for Mucosal Immunity

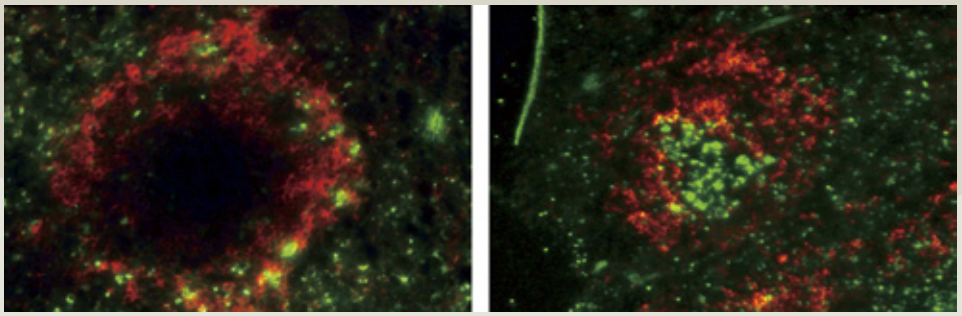




Front page of Part 2 Technology and Innovation

The transition from an early innate response to the adaptive immune response can be orchestrated by a special subset of B cells named B1 cells. The Laboratory for Mucosal Immunity showed that B1 cells sense the retinoic acid (RA) produced by peritoneum associated adipose tissues such as omentum, and activate the transcription factor NFATc1, which is critical for the survival, proliferation and antibody production of B1 cells (Maruya et al. *Proc. Natl. Acad. Sci. USA*, 2011). (See Part 6 Laboratory Activities, p. 74-75)

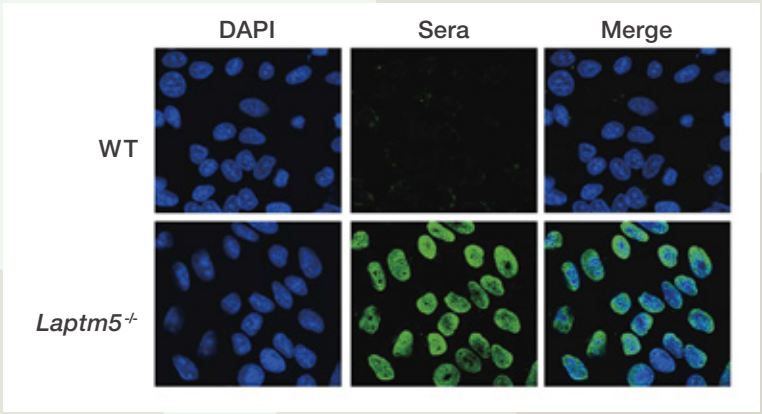
Aggregates of peritoneal B1 cells (stained for IgM (*green*)) in the omentum. Image courtesy of the Laboratory for Mucosal Immunity



Front page of Part 3 Nurturing Young Scientists

To elucidate the mechanism by which a candidate allergy vaccine mediates suppression of IgE antibody responses, the Laboratory for Vaccine Design identified the target cells of the liposome vaccine. Splenic $B220^{+}CD1d$ high cells in the marginal zone preferentially incorporate liposomes and express CXCL16, which then recruits iNKT cells that express CXCR6, the receptor for CXCL16. (See Part 6 Laboratory Activities, p. 100-101)

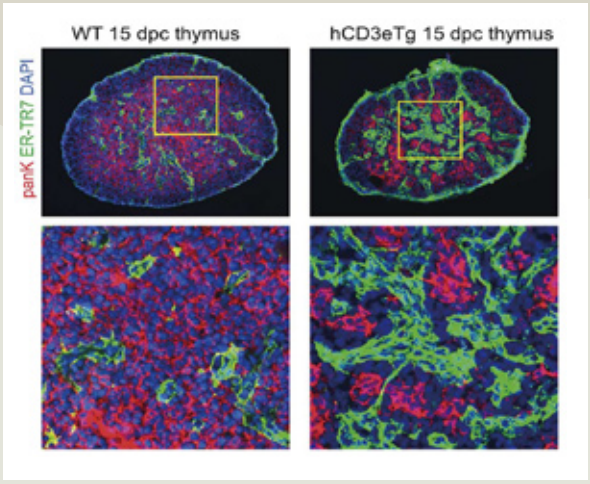
iNKT cells (*green*) failed to traffic to liposome antigen-capturing $B220^{+}$ cells (*red*) in the marginal zone when anti-CXCL16 antibody was co-administrated with the liposomes. No Ab treatment (*left*), treated with anti-CXCL16 Ab (*right*). Image courtesy of the Laboratory for Vaccine Design



Front page of Part 4 Collaborative Networks

LAPTM5 is a lysosomal protein highly expressed in resting B cells but down regulated in germinal center B cells. The Laboratory for Immune Diversity found that LAPTM5 negatively regulates cell surface expression of the B cell antigen receptor by promoting its lysosomal degradation. (See Part 6 Laboratory Activities, p. 68-69)

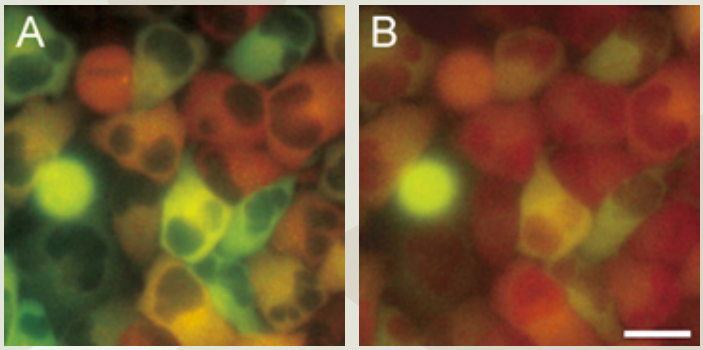
Increased levels of anti-nuclear autoantibodies (*green*) in the sera of *Laptm5*$^{-/-}$ mice. Hep2 cells were stained with sera from WT (*upper panel*) or *Laptm5*$^{-/-}$ mice (*lower panel*). Image courtesy of the Laboratory for Immune Diversity



Front page of Part 5 Outreach Activities

The Research Unit for Thymic Environment found that loss of thymic crosstalk signaling leads to expansion of mesenchymal cells in the thymus (See Part 6 Laboratory Activities, p. 116-117).

In the thymus of fetal hCD3ε transgenic mice, in which T cell development is blocked, the organization of thymic epithelial cells is disturbed, increasing mesenchymal cells detected by ERTR7 expression (*green*). Image courtesy of the Research Unit for Thymic Environment



Front page of Part 6 Laboratory Activities

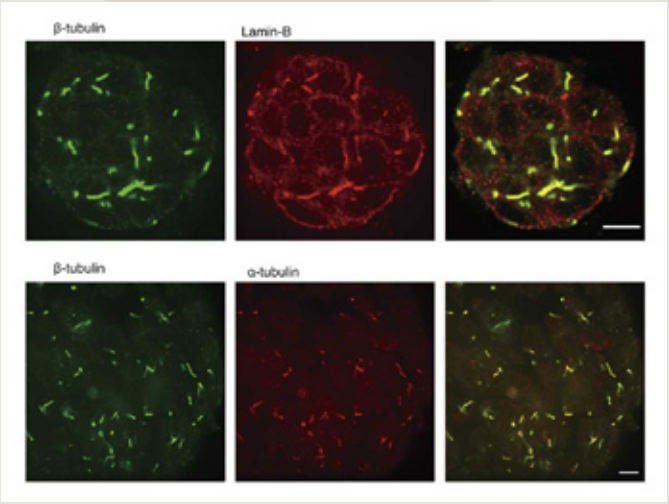
Using single molecule fluorescence microscopy, the Research Unit for Single Molecule Imaging, in collaboration with the Research Units for Molecular Systems Immunology, visualized the dynamics of NF-κB and its suppressor IκBα. In resting cells, both proteins were localized in the cytoplasm. Upon stimulation, degradation of IκBα caused translocation of NF-κB to the nucleus (See Part 6 Laboratory Activities, p. 64-65).

HeLa cells expressing both NF-κB-RFP (*red*) and IκBα-GFP (*green*) were stimulated with human TNF-α. (A) Before stimulation. (B) 30min after stimulation. Scale bar: 20 μm. Image courtesy of the Research Unit for Single Molecule Imaging.

Front page of Part 7 Data and Statistics

The laboratory for Developmental Genetics identified a fraction of tubulins that persistently associated with the nuclear lamina and exhibited a filamentous structure (See Part 6 Laboratory Activities, p. 56-57)

Subnuclear colocalization of α (*red*) and β tubulin (*green*) with Lamin-B (*red*) in mouse ES cells. Image courtesy of the Laboratory for Developmental Genetics.



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