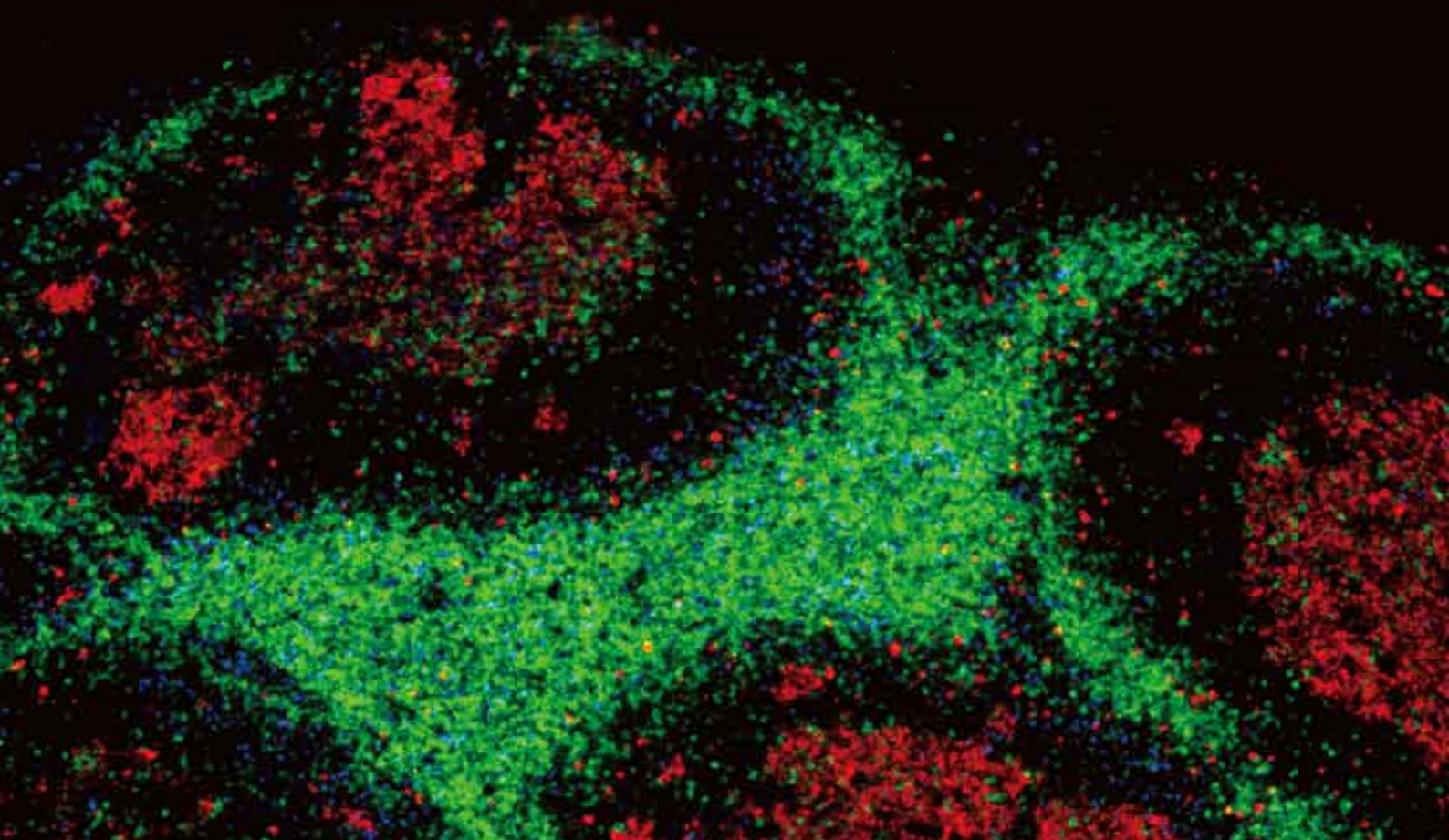
RIKEN RCAI Annual Report 2008

RIKEN Research Center for Allergy and Immunology



RIKEN
Research Center for Allergy and Immunology (RCAI)

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Masaru Taniguchi M.D., Ph.D.

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

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Lab. for Signal Network **Masato Kubo Ph.D.**
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Lab. for Mucosal Immunity **Sidonia Fagarasan M.D., Ph.D.**
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Unit for Immunodynamics **Takaharu Okada Ph.D.**

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Unit for Immunogenetics **Miguel Vidal Ph.D.**

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Director's Report

This is the fifth annual report of the RIKEN Research Center for Allergy and Immunology (RCAI). Five years have passed since all research groups moved from universities into a new building on the RIKEN Yokohama campus. As we stand at this juncture, it is important to critically evaluate what we have accomplished thus far in our goal to becoming an international center of excellence in immunology research, as well as to contemplate our goal and plan for the future.

RCAI's mission and strategic focus

The mission of the RCAI is to perform fundamental and translational research in immunology with particular emphasis on immune regulation and allergic/asthmatic diseases. During its short existence, RCAI has developed into an internationally renowned center for immunology research. One objective measure of this success can be found in the Citation Index compiled by Thompson Essential Science Indicators. According to their data, the average number of citations per paper published by RCAI investigators is 42.67. This ranks very well ahead with other influential universities and research institutes: Osaka (45.79), Yale (42.57), Caltech (40.39), Kyoto (40.28), MIT (40.03), Stanford (39.38), Harvard (33.87), Oxford (32.01), Max Planck Society (27.78), Tokyo (23.13), and Cambridge (22.87). Within RIKEN, immunology (42.67) was ranked as the strongest field compared to Molecular Biology & Genetics (29.18), Plant & Animal Sciences (22.94), Neuroscience & Behavior (20.52), Biology & Biochemistry (18.29), Clinical Medicine (16.37), Microbiology (11.89), Chemistry (9.52), Physics (9.26), Materials Science (7.05), and Engineering (4.71).

2008 Significant Publications

When I think of the research activities in the Center, most of the research groups led by senior investigators are very productive and doing well. More importantly, many of the younger investigators have blossomed during the past five years. The Center's publications can be categorized based on two of the RCAI's missions, creation of new paradigms in immunology and establishment of platforms for clinical applications.

Creation of new paradigms

In 2008, Drs. Sho Yamasaki and Takashi Saito in the Cell Signaling Research Group published two important papers on Mincle, a transmembrane C-type lectin receptor. Mincle was shown to recognize a soluble factor released by necrotic cells, spliceosome-assoicated protein 130, and be involved in the inflammatory

response triggered by necrotic cells. In addition to this endogenous ligand, the group identified an exogenous ligand, alpha-mannosyl residues on *Malassezia* species of fungus (*Nature Immunology*, 2008 and *PNAS,* 2009). Dr. Saito's group also used single molecule imaging to understand the spatiotemporal regulation of T cell costimulation by TCR-CD28 microclusters (*Immunity,* 2008). In terms of regulation of TCR expression levels, Dr Wang found that a lysosomal protein could negatively regulate these levels by preferentially degrading CD3ζ chain (*Immunity*). Dr. Koseki of the Developmental Genetics Research Group identified a mutation in one of the fibroblasts growth factors (FGF9) that causes a defect in the developing skeleton. This mutation was unique in that it exerted its effect due to increased FGF diffusion because of decreased avidity for the extracellular matrix (*Nature Genetics*, 2009). In related developemetal studies, Dr. Hirano found a critical role for a zinc transporter in connective tissue development (*PLoS ONE*, 2008). Dr. Kawamoto published a *Nature* paper challenging the textbook models of hematopoiesis that include a common lymphoid progenitor of T and B cells, instead showing that myeloid potential is maintained until very late in lymphoid progenitors. In progenitors of B cells, Dr. Kurosaki was able to link the pre-B cell receptor with downstream transcriptional events via the Erk kinases (*Immunity,* 2008). In early T cell development, Dr. Taniuchi showed how helper versus cytotoxic T lineage fate decisions in the thymus can be controlled by cascading suppression of transcriptional silencers (*Nature Immunology*, 2008). Dr. Fagarasan helped explain a long-standing question, what are the sites and mechanisms of T cell independent switching to IgA in the gut? She found a crucial role for lymphoid tissue inducer cells in their role in isolated lymphoid follicle formation in this process (*Immunity,* 2008).

Establishment of platforms for clinical applications

Dr. Watarai and I identified a new subset of NKT cells that respond to Il-25 and contribute to airway hypersensitivity in mice, a model for human asthma (*JEM*, 2008). Dr. Fagarasan and Dr. Hori's paper on the Tregs in the gut and their ability to differentiate into T follicular helper cells for IgA switching will have implications for how the suppression of inflammatory reactions and induction of IgA synthesis occur in the gut. (*Science,* 2009). Dr. Hori has another paper dealing with the stability of the Treg phenotype as assessed by Foxp3 expression, an issue of potential clinical relevance (*PNAS,* 2009).

Strategic projects

The Center's strategic projects can be categorized based on the RCAI's three missions, 1) creation of new paradigms in immunology, 2) establishment of platforms for clinical applications, and 3) clinical applications.

The first mission, the creation of new paradigms is important for fundamental immunology and science advancement in general. Three major projects are ongoing in this area. The first is on single molecular movement and includes the development of HILO microscope and the detection of molecular movement in living cells. The second project, intravital single cell movement, includes the development of a deep-two photon microscope and the detection of single cell movement *in vivo*. This project is supported by the RIKEN President's Fund and our new unit leader, Dr. Okada, collaborates with Dr. Midorikawa in RIKEN Advanced Science Institute (ASI) and Dr. Miyawaki in RIKEN Brain Science Institute (BSI). The third project, systems biology, was launched in 2008. We are establishing an Open Laboratory for Systems Immunology where multidisciplinary researchers can interact with each other at RCAI. We have invited the following top researchers in the fields of mathematical/theoretical biology and systems engineering of biological systems: Drs. Kazuyuki Aihara (The Univ. of Tokyo), Shinji Hara (The Univ. of Tokyo), Hidenori Kimura (Toyota-RIKEN BSI Center), Yohiharu Yamamoto (The Univ. of Tokyo), and Kenko Uchida (Waseda Univ.). We have begun in-depth face-to-face discussions concerning various immune events with these non-immunologists in order to establish a common ground to harmonize both experimental and theoretical/computational viewpoints.

The second mission, the establishment of platforms for clinical applications, includes four major projects: humanized mice, induced pluripotent stem (iPS) cells, Primary Immunodeficiency Diseases (PID), and the Allergy Network. This year, Dr. Koseki received iPS CREST (Core Research for Evolutional Science and Technology) funding (supported by the Japan Science and Technology Agency) and an Immune iPS Project was launched in RCAI. We have succeeded in generating iPS cells derived from NKT cells, and NKT cells were successfully developed from these NKT-iPS cells. The PID project made significant progress in expanding the network. We organized an international symposium for PID Asia in December, 2008. PID experts gathered from Australia, India, Iran, China, Korea, Japan, Taiwan, Thailand, and the USA. We agreed to establish the PID Asia

Network and collaborate for PID diagnosis and therapies. This year, the Allergy Network was established with seven universities (Univ. of Yamanashi, Nippon Medical School, Univ. of Fukui, Kagoshima Univ., Okayama Univ., Mie Univ., and Chiba Univ.) and two hospitals (Sagamihara National Hospital and Kanagawa Children's Medical Center). The network will cooperate for microarray analyses of allergy-related genes using clinical samples. One goal of the network is to discover novel biomarkers for allergy, which we expect will then be useful tools to determine the efficacy of allergy therapies.

The third mission, clinical applications, includes Allergy Vaccine Development and Clinical Studies of NKT Adjuvant Cell Therapy. In June, 2008, the Allergy Vaccine project was designated as the first RIKEN translational research project. Supported by the RIKEN President Fund, we are now in negotiations with pharmaceutical companies to collaborate for clinical application of two types of cedar polinosis vaccines developed by Dr. Ishii (RCAI). One of them is a recombinant fusion protein of two cedar antigens (Cry j1/Cry j2) and the other is liposome vaccine containing the cedar antigens and α-GalCer glycolipids. Our collaboration design is that using GMP samples generated by a pharmaceutical company, RIKEN, the universities and the hospitals in the Allergy Network will cooperate for pre-translational/ translational research and proof of concept studies. The NKT Adjuvant Cell Therapy proceeded to Phase IIa Clinical studies in 2008 in collaboration with Professors Motohashi and Nakayama at Chiba University Hospital. We treated 23 advanced lung cancer outpatients with α-GalCer-pulsed autologous dendritic cells. 60% of patients with high IFNγ production survived 3 years only with primary treatment without tumor progression and metastasis. On the whole, 40% of patients survived 3 years or more only with primary treatment.

RCAI's new directions

In spite of a severe reduction in the research budget, the Center is requested to strengthen its research activities and contributions to society. To face this challenge, an RCAI internal committee, a so-called "dream team", was organized to discuss the future directions of the Center. Independent external input and advice was provided by the Japanese Society for Immunology "Future Directions of Immunology" committee chaired by Drs. Masayuki Miyasaka and Shigeo Koyasu. The dream team ultimately suggested a new direction for RCAI focused on molecular and cellular movement (single molecule imaging and in vivo imaging), human immunology, and systems biology. They also discussed details concerning the criteria for evaluating the 5-year performance of RCAI Principal Investigators (PIs).

At their meeting held on Dec 4-5, 2008, the RCAI External Advisory Council supported the RCAI future directions and reorganization plans. The reorganization will be based on the evaluation (scientific achievements as well as the contributions to RCAI and society) of PIs after their initial 5-year appointment and how their research matches with the RCAI's future plan.

The PI evaluation process occurred at several levels that included the RCAI Advisory Council (AC) and an RCAI executive committee.

After presenting these RCAI reorganization plans, the AC concurred with the fundamental RCAI plan for the tertiary term of the Independent Administrative Institutions as well as its reorganization plan and offered strong endorsement of the plans.

International collaborations

I would like to specifically comment on three new international programs held at RCAI in 2008/2009. In the summer of 2008, an RCAI Undergraduate Immunology Program started as an official credit earning program of Harvard University (http://www.summer.harvard.edu/2009/programs/abroad/yokohama/). Iddoshe Hirpa and Tzu Ying Chaung spent two months at RCAI and experienced internships at laboratories, immunology lectures, and basic Japanese classes. They were also able to participate in the RCAI international summer program (RISP) and the RCAI-JSI international symposium. This year's RISP was combined with international symposium and it gathered wide international attention. There were 39 students from 19 countries in attendance.

In January, La Jolla Institute for Allergy and Immunology (LIAI)-RCAI joint Workshop was held at RCAI. Sixty-five participants, including five speakers from LIAI discussed Regulation of Lymphocyte Function and Immune Responses.

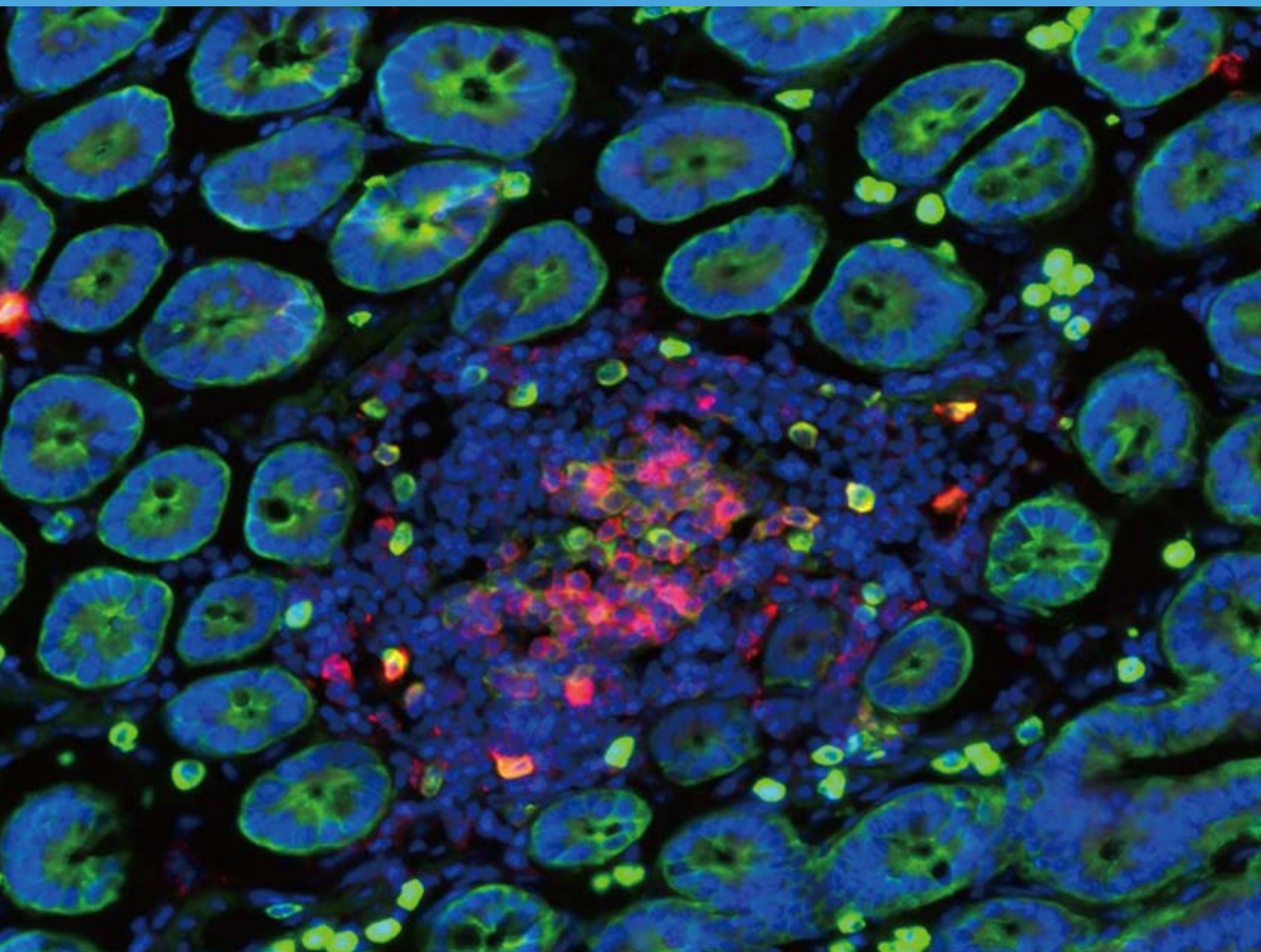
In March 23-27th, 2009, the International Symposium on CD1/NKT cells was held in Kamakura, Yokohama. There were about 150 attendees from Europe and the United States.

My hope is that these and similar RCAI activities will be widely supported and contribute to future immunology.

Masaru Taniguchi
March, 2009

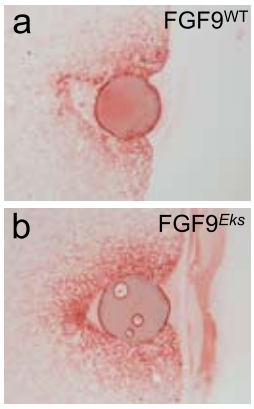
2008

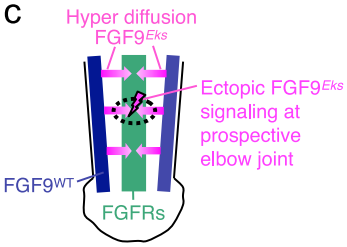
Research Highlights



Impaired dimerization of FGF9 causes its increased tissue diffusion and skeletal abnormalities

Haruhiko Koseki

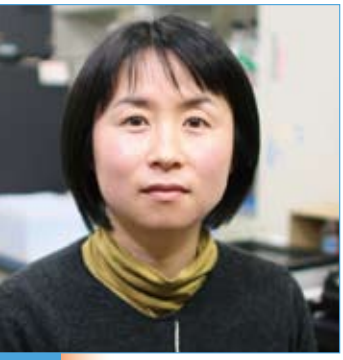




Figure

(a, b) **Increased diffusibility of FGF9^{Eks} in the forelimb bud.** FGF9^{WT} or FGF9^{Eks} beads were implanted into forelimb buds of Fgf9$^{-/-}$ embryos of E10.5 mice. Diffusion of exogenous FGF9^{WT} (*a*) and FGF9^{Eks} (*b*) after 2 hours was immunodetected using a FGF9 antibody.

(c) **A model for the pathogenic mechanism underlying elbow joint synostosis in Fgf9$^{Eks/Eks}$ mice.** In Fgf9$^{Eks/Eks}$ mice, ectopic FGF9 signaling due to hyperdiffusion of FGF9^{Eks} at the prospective elbow joint may inhibit the initiation of joint development.



Masayo Harada

An RCAI research team led by Haruhiko Koseki discovered that a mutation in the fibroblast growth factor 9 (*FGF9*) gene causes an FGF9 dimerization defect. The monomeric form of FGF9 protein can diffuse more widely in the developing tissues than the dimer form due to decreased interaction with the extracellular matrix heparan sulfate proteoglycans. This has profound developmental consequences and induces joint fusions in elbows and knees. The research was conducted in collaboration with Makoto Taiji (RIKEN Advanced Science Institute), Shigeyuki Yokoyama (RIKEN Systems and Structural Biology Center), Akihiko Okawa (Chiba Univ.), Sachiko Iseki (Tokyo Medical and Dental Univ.), Atsushi Kuroiwa (Nagoya Univ.) and David Ornitz (Washington Univ. School of Medicine).

In this study, Koseki's team identified a missense genetic mutation in *FGF9* of Elbow knee synostosis (Eks) mutant mice, which have an abnormal gait and joint bone adhesions in the elbows and knees. The mutated residue was predicted to participate in homodimerization and receptor activation. Indeed, biochemical and biophysical analysis showed that the mutant protein (FGF9^{Eks}) existed primarily as a monomer, while normal FGF9 (FGF9^{WT}) was present primarily as a dimer. Furthermore, FGF9^{Eks} mediates less potent signaling via several FGF receptors and shows decreased heparin affinity.

Heparan sulfate proteoglycans (HSPGs) are present in most tissues and regulate the distribution of FGF9 and stabilize its interaction with FGF receptors. Using molecular-dynamics simulations, the authors analyzed the configuration of heparin binding domains in monomeric and dimeric FGF9 and calculated the binding affinity with heparin, which is functionally very similar to HSPGs. They found that the dimeric FGF9 had higher affinity for heparin than the monomeric form.

The Eks mutation affected homodimerization of FGF9, and monomeric FGF9 had lower affinity for heparin. Furthermore, the joint synostosis in Eks mice is similar to that in gain-of-function FGF receptor mutants. Thus, the authors hypothesized that diffusibility of FGF9^{Eks} would be increased because of its lower affinity for HSPG. This could lead to ectopic localization of FGF9 and ectopic activation of FGF receptors in the prospective joint, preventing joint formation. To test this hypothesis, they examined the 1) expression of FGF9 and FGF receptors, 2) ability of FGF9^{Eks} to induce bony fusions, 3) diffusibility of FGF9^{Eks}, and 4) ectopic FGF signaling in Eks mice. Indeed, FGF receptors were expressed in prospective joint regions, and FGF9 was expressed in myoblasts. Importantly, ectopic expression of both FGF9^{WT} and FGF9^{Eks} by viral transduction caused knee joint fusions, clearly demonstrating that both FGF9^{WT} and FGF9^{Eks} can inhibit joint development. The diffusibility of FGF9 was assessed by grafting FGF9^{WT}- or FGF9^{Eks}-soaked beads into the limb buds of FGF9 deficient embryos, and FGF9^{Eks} was shown to diffuse farther than FGF9^{WT} through the tissue. Finally, they confirmed ectopic FGF signaling in the prospective elbow joint in Eks mice.

Based on these studies, the authors have developed an important new paradigm in FGF biology. Previously, one might expect that an FGF mutation would affect the bioactivity of FGF by increasing or decreasing its affinity for the FGF receptor. However, the mutation characterized here instead affects the affinity for the extracellular matrix HSPGs, consequently making the FGF more diffusible and bioavailable in developing tissues, leading to developmental abnormalities. These findings could have far-reaching implications for the pharmacologic manipulation of FGF signaling under a variety of circumstances and in a wide range of tissues.

The study was featured in the "News and Views" section in the same issue of Nature Genetics. In the article, Douglas Spicer commented that the findings are an important step toward a better understanding of FGF biology and may lead to new therapeutic strategies to manipulate this pathway (*Nat. Genet.*, 2009).

ORIGINAL RESEARCH PAPER

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. *Nature Genetics*, 41, 289-298 (2009)

RELATED READING

Spicer D. FGF9 on the move. *Nature Genetics*, 41, 272-273 (2009)

Dysfunction of a zinc transporter causes a connective tissue disorder in mouse and human

Toshio Hirano

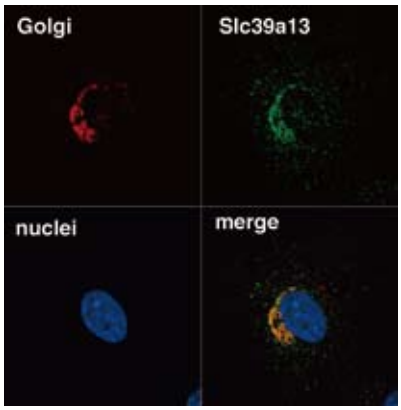
Zinc is an essential trace element that is relatively abundant in connective tissues, however the biological role of zinc and its transporters in these tissues has been unknown. The RCAI research team led by Toshio Hirano, in collaboration with experts in skeletal and dental medicine, discovered that zinc transporter dysfunction causes a novel type of Ehlers-Danlos syndrome, which presents with short stature and connective tissue abnormalities.

Hirano's team had previously found that a zinc transporter mediates cell migration during early development of zebrafish (*Nature*. 2004), and has an important role in cell maturation and signaling in immune cells (*Nature Immunology*. 2006). To examine its physiologic role *in vivo*, the team generated mice deficient in a zinc transporter: Slc39a13/Zip13. Surprisingly, the *Slc39a13* knockout (*Slc39a13*-KO) mice showed severe growth retardation, fragile skin and eyes, abnormal cartilage formation and tooth development. When they described the appearance of the *Slc39a13*-KO mice to Shiro Ikegawa, an expert of inherited skeletal diseases in RIKEN's Center of Genomic Medicine, and Ichiro Saito, a dental pathologist in Tsurumi University School of Dental Medicine, they predicted its high correlation with certain human diseases. Then Ikegawa introduced Andrea Superti-Furga, a clinical expert of the field. "This research was realized because of RIKEN's great collaborative and cooperative atmosphere" Toshiyuki Fukada, a researcher in Hirano's team, recalls. Superti-Furga selected two siblings with Ehlers-Danlos syndrome whose symptoms were very similar to those of the *Slc39a13*-KO mice. They found a homozygous point mutation in the *SLC39A13* gene, which caused a non-conservative amino acid substitution G74D in one of eight transmembrane domains, resulting in the loss of function.

Hirano's team further investigated the intracellular localization, and Zn-transporting function of the Slc39a13 protein. They found Slc39a13 in the Golgi (Figure) and, using a technique called Electron Probe X-ray Micro Analysis, revealed that it transports zinc from the Golgi to the cytosol, thus Slc39a13 controls intracellular zinc distribution.

They also analyzed in which signaling pathways Slc39a13 is involved. "RCAI's collaborative environment and cooperative atmosphere greatly helped us again. Hiroshi Kitamura of the Immunogenomics Group gave us critical advice about bioinformatics" says Fukada. Using microarray analysis, they found that the BMP/TGF-β pathway, one of the major signaling cascades controlling cell differentiation, adhesion and polarity, was perturbed in the *Slc39a13*-KO mice.

Their findings suggests that Slc39a13/Zip13 controls intracellular zinc distribution, is involved in the BMP/TGF-β signaling pathway, and regulates the development of connective tissues in mouse and man. The *Slc39a13*-KO mice are a new model for Ehlers-Danlos syndrome and are expected to contribute for the development of new therapies and understanding of the disease. This paper was selected by the *Faculty of 1000 Biology*; "I think we could show the scientific power of RIKEN to establish international collaborative studies that opened a new field," Fukada commented.



Figure

Slc39a13/Zip13 protein is localized in the Golgi. Confocal microscopic analysis of dermal fibroblasts. Golgi and Slc39a13 were stained with antibodies to GM130 (*red*) and Slc39a13 (*green*), respectively. Regions of co-localization (merge) are yellow. Nuclei were stained with DAPI (*blue*).

ORIGINAL RESEARCH PAPER

Fukada, T., Civic, N., Furuichi, T., Shimoda, S., Mishima K., Higashiyama, H., Idaira, Y., Asada, Y., Kitamura, H., Yamasaki, S., Hojyo, S., Nakayama, M., Ohara, O., Koseki, H., dos Santos, H. G., Bonafe, L., Ha-Vinh, R., Zankl, A., Unger, S., Kraenzlin, M. E., Beckmann, J. S., Saito, I., Rivolta, C., Ikegawa, S., Superti-Furga, A., and Hirano, T. The Zinc Transporter SLC39A13/ZIP13 is Required for Connective Tissue Development; Its Involvement in BMP/TGF-β Signaling Pathways. *PLoS ONE* 3, e3642 (2008)

RELATED READINGS

Yamashita, S., Miyagi, C., Fukada, T., Kagara, N., Che, Y.S. & Hirano, T. Zinc transporter LIVI controls epithelial-mesenchymal transition in zebrafish gastrula organizer. *Nature* 429, 298–302 (2004).

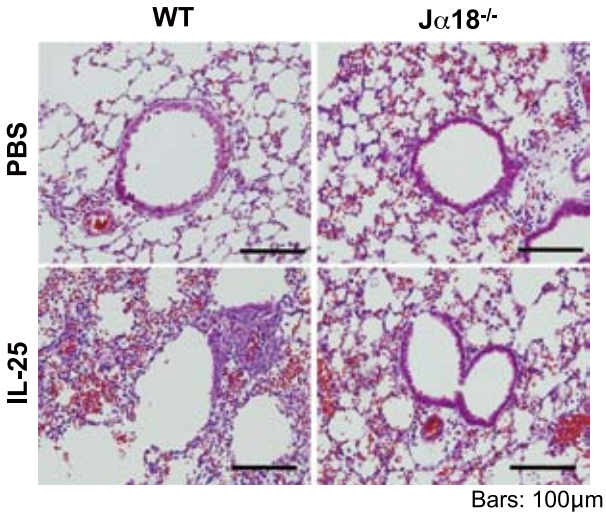
Kitamura, H., Morikawa, H., Kamon, H., Iguchi, M. Hojyo, S., Fukada, T., Yamashita, S., Kaisho, T., Akira, S., Murakami, M. & Hirano, T. Toll-like receptore-mediated regulation of zinc homeostasis influences dendritic cell function. *Nature Immunology* 7, 971–977 (2006).

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

Toshiyuki Fukada *(left)* **and Hiroshi Kitamura** *(right)*

A novel subset of NKT cells contributes to airway hyperreactivity

Masaru Taniguchi



Figure

Histological analysis of lung tissues with H&E staining. IL-25-treated WT or Ja18$^{-/-}$ mice were compared with WT or Jα18$^{-/-}$ mice from control. The levels of infiltration of inflammatory mononuclear cells into the peribronchiolar region were higher in WT mice with severe tissue destruction compared to those in Jα18$^{-/-}$ mice.

An RCAI research team led by Masaru Taniguchi and Hiroshi Watarai discovered a novel subset of NKT cells that contributes to the airway hypersensitive reaction (AHR). AHR is an animal model for asthma, which is caused or enhanced by environmental factors such as allergen exposure. AHR was known to be associated with cytokines produced by Type 2 helper T (Th2) cells, but the precise mechanisms that drive AHR were unclear. Recently, Interleukin-25 (IL-25), also known as IL-17E, was reported to be produced by activated Th2 cells and mast cells, resulting in enhanced AHR. NKT cells are also involved in the development of asthma. NKT cell-deficient mice fail to develop antigen-induced AHR. Moreover, activation of NKT cells can induce AHR in the absence of Th2 cells, suggesting that NKT cells are directly involved in the AHR pathogenesis.

When the authors investigated the expression of IL-17RB, a receptor for IL-25, they found that IL-17RB was selectively expressed by a fraction of NKT cells. This differential expression of IL-17RB led to an investigation of the phenotypic and functional characteristics of IL-17RB^{+} NKT cells. When the response of IL-17RB^{+} NKT cells to IL-25 was analyzed *in vitro*, the cells were found to produce Th2 cytokines and chemokines known to be associated with AHR. In addition, the IL-17RB^{+} NKT cells were found to be abundant in the lungs of Th2-prone mice.

Given these intriguing observations, the authors used a genetic approach to directly investigate the contribution of IL-17RB^{+} NKT cells to IL-25-triggerd AHR. Normal mice treated with IL-25 and immunized developed AHR; they had increased lung resistance, infiltration of inflammatory cells, abundant mucus-producing cells, and inflammatory cytokine production. Similarly treated NKT-deficient mice, by contrast, had no evidence of AHR. Furthermore, depletion of IL-17RB^{+} NKT cells by IL-17RB antibodies suppressed the development of AHR. These findings strongly suggest that IL-25 directly acts on NKT cells to induce AHR.

Allergy has become an increasing health and economic burden in industrial countries. In Japan, an estimated 30% of the population suffers from allergies such as pollinosis, asthma, or atopic diseases. According to the World Health Organization, 300 million people worldwide are afflicted with asthma, and there are 3 million patients in Japan. It has also been reported that IL-25 producing cells are present in patients with asthma. In this study, IL-17RB neutralizing antibodies prevented AHR and reduced inflammation, suggesting that IL-17RB will be a promising therapeutic target for asthma intervention.

Asuka Terashima *(left)* **and Sayo Inoue** *(right)*

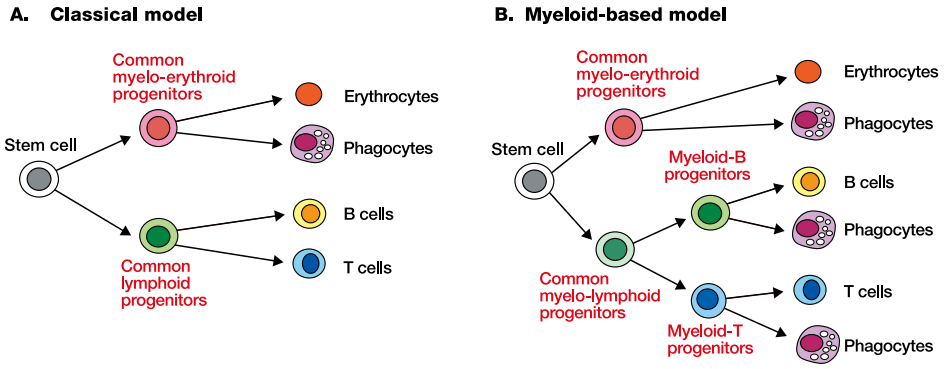
ORIGINAL RESEARCH PAPER

Terashima A, Watarai H, Inoue S, Sekine E, Nakagawa R, Hase K, Iwamura C, Nakajima H, Nakayama T, Taniguchi M. A novel subset of mouse NKT cells bearing the IL-17 receptor B responds to IL-25 and contributes to airway hyperreactivity. *J Exp Med*. 24, 2727-2733. (2008)

Choosing the right path

A new study of blood cell development could help put an end to a decade-old controversy about immune cell differentiation

Hiroshi Kawamoto



Figure

Two models of blood cell differentiation. (A) In the long-standing 'classical model', stem cells can differentiate into myelo-erythroid progenitors, which can form myeloid cells and erythrocytes (red blood cells), or CLPs, which can form B and T cells but not myeloid cells. (B) Kawamoto and colleagues' findings support a 'myeloid-based model', in which CLPs are not a requisite intermediate stage. Instead, stem cells develop into common myelo-lymphoid progenitors and then into myeloid-B or myeloid-T progenitors, all of which retain the ability to form myeloid as well as lymphoid cells.

Blood contains many different cell types, all of which develop from a common stem cell precursor via a multi-stage differentiation process. T cells and B cells, immune cells that respond to foreign substances in the body, belong to a class known as lymphocytes, and have long been assumed to follow a similar developmental course. This 'classical model' of differentiation predicts that stem cells will differentiate into common lymphoid progenitors (CLPs), which become T and B cells, or myelo-erythroid progenitors, which form myeloid cells—such as macrophages—and red blood cells (Figure A).

It was therefore quite surprising when immunologists Hiroshi Kawamoto and Yoshimoto Katsura were unable to detect CLPs from blood cell progenitors in the fetal liver. Their 1997 study suggested a 'myeloid-based model' of differentiation (Figure B), in which the ability to form myeloid cells is retained by all blood cell progenitors. However, another group's subsequent discovery of putative CLPs in adult bone marrow left immunologists with a controversy on their hands: do fetal and adult blood cells develop along different pathways?

New work from Kawamoto's group at RCAI represents important progress towards resolving this mystery. Kawamoto and colleagues developed a new culture system that enabled them to gauge the ability of progenitor cells from different developmental stages to form T and myeloid cells. The researchers found that these progenitors could form macrophages even at stages of differentiation where B cell formation was no longer a possibility, regardless of whether the cells were adult or fetal in origin. Similar results were obtained in live mice when these later-stage progenitor cells were grafted into the thymus.

Kawamoto believes that these findings support a myeloid-based model of development for both fetal and adult immune cells, and argues against the need for CLPs. "Many researchers have taken it for granted that T cells and B cells are very closely related, and thus both lineage cells are generated through a common pathway," he says. "Our finding—that T cells and macrophages are generated from progenitors that have lost B cell potential—refutes this dogma."

These findings should shed new light on T cell development. "Now that we have proven that the myeloid-T bipotential stage exists in hematopoiesis, we are in the ideal place to start to clarify the molecular mechanisms of myeloid-T lineage commitment," he says. "These studies will give us answers about what environmental and intrinsic factors determine the identity of the T cell lineage."

Haruka Wada

ORIGINAL RESEARCH PAPER

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).

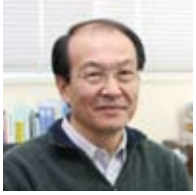
RELATED READINGS

Kawamoto, H., Ohmura, K. & Katsura, Y. Direct evidence for the commitment of hematopoietic stem cells to T, B and myeloid lineages in murine fetal liver. *International Immunology* 9, 1011–1019 (1997).

Kondo, M., Weissman, I.L. & Akashi, K. Identification of clonogenic common lymphoid progenitors in mouse bone marrow. *Cell* 91, 661–672 (1997).

How the body senses emergency

A receptor on macrophages can detect excessive cell death and recruit help



Takashi Saito

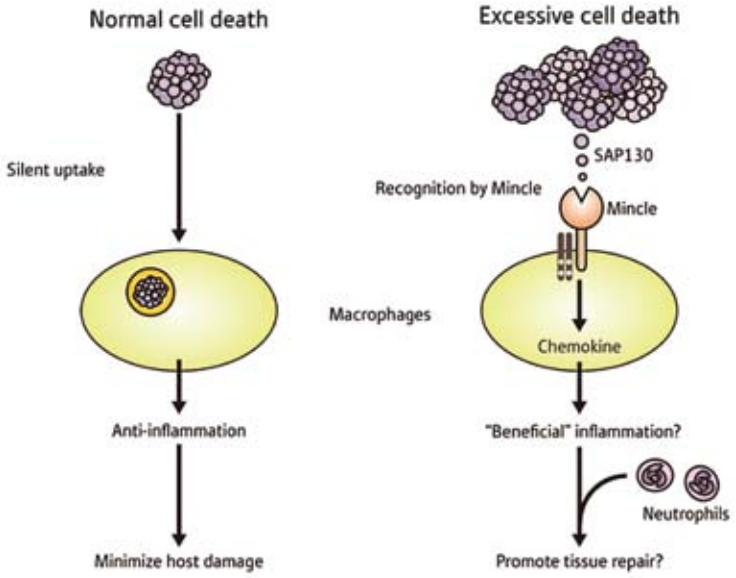


Figure How Mincle senses tissue damage and induces inflammation.

A receptor induced on the surface of macrophages under stressful conditions can detect tissue injury, stimulating inflammation and possibly repair, a RIKEN-led team of molecular biologists has discovered. Their work could provide new leads for anti-inflammatory drugs and healing.

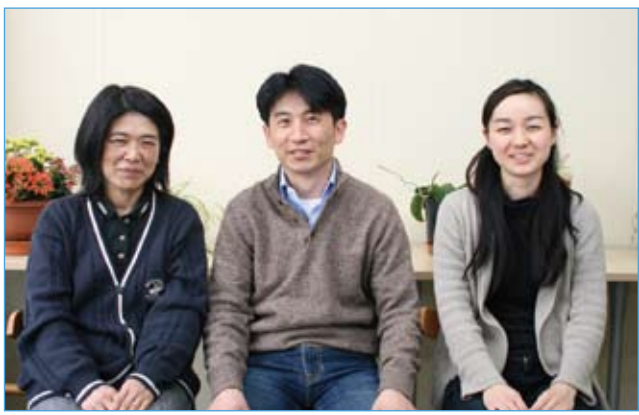
Stress, age and body maintenance generate a continuous supply of dead cells, which normally are cleaned up by the macrophages that engulf pathogens and cellular debris. This mechanism, however, becomes overwhelmed at times of large-scale tissue damage, such as that caused by radiation or injury. To deal with such emergencies, the body needs a sensor that not only can detect the scale of the problem, but also that the dead tissue is not foreign.

Earlier research by another group had suggested that cellular stress leads to an upsurge in the activity of a gene, *Mincle*, which encodes a surface receptor on macrophages, an observation that promoted the RIKEN-led research team to investigate the function of this receptor. Their findings were published recently in *Nature Immunology*.

Initially, the researchers found that the Mincle receptor is associated with another signaling receptor chain, Fc receptor common γ chain (FcRγ) and triggers macrophage activation through a specific sequence known as the immunoreceptor tyrosine-based activation motif (ITAM). This stimulates the release of cellular hormones—cytokines and chemokines—that summon neutrophils to take part in inflammation and possibly tissue repair. Using a system involving green fluorescent protein to detect ITAM-mediated cell activation, the researchers found that Mincle responds to the presence of dead cells.

They then purified protein material from dead cells bound to the Mincle receptor, and discovered it was SAP130, a protein found in cell nuclei. SAP130 is released from cells where it can come into contact with the Mincle receptor only after they die and break down (Figure). In further experiments, the researchers determined that the Mincle alert system works in mammals by showing that in living mice in which thymus cells had been killed by irradiation, the recruitment of neutrophils to the site of the damage was prevented by Mincle-specific antibody.

According to the team leader, Takashi Saito, the research group now wants to determine the role of the alert system in diseases involving tissue damage; how activation of Mincle is related to the induction of autoimmune diseases such as rheumatoid arthritis; and whether it is possible to inhibit or cure inflammation and/or autoimmune diseases by blocking Mincle.

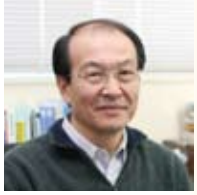


Machie Sakuma *(left)*, **Sho Yamasaki** *(middle)* **and Eri Ishikawa** *(right)*

ORIGINAL RESEARCH PAPER

Yamasaki, S., Ishikawa, E., Sakuma, M., Hara, H., Ogata, K. & Saito, T. Mincle is an ITAM-coupled activating receptor that senses damaged cells. *Nature Immunology* 9, 1179–1188 (2008).

Mincle is an activating receptor for the pathogenic fungus *Malassezia*



Takashi Saito

Mincle (Macrophage-inducible C-type lectin) is primarily expressed by macrophages and is induced after exposure to various stimuli and stresses. Takashi Saito and his team recently found that Mincle is an Fc receptor common γ chain (FcRγ) -associated activating receptor that senses damaged cells by recognizing a nuclear protein released from dead cells. Mincle contains an ITAM (immunoreceptor tyrosine-based activation motif) signaling motif, that, when phosphorylated, transmits a signal to the associated FcRγ molecule, triggering macrophage activation.

ITAM-coupled receptors are often called "multi-task" receptors because they have the ability to discriminate the quality of the ligands. Some ITAM-coupled receptors, such as the antigen receptors on T cells and B cells, can recognize differences in antigens to determine the appropriate cellular responses. Some C-type lectins have also been reported to recognize various self and non-self ligands. To explore the possibility that Mincle is a multi-task receptor, Saito's team searched for the exogenous Mincle ligand.

Because Mincle is structurally similar to other C-type lectins that recognize non-self ligands such as fungi, they first screened pathogenic fungi for Mincle-ligand activity. Of the more than 50 species tested, only *Malassezia* species induced activation of a reporter gene (figure). The authors then obtained Mincle deficient mice and found that cytokine/chemokine production by macrophages in response to *Malasezzia* was impaired, as was the *in vivo* inflammatory response to the fungus. These results indicated that Mincle is a specific receptor for *Malassezia* species and plays a crucial role in immune responses to this fungus. They further investigated the structure of *Malassezia* recognized by Mincle.

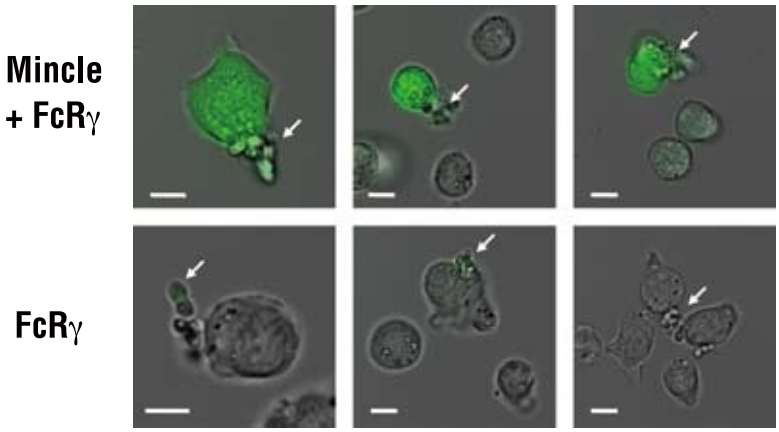


Figure **Mincle directly recognizes *Malassezia***
When Mincle and the Fcγ receptor (FcR γ) were both expressed in a T cell line, the NFAT-GFP reporter gene was activated by *Malassezia* stimulation, resulting in GFP expression (*green*).

Since mutation of the mannose-binding motif in the carbohydrate recognition domain of Mincle inhibited responses to *Malassezia*, mannose binding seemed to be crucial for Mincle to sense this organism.

Malassezia species are ubiquitous residents of human skin, but under some conditions they induce invasive infections, such as tinea versicolor, atopic dermatitis, and lethal sepsis. The identification of Mincle as a specific receptor for *Malassezia* will provide valuable information for the development of therapy and effective drugs for *Malassezia*-related diseases.

ORIGINAL RESEARCH PAPER

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.* 106, 1897-1902 (2009)

RELATED READING

Yamasaki, S., Ishikawa, E., Sakuma, M., Hara, H., Ogata, K. & Saito, T. Mincle is an ITAM-coupled activating receptor that senses damaged cells. *Nature Immunology* 9, 1179–1188 (2008).

How to halt immune cell activation

A new study sheds light on the molecular machinery required for reining in cellular signals that, if unleashed, could result in pathological inflammation

Ji-Yang Wang

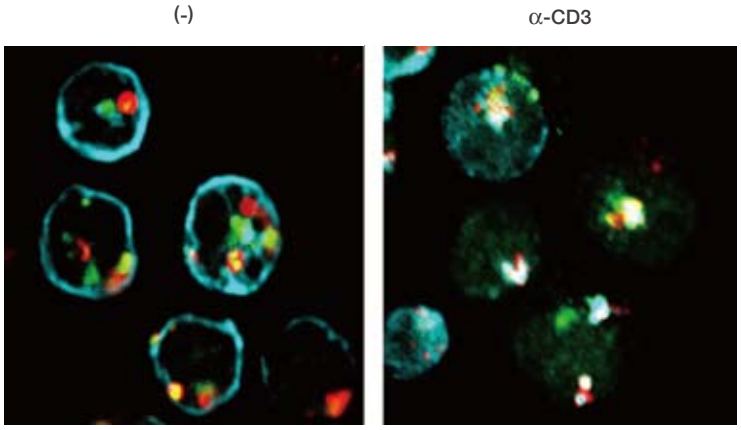


Figure **Interaction between TCR, CD3 and LAPTM5.** *Left*, CD3ζ (*blue*) is localized on the plasma membrane whereas LAPTM5 (*green*) and the lysosome-associated protein LAMP1 (*red*) are in the lysosomes in T cells before stimulation. *Right*, after TCR stimulation (α-CD3), CD3ζ moves to the lysosomal compartment where it co-localizes with LAPTM5 and LAMP1 and is degraded.

Researchers in RCAI have identified part of the mechanism responsible for preventing prolonged—and potentially dangerous—activation of immune cells called T lymphocytes. Each decorated with a unique surface receptor (TCR) capable of detecting foreign proteins, T lymphocytes circulate throughout the body patrolling for invading microorganisms. Upon encounter with rogue proteins, TCRs trigger—via a complex of CD3 signaling proteins—intracellular events that orchestrate release of biologically active mediators called cytokines, some of which are proinflammatory.

As unrestrained inflammation can cause tissue damage, the immune system exerts tight control over T lymphocyte activation. In a healthy state, TCR and CD3 proteins are constantly internalized and released back to the lymphocyte surface; this 'recycling' maintains a low level of TCR expression and thus a high 'threshold' precluding unwarranted activation. After stimulation, however, TCRs and CD3 subunits are routed towards destructive intracellular compartments called lysosomes, where they are degraded as part of a signal 'shut off' mechanism.

A team led by Ji-Yang Wang of the RIKEN Center for Allergy and Immunology in Yokohama sought to identify proteins underpinning this 'fail safe' TCR signal termination process.

Having noted in previous experiments that expression of the lysosomal protein LAPTM5 is altered after TCR stimulation, the researchers tested whether LAPTM5 is involved in turning off TCR signals. They used genetic manipulation techniques to generate mutant mice in which the *Laptm5* gene is not expressed. These *Laptm5*-deficient animals exhibited excessive T lymphocyte-driven responses to skin sensitization.

The team also found that, compared to normal T lymphocytes, LAPTM5-deficient T lymphocytes underwent more cell divisions, and released more cytokines interferon-γ and interleukin-2 after TCR stimulation. After activation, T lymphocytes lacking LAPTM5 expressed higher amounts of surface and intracellular TCR and a CD3 subunit, CD3ζ, than did wild-type T lymphocytes. Conversely, overexpression of LAPTM5 dampened CD3ζ expression.

TCR and CD3ζ proteins co-localized with LAPTM5 in lysosomes of activated T cells, and LAPTM5 physically interacted with CD3ζ (Figure). These findings suggest that LAPTM5 promotes CD3ζ degradation by binding to and shuttling this protein to lysosomes.

Whether LAPTM5 cooperates with other lysosomal proteins to orchestrate CD3ζ destruction, and whether any human immune disorders are associated with mutations in *Laptm5*, remains to be determined.

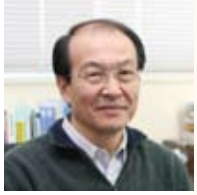
LAPTM5 is the first lysosomal protein known to be specifically expressed in blood generating (hematopoietic) cells. "In addition to its role in the negative regulation of TCR signaling, preliminary studies indicate that LAPTM5 may regulate the cell surface expression of additional immune receptors and may also function to prevent hematopoietic malignancies," says Wang.

Rika Ouchida

ORIGINAL RESEARCH PAPER

Ouchida, R., Yamasaki, S., Hikida, M., Masuda, K., Kawamura, K., Wada, A., Mochizuki, S., Tagawa, M., Sakamoto, A., Hatano, M., Tokuhisa, T., Koseki, H., Saito, T., Kurosaki, T. & Wang, J.Y. A lysosomal protein negatively regulates surface T cell antigen receptor expression by promoting CD3ζ-chain degradation. *Immunity* **29**, 33–43 (2008).

Immune cell activation under the microscope



Takashi Saito

The application of sophisticated imaging techniques illuminates spatio-temporal regulation critical for T cell activation

T cells are central to an organism's defense against invading pathogens. But scientists have long puzzled over how they are activated and regulated after pathogen recognition. Now a team of researchers, led by Takashi Saito from the RIKEN RCAI in Yokohama, has succeeded in imaging molecular events that are crucial for these processes.

Full activation and differentiation of T cells requires a primary signal from T cell receptors (TCRs) upon interaction with an antigen-presenting cell (APC), and a second, distinct signal transmitted through 'costimulatory' receptors.

The receptor CD28 plays a predominant role in T cell costimulation. CD28-mediated signals augment many T cell functions, such as cytokine production and cell proliferation.

Modulation of these costimulatory signals has been applied in clinical trials by increasing tumor immunity and reducing autoimmune diseases. But the precise roles of molecules implicated in CD28-mediated costimulatory signals and their relationship with TCR signals require clarification.

Antigen-specific T cells 'communicate' with APCs through an 'immunological synapse', which forms at their interface and contains a central (c) and a peripheral (p) supramolecular activation cluster (SMAC).

At initial activation, TCRs form microclusters, which contain receptors, kinases, and adaptor proteins to induce activation signals at the interface between a T cell and an APC. These microclusters translocate to the center of the interface, resulting in cSMAC formation.

The role of microcluster translocation in T cell signaling has been unclear, and the concept that they function as signaling centers for T cell activation has raised questions as to how CD28-mediated costimulation is regulated.

Using sophisticated fluorescence microscopy techniques to study CD28-mediated costimulation at the single molecule level, Saito and colleagues have found that the accumulation of microclusters at cSMAC is important for T cell costimulation. CD28 is initially recruited together with TCRs to microclusters. PKCθ—a protein kinase acting downstream of CD28—is also recruited to microclusters by association with CD28 (Figure), thereby resulting in the initial activation of T cells.

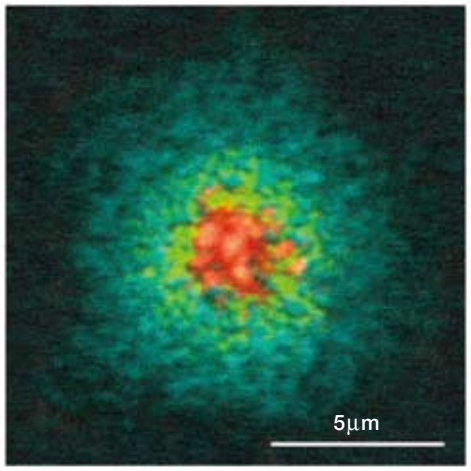
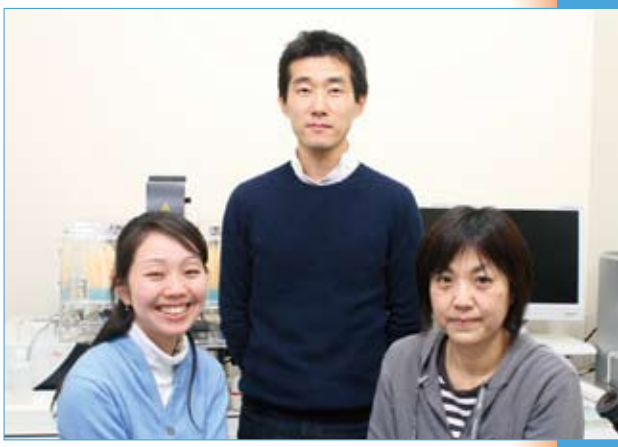


Figure
An immunological synapse showing the central supramolecular activation cluster (SMAC) containing both T cell receptors (*red*) and PKCθ (*green*) and the peripheral SMAC (*blue*).

CD28 also plays a role in retaining PKCθ at a spatially unique subregion of cSMAC, leading to sustained signals for T cell activation. "Thus, costimulation is mediated by the generation of a unique costimulatory compartment in the cSMAC via the dynamic regulation of microcluster translocation," say the researchers.

Establishing the underlying mechanisms should lead to new treatments for autoimmune diseases, such as rheumatoid arthritis and psoriasis, as well as the prevention of graft versus host disease in transplantation, more effective vaccinations, and augmented anti-tumor immunity.



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

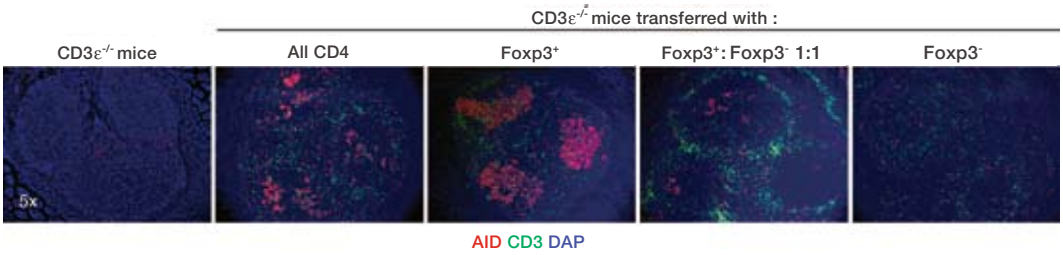
ORIGINAL RESEARCH PAPER

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

Foxp3$^+$ T cells preferentially differentiate to follicular B helper T cells in the gut

Shohei Hori

Sidonia Fagarasan



Figure

T cell deficient mice were untreated or transplanted with the indicated cell types. Four weeks later, sections of Peyer's patches were examined by immunofluorescence microscopy. AID, activation induced cytidine deaminase, a marker for GC B cells undergoing isotype switching/somatic hypermutation (*red*); CD3, a T cell marker (*green*); DAPI, a DNA stain to reveal cell nuclei (*blue*).

Collaborative research by RCAI teams led by Sidonia Fagarasan and Shohei Hori resulted in a surprising discovery. Foxp3$^+$ T cells, called T_{regs} because of their ability to suppress immune responses, can differentiate into follicular B helper T cells in the gut.

The transcription factor Foxp3 is a marker for T_{regs}, which play a central role in preventing pathological immune responses and ensuring tolerance to self- and innocuous environmental antigens. However, Hori recently reported unstable expression of Foxp3 and developmental plasticity in some of Foxp3$^+$ T cells.

IgA in the gut is critical for maintaining the immune system homeostasis, by controlling the vast community of intestinal bacteria. IgA is mostly produced by B cells in gut Peyer's patches (PP), and requires the presence of follicular B helper T cells (T_{FH}). These B cells and T_{FH} co-localize in a specialized structure, the germinal center (GC), where B cells undergo isotype class switching and somatic hypermutation, upon induction of activation-induced cytidine-deaminase (AID). However, the origin of T_{FH} cells in PP was unknown.

To investigate the origins of T_{FH} in PPs, the teams transferred Foxp3$^+$ and/or Foxp3$^-$ T cells into T cell deficient mice. To their surprise, typical GCs were formed moslty when Foxp3$^+$ T cells were transferred. Furthermore, the induction of GCs (Figure) and generation of IgA-producing cells in PPs were more effective than that induced by unfractionated T cells or a 1:1 mixture of Foxp3$^+$ and Foxp3$^-$ T cells.

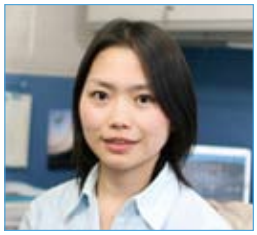
A large fraction of the transferred Foxp3$^+$ T cells lost Foxp3 expression and preferentially localized to the B cell follicles. These cells expressed chemokine receptors, transcription factors, and cytokines critical for migration, generation and function of T_{FH}. Thus, Foxp3 expression is plastic and cells that previously expressed Foxp3 are effective precursors for T_{FH} cells.

To determine whether B cells are required for the differentiation to T_{FH} cells, they transferred Foxp3$^+$ T cells to mice deficient in both T and B cells. In the absence of B cells, Foxp3$^+$ T cells failed to differentiate into T_{FH} cells, although Foxp3 expression was down regulated. Thus, differentiation of Foxp3$^+$ T cells into T_{FH} cells requires interaction with B cells while down-regulation of Foxp3 expression does not.

Foxp3$^+$ T cells were converted into T_{FH} cells only in PPs. When they transferred Foxp3$^+$ T cells into T cell deficient mice, neither T_{FH} cells nor GCs could be detected in spleen or lymph nodes after immunization. In contrast, Foxp3$^-$ T cells generated GCs in the spleen.

The study suggests that, depending on the environment, TCR stimulation induces either "suppressor" or "helper" T cells. The studies have implications for understanding how suppression of inflammatory reactions and induction of IgA synthesis occur in the gut.

Masayuki Tsuji



Noriko Komatsu

Shinpei Kawamoto

ORIGINAL RESEARCH PAPER

Tsuji, M., * Komatsu, N.,* Kawamoto, S.,* Suzuki K., Kanagawa, O., Honjo, T., Hori, S.,† Fagarasan, S.† Preferential Generation of Follicular B Helper T (T_{FH}) Cells from Foxp3+ T Cells in Gut Peyer's Patches. Science 323, 1488-1492 (2009)

How the gut manages bacteria

A previously unknown mechanism enables the immune system in the gut to respond rapidly to changes in bacteria

Sidonia Fagarasan

A RIKEN-led international research group has puzzled out details of the intricate mechanism by which the immune system in the gut can respond rapidly to changes in its bacterial environment. Eventually, the work could lead to better treatment and control of gut infections and inflammatory bowel diseases.

The gut is in direct contact with the external environment and houses at least 400 different species of bacteria in vast numbers. It maintains a finely tuned immune system built around immunoglobulin A (IgA) antibodies produced by B cells to protect the body against pathogens and manage the growth of benign organisms. Previous research by other researchers unraveled a mechanism whereby T cells control the formation of these IgA-producing B cells in organized multi-cellular structures called Peyer's patches, which develop in the embryo. But such a system could take weeks to respond to invasive bacteria.

The latest work reveals a second mechanism that operates without intervention of T cells, and develops only after colonization of the intestine with bacteria, hence after birth. It involves another set of cellular structures called isolated lymphoid follicles (ILFs).

In a recent paper in the journal *Immunity*, the researchers, led by Sidonia Fagarasan of RCAI, detail how these ILFs piece together, providing an understanding of the newly identified mechanism. They used strains of mice bred to lack compounds significant to the development of ILFs.

The researchers noticed that the numbers and size of ILFs in the gut paralleled the level of bacteria, increasing with bacterial colonization and decreasing with the use of antibiotics. Recently, they also discovered cells in adults similar to embryonic lymphoid tissue-inducer (LTi) cells essential to the development of immune centers, such as lymph nodes and Peyer's patches.

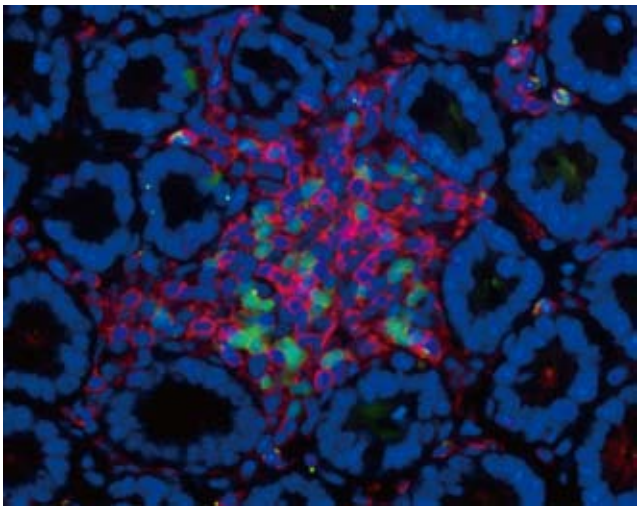


Figure Adult LTi cells (*green*) interact with underlying stromal cells in the gut to recruit lymphocytes (*red*) in the small intestine. The nuclei of cells are stained in blue to reveal the gut structure.

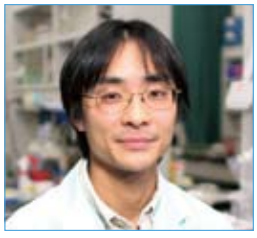
Fagarasan and her colleagues showed that these adult LTi cells could interact with underlying stromal cells in the gut to recruit the cellular components of ILFs—B cells and antigen presenting dendritic cells (Figure). But the adult LTi cells only did this effectively in the presence of bacterial cells which stimulate an immune response, partly through the production of tumor necrosis factor. So ILFs are only formed when bacteria are present. The researchers also demonstrated that functioning ILFs could transform typical B cells that make immunoglobulin M into those that produce IgA.

"If we can understand more about these LTi cells and their interactions," says Fagarasan, "it could provide us with the potential to manipulate the gut immune system."

ORIGINAL RESEARCH PAPER

Tsuji, M., Suzuki, K., Kitamura, H., Maruya, M., Kinoshita, K., Ivanov, I.I., Itoh, K., Littman, D.R. & Fagarasan, S. Requirement for lymphoid tissue-inducer cells in isolated follicle formation and T cell-independent Immunoglobulin A generation in the gut. *Immunity* **29**, 261–271 (2008).

Masayuki Tsuji



Keiichiro Suzuki

PLC-γ2 is essential for formation and maintenance of memory B cells

Tomohiro Kurosaki

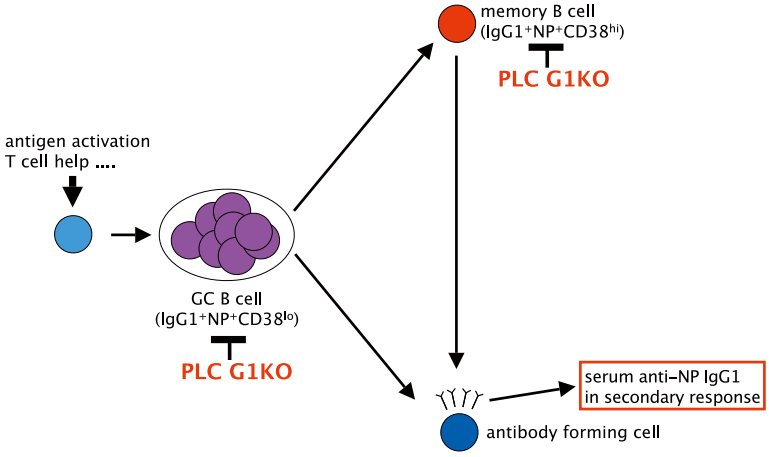


Figure Regulation of formation and survival of memory B cells by PLCγ2

Immunological memory is the hallmark of the adaptive immune response. Memory exists in both T and B cell compartments, but B cell memory is the focus of the studies by Hikida and colleagues. Humoral, antibody-mediated memory has several distinguishing characteristics. Immune responses on secondary antigen encounter are more rapid than the primary response, the antibodies, mostly IgG, have higher titers and higher affinity due to somatic hypermutation.

Most memory B cells are thought to originate in the germinal center (GC), where somatic hypermutation and selection based on the affinity of the BCR for the antigen are responsible for the generation of high-affinity antibody variants. These ultimately differentiate into long-lived plasma cells or long-lived memory B cells. The GC is also the site of antibody class switching, so that the memory B cells express an IgG BCR. Memory B cells generated are thought to acquire intrinsically different traits from their naïve predecessors, including longevity, that account for more rapid and heightened secondary antibody responses.

The molecular basis for the development and survival of memory B cells is poorly understood. It was known from previous studies that the cytoplasmic tail of IgG1 is required for normal secondary antibody responses, however the mechanism was unknown. The studies reported here identified a requirement for phospholipase C (PLC) γ2, an enzyme known to be important in BCR signaling, in the formation of GC and memory B cells. Genetic ablation of PLC-γ2 by conventional knockout strategies results in a premature block in B cell development, making these mice unsuitable for studying memory B cells. The authors used a clever strategy of crossing floxed PLC-γ2 mice with Cγ1-Cre knockin mice. In this inducible system, the Cre recombinase is expressed and the PLC-γ2 gene is deleted only when the endogenous Cγ1 locus becomes transcriptionally active, i.e. just prior to isotype switching. Using this system the authors found that primary immune responses were normal, however there was an almost complete abrogation of the secondary antibody response. These and other observations demonstrated that PLC-γ2 is required for the efficient generation of GC and memory B cells as well as for their survival (Figure).

These studies provide the first clear cut definition of a signaling defect that differentially affects naïve versus memory B cells and have potential clinical importance. An understanding of what regulates memory B cell generation and survival is important in the vaccine field, where the goal is to generate effective memory responses to pathogens, as well as in autoimmunity, where elimination of autoreactive memory cells might alleviate disease.

Masaki Hikida *(left)* **and Noriko Takahashi** *(right)*

ORIGINAL RESEARCH PAPER

Hikida, M., Casola S., Takahashi, N., Kaji, T., Takemori T., Rajewsky K., and Kurosaki, T. PLC- γ2 is essential for formation and maintenance of memory B cells. *JEM* 206, 681-689 (2009)

Tracing lymphocyte development signaling pathways

Two related proteins are essential for proliferation and survival of immune cell precursors

Tomohiro Kurosaki

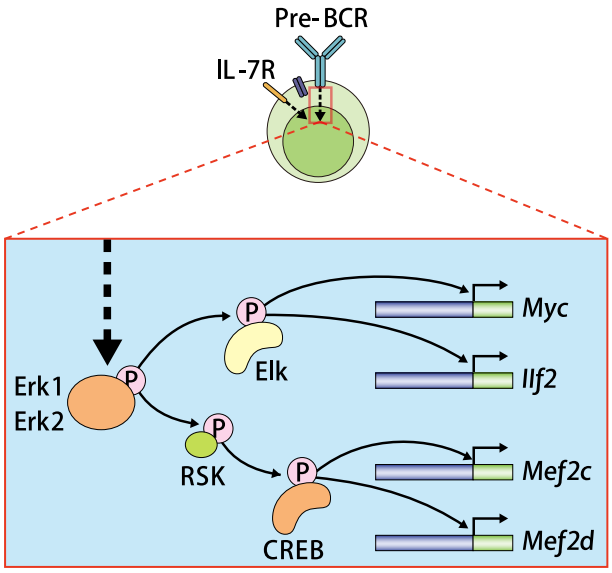


Figure The proteins Erk1 and Erk2 control a transcription factor network that allows B lymphocyte progenitors to traverse the pre-BCR developmental checkpoint.

Researchers have pinpointed the proteins required for transduction of signals directing the development of B lymphocytes, a type of immune cell. Responsible for producing antibodies capable of neutralizing invading microbes, B lymphocytes develop in the bone marrow and, in mature form, circulate throughout the body through the blood and lymphoid organs.

Before exiting the bone marrow, B lymphocyte precursors are propelled through a series of distinct developmental stages. Progression through early stages depends largely on signals triggered by extracellular growth factors, whereas passage through some later stages requires a signal from the pre-BCR, a receptor expressed on the surface of B lymphocyte precursors.

Pre-BCR signals culminate in the induction of gene transcription in the nucleus. Although the cell-surface proteins responsible for initiating pre-BCR signals have been identified, Tomoharu Yasuda and co-workers at RIKEN RCAI set out to identify proteins further down the pre-BCR signaling pathway.

The team focused on the related proteins Erk1 and Erk2, which transduce signals by phosphorylating, or 'tagging' downstream target proteins. Using genetic techniques, the researchers generated mice in which Erk1, Erk2 or both Erk1 and Erk2 are deleted or 'knocked out' on demand.

Compared to normal mice or those lacking either Erk1 or Erk2, mice lacking both Erk1 and Erk2 exhibited an almost complete block in development beyond the stage during which pre-BCR signaling occurs. Despite normal surface pre-BCR expression, these 'double knockout' precursors showed impaired proliferation and survival.

Consistent with the defects of the double-knockout cells, B lymphocyte precursors treated with an inhibitor of Erk activity expressed lower amounts of immediate early genes (IEG), which are required for population expansion.

As Erk1 and Erk2 do not directly bind to DNA, the team searched for DNA-binding factors that link Erk proteins with IEG upregulation. Noting the presence of binding sites for the factors Elk and CREB in the DNA regions controlling IEG expression, the team showed that pre-BCR-induced Erk activation results in Elk and CREB phosphorylation (Figure).

Illustrating the importance of Erk activity in B lymphocyte development, forced expression of mutant forms of Elk and CREB that lack Erk phosphorylation sites blocked pre-BCR-induced population expansion.

"Pre-BCR signals transmitted through Erk and downstream transcription factors are critical for pre-B cell proliferation," says Yasuda. "Drugs able to promote or suppress such molecular activity may be useful for treatment of acute lymphoblastic leukemia or immune deficiency disorders."

Tomoharu Yasuda

ORIGINAL RESEARCH PAPER

Yasuda, T., Sanjo, H., Pagès, G., Kawano, Y., Karasuyama, H., Pouysségur, J., Ogata, M. & Kurosaki, T. Erk kinases link pre-B cell receptor signaling to transcriptional events required for early B cell expansion. *Immunity* 28, 499–508 (2008).

Looping-the-loop

A unique model may describe the genetic switch that controls whether a T cell becomes a helper or a killer

Ichiro Taniuchi

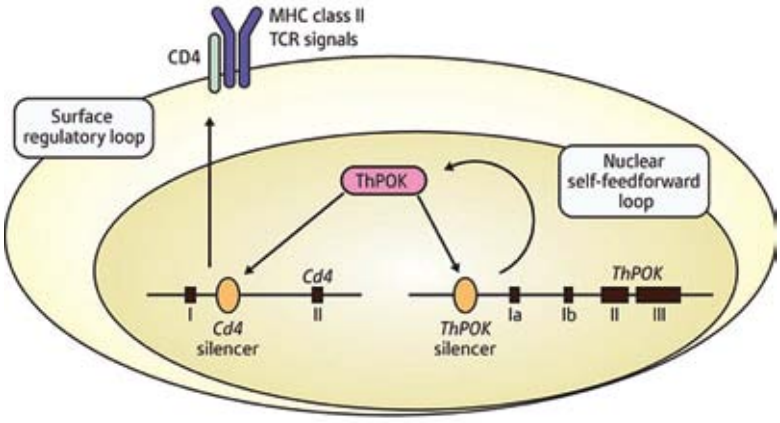


Figure Regulation of ThPOK expression in helper T cells. ThPOK (*magenta*) reverses the silencing of the *Cd4* gene as well as its own gene in a positive auto-feedforward mechanism. (TCR, T Cell Receptor; roman numerals denote segments of the *Cd4* and *ThPOK* genes).

Immunologists have come a step closer to understanding the genetic controls that direct the fate of the lynchpins of the immune system — T lymphocytes.

As T lymphocytes mature, they develop into different types, or lineages, which are characterized by different cell surface markers and have different roles in the immune response.

Helper T cells, which coordinate the immune response and promote antibody production, carry the CD4 surface marker protein, and require interaction with immune proteins called MHC class II molecules during development and differentiation. Killer T cells, which are cytotoxic and capable of directly killing infected or cancerous cells, carry the CD8 surface marker protein and must interact with MHC class I molecules during development and differentiation.

But the molecular mechanisms governing lineage decision of an immature T cell, or thymocyte, expressing both the CD4 and the CD8 markers to differentiate into CD4-positive, CD8-negative T helper or CD4-negative, CD8-positive T cytotoxic lineages are largely unknown.

Now, a team led by Ichiro Taniuchi at RIKEN RCAI has started to unravel some of the genetic controls directing the expression of lineage-specific genes.

A transcription factor called ThPOK has been identified in previous studies as being essential for the differentiation of MHC class II-selected thymocytes into helper T cells.

Taniuchi and colleagues used a fluorescent marker protein to visualize the expression of the *ThPOK* gene during the process of commitment to a specific T cell lineage. The gene has several regulatory elements, known as enhancers and silencers, which were modified in turn to assess the impact on lineage determination.

From these results, the researchers have developed a model that suggests stimulation of the T cell receptor during the initial stage of thymocyte differentiation induces ThPOK expression by reversing the activity of the *ThPOK* silencer. ThPOK then antagonizes the *CD4* silencer element, allowing expression of CD4, which in turn allows continued MHC class II-stimulation of the cell.

This has the effect of increasing ThPOK expression, generating a second auto-regulatory loop through the action of ThPOK to antagonize the silencer element within its own gene. The positive auto-feedforward mechanism amplifies and then stabilizes ThPOK expression in fully committed helper T cells (Figure).

"Our model is unique and could be the first one that describes an auto-regulatory loop by antagonizing a transcriptional silencer within its own locus," says Taniuchi. "I do expect that a similar mechanism may function in other lineage decision processes."

Sawako Muroi

ORIGINAL RESEARCH PAPER

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. *Nature Immunology* **9**, 1113–1121 (2008).

Outstanding Contribution of the Year 2008

RIKEN RCAI's Outstanding Contribution of the Year Award was established in 2006 to recognize staff members who make outstanding contributions to the Center. The awardees are determined by the Director and receive trophies and a monetary prize. In order to celebrate young people's efforts through this award and to develop the potential of young RCAI investigators, the Director and Group Directors agreed to exclude themselves from consideration for this award. The awards ceremony was held on March 13, 2009. Dr. Yasuyuki Ishii (Photo 1), Dr. Sho Yamasaki (Photo 2) and three researchers from the RefDIC project, Mr. Atsushi Hijikata, Dr. Yayoi Kimura and Dr. Hiroshi Kitamura (Photo 3) received the award.

Dr. Yasuyuki Ishii, the leader of the Laboratory for Vaccine Design received the award for his research to develop allergy vaccines for cedar pollinosis. This study was designated as the first RIKEN Translational Research project in June, 2008.

Photo 1 **Yasuyuki Ishii**

Most allergy medications available today treat the symptoms since their targets, for example histamine, are downstream of the allergic response. However, for a long-term cure it is necessary to regulate events upstream of the allergic response and induce allergen-specific tolerance. In association with other RCAI scientists, including Dr. Ohara and the members of the allergy vaccine project, Dr. Ishii developed two types of vaccines. One is a protective vaccine, and is a recombinant fusion protein composed of two cedar pollen antigens, while the other is a therapeutic vaccine, a liposome vaccine containing the cedar pollen antigens and α-GalCer glycolipids (Figure 1).

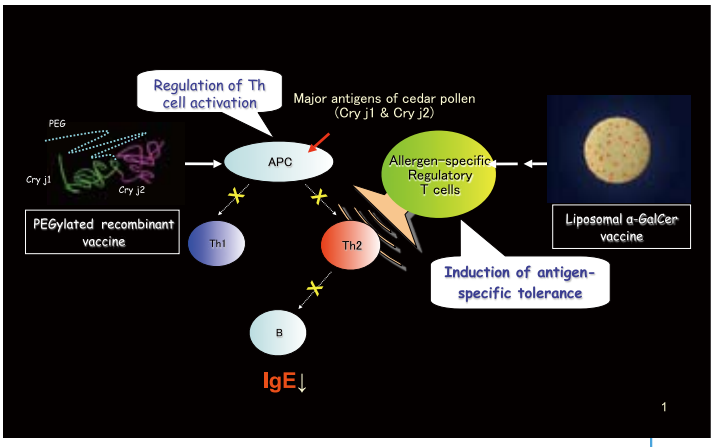


Figure 1 Two types of vaccines for cedar pollinosis. The first would be protective since it induces IgG rather than IgE responses, whereas the second liposomal vaccine would be therapeutic since it induces regulatory T cells.

To generate the fusion protein, there were two issues to overcome. The protein should include all the possible T cell epitopes, but not antibody epitopes recognized by IgE, since it must not induce anaphylactic shock, an acute, sometimes fatal allergic reaction induced by IgE antibodies preexisting in patients with allergies. Dr. Ishii and his team generated a recombinant fusion protein containing two cedar (*cryptomeria japonica*) pollen antigens (Cry j1/Cry j2). The protein encodes all potential human and mouse T-cell epitopes, but it does not have any structural IgE-binding epitopes. Further, they attached polyethylene glycol (PEG) to the protein to allow for a slow release and longer acting effect of the vaccine. When they analyzed the reactivity of IgE antibodies from 100 serum samples of cedar pollinosis patients, the PEG- fusion protein induced almost no reactions, although most patients'

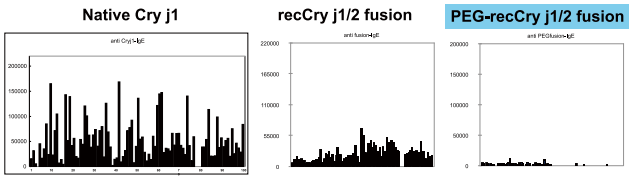


Figure 2 Binding of recCry j1/2 fusion protein to patient IgE antibodies
Binding capability of PEGylated recCry j1/2 fusion protein to IgE antibodies in sera of 100 patients of Japanese cedar pollinosis was remarkably lost, suggesting that the protein would not induce anaphylaxis.

sera were strongly reactive with the native cedar pollen antigen, Cry j1. Thus, this PEG- fusion protein has a low risk of inducing anaphylactic shock. (Figure 2). The investigators next examined the effect of the vaccine on the antibody

response against native cedar pollen. When mice were immunized with the fusion protein and boosted with native Cry j1 antigen, no IgE antibody against native Cry j1 was generated. Thus, this PEG-recombinant Cry j1/ Cryj 2 fusion protein is expected to have therapeutic potential as protective vaccine.

The idea behind the other type of vaccine, the liposome containing the cedar pollen antigens and α-GalCer glycolipids, is to activate immunoregulatory cells., The recombinant Cry j1/ Cry j2 fusion protein is encapsulated in the liposome, which is as small as 100nm in diameter. The NKT cell-ligand α-GalCer is embedded in lipid bilayer of the liposome, so it can activate NKT cells to induce NKT cell-mediated antigen-specific tolerance. To evaluate the antigen-specific suppressive activity of this liposomal vaccine, the mice were first immunized with Cry j1 and then injected with the liposomal vaccine. Anti-Cry j1 antibody responses were markedly suppressed after native Cry j1 antigen challenge. Thus, this liposomal vaccine is expected to be a useful therapy for cedar pollinosis.

Dr. Sho Yamasaki, a senior research scientist in the Laboratory for Cell Signaling, received the award for his research on signal regulation through immunoreceptor tyrosine-based activation motif (ITAM) containing receptors. He made two major findings: 1) the activation of the pre-TCR is ligand-independent and instead due to self-oligomerization of the receptor, and 2) the mechanism for the recognition of both damaged self and non-self pathogens by the C-type lectin, Mincle, in macrophages.

Photo 2 **Sho Yamasaki** (*left*)

ITAMs have been identified in the cytoplasmic domain of many immune receptors. ITAM-coupled receptors are often called "multi-task" receptors because they can discriminate the quality of the ligand, resulting in multiple levels and types of immune responses. However, the precise molecular mechanisms regulating these divergent responses are unclear. In order to understand the general principles which generate these ITAM-mediated responses, Dr. Yamasaki has been focusing on ITAM-containing receptors such as the T cell and pre-T cell receptors, Fc receptors, and C-type lectins.

The pre-T cell receptor is critical for T cell development, but the pre-TCR ligand had not been identified despite two decades of effort. In 2006, Dr. Yamasaki found the surprising solution: no ligand was required for pre-TCR signaling. Self-oligomerization after cell surface expression was the key event in mediating the pre-TCR signal (Yamasaki et al. *Nature Immunol*., 2006).

Recently, some C-type lectins have been reported to utilize ITAMs, but their functions and ligands were largely unknown. Among them was the Macrophage-inducible C-type lectin (Mincle). This year, Dr. Yamasaki reported that Mincle recognizes a nuclear protein, SAP130, released from dead cells (Yamasaki et al. *Nature Immunol*., 2008). Then, he discoverd that Mincle also recognizes a pathogenic fungus called *Malasezzia* (Yamasaki et al. *PNAS*., 2009). Thus, his discoveries indicate that Mincle is a dual receptor for danger, by recognizing both damaged self and harmful non-self. He is now focusing on the physiological significance of this dual recognition (Figure 3).

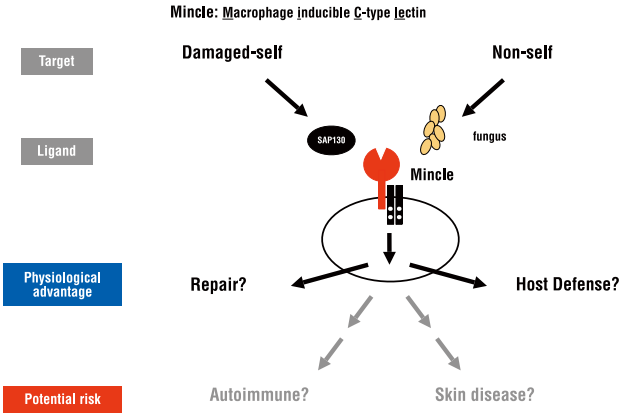


Figure 3 Mincle is a dual receptor for danger, damaged self and pathogenic non-self

Because of these achievements, Dr, Yamasaki has been selected to become a full professor at the Institute of Bioregulation, Kyusyu University. This promotion is the first case where a postdoctoral fellow of RCAI directly became a professor of a university. "I am given a chance to move to Kyusyu next April. Actually I hesitated to accept this award at first. I still think I don't deserve it. But because I don't have any research grants to establish a new laboratory, I really appreciate the funding and the opportunity. I'd like to thank the members of RCAI for the helpful discussions and advice during these 5 years," commented Dr. Yamasaki.

Photo 3 **Hiroshi Kitamura** (left), **Atsushi Hijikata** (middle) and **Yayoi Kimura** (right)

Mr. Atsushi Hijikata, Dr. Yayoi Kimura and Dr. Hiroshi Kitamura of Laboratory for Immunogenomics, received the award for their work on their RefDIC Project, which is an important database for immunological research.

A large amount of genomics data have become available in recent years, however, there are many hurdles for immunologists to fully exploit these data. Thus, soon after RCAI started its activity at Yokohama Institute in 2004, they began to develop a database named RefDIC to share omics data with immunologists.

RefDIC (Reference genomics Database of Immune Cells) is an immunogenomics database that integrates transcriptomic and proteomic data derived from immune cells in a user-friendly manner. As many as 50 researchers in RCAI cooperated to provide various immune cell and tissue samples. To collect mRNA and protein expression profiles on various types of immune cells in a cross-referenced manner, the RefDIC group performed DNA microarray analysis and two-dimensional gel electrophoresis (2DE) -based proteomics. The mRNA and protein profiling data were cross-referenced *in silico*. The group also annotated sample information with a controlled vocabulary, and all of those data were then integrated and stored in a relational database (Hijikata et al. *Bioinformatics*, 2007).

Since it launched as an open-access database in 2006 (http://refdic.rcai.riken.jp/), RefDIC quickly gathered attention from immunologists. At the end of 2008, RefDIC was accessed more than 20,000 times, and currently RefDIC is accessed by about 30 world-wide institutes per day. More importantly, RefDIC served to identify several important cell-specific molecules. Cd200r3 in regulatory dendritic cells (Sato et al. *Blood*, 2009), Il17rb in a subset of NKT cells (Terashima et al. *JEM*, 2008), and Psg18 in follicle-associated epithelium (Kawano et al., *Cell Struct. Funct*., 2007) are a few examples.

RefDIC currently contains: (i) 354 quantitative mRNA profiles for human and mouse immune cells/tissues obtained using Affymetrix GeneChip technology; (ii) 23 quantitative protein profiles for mouse immune cells obtained using 2DE followed by image analysis and mass spectrometry; and (iii) various visualization tools to cross-reference the mRNA and protein profiles of immune cells. In addition, the RefDIC group is collecting more informative genomics data. Using the Luminex system, they have begun to collect cytokine profiling of various immune cell types. The data will be added into RefDIC and will be open to the public in the near future.

Figure 4 The RefDIC Web interface. http://refdic.rcai.riken.jp/

References

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

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

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.* 106, 1897-1902 (2009)

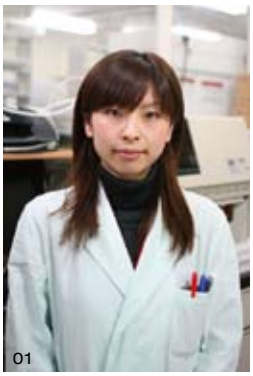
Hijikata A, Kitamura H, Kimura Y, Yokoyama R, Aiba Y, Bao Y, Fujita S, Hase K, Hori S, Ishii Y, Kanagawa O, Kawamoto H, Kawano K, Koseki H, Kubo M, Kurita-Miki A, Kurosaki T, Masuda K, Nakata M, Oboki K, Ohno H, Okamoto M, Okayama Y, O-Wang J, Saito H, Saito T, Sakuma M, Sato K, Sato K, Seino K, Setoguchi R, Tamura Y, Tanaka M, Taniguchi M, Taniuchi I, Teng A, Watanabe T, Watarai H, Yamasaki S, Ohara O. Construction of an open-access database that integrates cross-reference information from the transcriptome and proteome of immune cells. *Bioinformatics*. 23, 2934-2941 (2007)

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*. in press (2009)

Terashima A, Watarai H, Inoue S, Sekine E, Nakagawa R, Hase K, Iwamura C, Nakajima H, Nakayama T, Taniguchi M. A novel subset of mouse NKT cells bearing the IL-17 receptor B responds to IL-25 and contributes to airway hyperreactivity. *J Exp Med*. 205, 2727-2733. (2008)

Kawano K, Ebisawa M, Hase K, Fukuda S, Hijikata A, Kawano S, Date Y, Tsuneda S, Itoh K, Ohno H. Psg18 is specifically expressed in follicle-associated epithelium. *Cell Struct Funct*. 32, 115-126 (2007)

Award Winners 2008


01

Asuka Terashima received the Dean's Award from Graduate School of Medicine and Pharmaceutical Sciences, Chiba University

Among the graduate students who will finish the Ph.D. program in March, 2009 at the Graduate School of Medicine and Pharmaceutical Sciences, Chiba University, Ms. Asuka Terashima (Photo 1), Laboratory for Immune Regulation, was selected as the most outstanding student. She was recognized as the individual with the best achievements during the Ph.D. course, and will receive the award at the Ph.D. commencement ceremony on March 25. Her paper, "A novel subset of mouse NKT cells bearing the IL-17 receptor B responds to IL-25 and contributes to airway hyperreactivity", was published recently in *the Journal of Experimental Medicine* (November, 2008).


02

Daisuke Takahashi received The Melchers' Travel Award of the Japanese Society for Immunology

Mr. Daisuke Takahashi (Photo 2), a graduate student in the Lab. for Epithelial Immunology, received The Melcher's Travel Award at the Japanese Society for Immunology held on Dec. 1-3, 2008. Mr. Takahashi received the award for his research on "Deficiency of epithelium-specific basolateral sorting factor AP-1B leads to spontaneous development of colitis".

The award is funded by the donation of former director of Basel Institute of Immunology (BII) and his wife in recognition of the outstanding activity of the Japanese alumni at the BII.

Excellent Paper of the Year 2008

The RCAI Award for Excellent Paper was established in 2004 with donations from Dr. Masaru Taniguchi and Dr. Toshio Hirano. The annual award aims to recognize exceptional publications by RCAI scientists. This year, 12 excellent papers were selected.

Masayo Harada and Haruhiko Koseki
FGF9 monomer–dimer equilibrium regulates extracellular matrix affinity and tissue diffusion. *Nature Genetics*, Vol 41, pp 289-298, 2009

Haruka Wada and Hiroshi Kawamoto
Adult T-cell progenitors retain myeloid potential. *Nature*, Vol. 452, pp 768–772, 2008

Sho Yamasaki and Takashi Saito
Mincle is an ITAM-coupled activating receptor that senses damaged cells. *Nature Immunology*, Vol. 9, pp 1179-1188, 2008
and
C-type lectin Mincle is an activating receptor for pathogenic fungus, *Malassezia. PNAS*, Vol. 106, pp 1897- 1902, 2009

Masayuki Tsuji, Noriko Komatsu, Shimpei Kawamoto, Shohei Hori and Sidonia Fagarasan
Preferential Generation of Follicular B Helper T (T_{FH}) Cells from Foxp3^{+} T Cells in Gut Peyer's Patches. *Science* 323, 1488-1492 (2009)

Toshiyuki Fukada and Toshio Hirano
The Zinc Transporter SLC39A13/ZIP13 is Required for Connective Tissue Development; Its Involvement in BMP/TGF-β Signaling Pathways. *PLoS ONE*, e3642, 2008

Tomoharu Yasuda and Tomohiro Kurosaki
Erk kinases link pre-B cell receptor signaling to transcriptional events required for early B cell expansion. *Immunity*, Vol. 28, pp 499-508, 2008

Sawako Muroi and Ichiro Taniuchi
Cascading suppression of transcriptional silencers by ThPOK seals helper T cell fate. *Nature Immunology*, Vol. 9, pp 1113-1121, 2008

Tadashi Yokosuka and Takashi Saito
Spatiotemporal regulation of T cell costimulation by TCR-CD28 microclusters through protein kinase C θ translocation.. *Immunity*, Vol. 29, pp 589-601, 2008

Rika Ouchida and Ji-Yang Wang
A lysosomal protein negatively regulates surface T cell antigen receptor expression by promoting CD3 ζ chain degradation. *Immunity*, Vol. 29, pp 33-43, 2008

Asuka Terashima and Hiroshi Watarai
A novel subset of mouse NKT cells bearing the IL-17 receptor B responds to IL-25 and contributes to airway hyperreactivity. *J Exp Med*. Vol. 24, pp 2727-2733, 2008

Masayuki Tsuji and Sidonia Fagarasan
Requirement for lymphoid tissue-inducer cells in isolated follicle formation and T cell-independent immunoglobulin A generation in the gut. *Immunity*, Vol. 29, pp 261–271, 2008

Masaki Hikida and Tomohiro Kurosaki
PLC- γ 2 is essential for formation and maintenance of memory B cells. *J Exp Med.* Vol. 206, pp 681-689, 2009

Excellent Poster Award 2008

On September 1, RCAI held two poster presentation sessions during its retreat meeting in Chiba. Dr. Katrina L. Kelner, Deputy Editor, Science magazine and Dr. Elizabeth Thompson, Associate Editor, JEM also participated in the sessions.

Among 88 posters presented, nine posters were selected for an “Excellent Poster Award 2008” by vote of laboratory heads. One-year subscription of *Science* was also given to the best poster winner, Tomokatsu Ikawa from Dr. Kelner.

Awardees of Excellent Poster Award 2008


Tomokatsu Ikawa
(Lab. for Lymphocyte Development)


Naomi Hongo
(Lab. for Immune Regulation)


Chun-Hong QIU
(Lab. for Innate Cellular Immunity)


Toshiyuki Fukada
(Lab. for Cytokine Signaling)


Kiyokazu Kakugawa
(Lab. for Lymphocyte Development)


Katsuaki Hoshino
(Lab. for Host Defence)


Arata Takeuchi
(Lab. for Cell Signaling)


Gaku Nakato
(Lab. for Epithelial Immunobiology)


Shinya Tanaka
(Lab. for Signal Network)

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

RIKEN Special Postdoctoral Researcher (SPDR) Program

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

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

Dr. Tomoyuki Suzuki (Lab. for Cytokine Signaling) (01)
Dr. Shinya Tanaka (Lab. for Signal Network) (02)
Dr. Keniti Asano (Lab. for Innate Cellular Immunity) (03)
Dr. Noriko Komatsu
(Research Unit for Immune Homeostasis) (04)
Dr. Kohei Kometani (Lab. for Lymphocyte Differentiation) (05)
Dr. Yuki Horisawa-Takada
(Lab. for Developmental Genetics) (06)
Dr. Masahiro Kitano
(Research Unit for Immunodynamics) (07)
Dr. Shinji Fukuda (Lab. for Epithelial Immunobiology) (08)

RIKEN's Junior Research Associate (JRA) Program

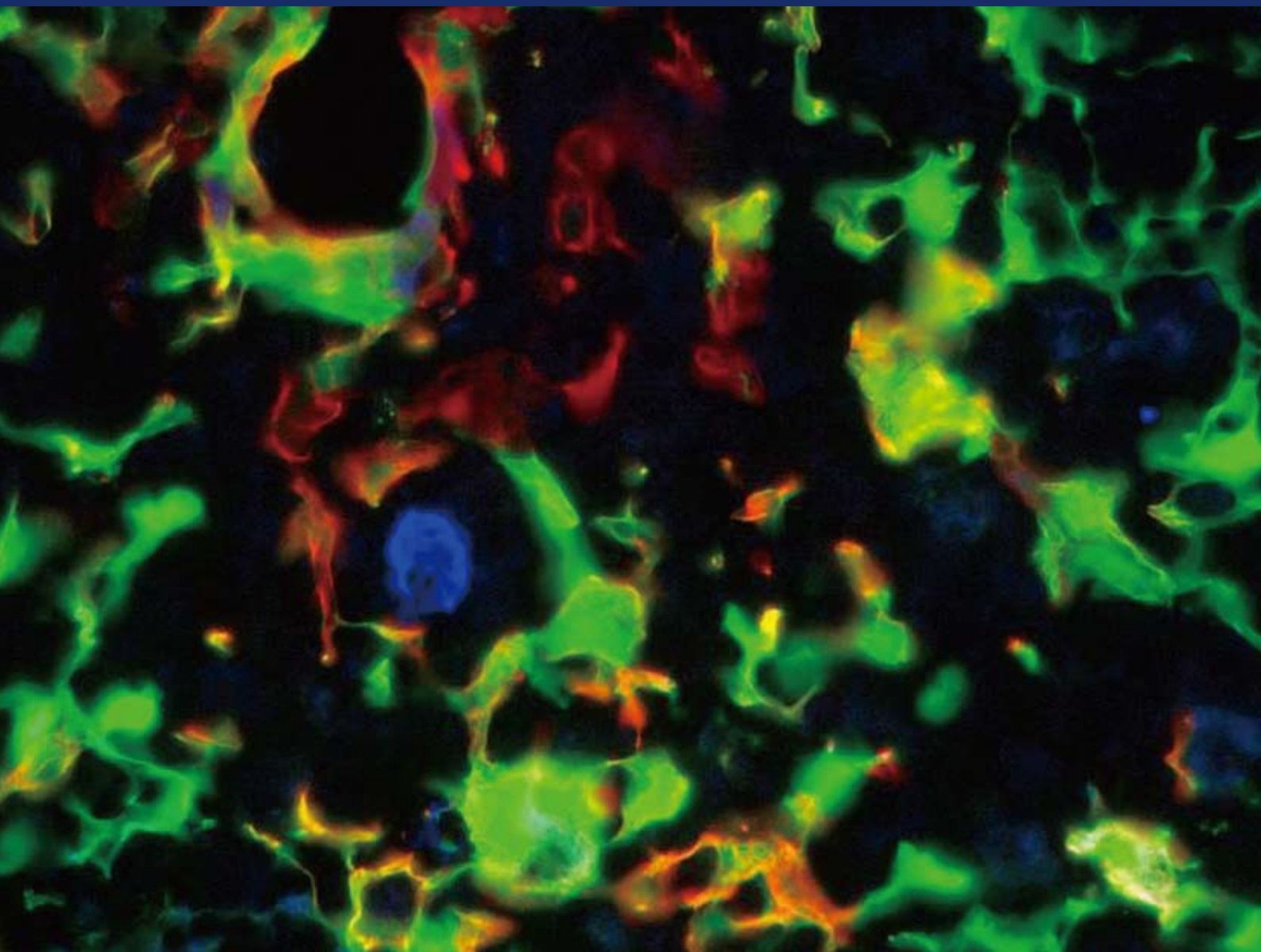
The Junior Research Associate program was launched in 1996 to encourage young scientists with fresh ideas and youthful enthusiasm to collaborate with, and learn from, veteran scientists with years of experience. This program provides part-time positions at RIKEN for young researchers enrolled in university 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, twelve JRA students studied in RCAI.

Mr. Masashi Ebisawa (Lab. for Epithelial Immunobiology) (09)
Mr. Gaku Nakato (Lab. for Epithelial Immunobiology) (10)
Mr. Daisuke Takahashi (Lab. for Epithelial Immunobiology) (11)
Mr. Takanori Sawaguchi
(Lab. for Developmental Genetics) (12)
Ms. Asuka Terashima (Lab. for Immune Regulation) (13)
Mr. Tomohiro Fukaya
(Lab. for Dendritic Cell Immunobiology) (14)
Mr. Shigeharu Fujita
(Lab. for Dendritic Cell Immunobiology) (15)
Mr. Shintaro Hojo (Lab. for Cytokine Signaling) (16)
Ms. Naomi Hongo (Lab. for Immune Regulation) (17)
Mr. Yasutaka Motomura (Lab. for Signal Network) (18)
Ms. Chihiro Yamazaki (Lab. for Host Defense) (19)
Mr. Izumi Sasaki (Lab. for Host Defense) (20)

2008

Laboratory Activities



Laboratory for Developmental Genetics

Group Director

Haruhiko Koseki, M.D., Ph. D.

Research Scientists : **Yuki Takada (SPDR)**
Yixin Dong
Mitsuhiro Endo
Yûichi Fujimura
Kyôichi Isono
Jun Shinga
Daisuke Yamada

Technical Staff : **Hiroshi Kajita**
Iyo Kataoka
Kayoko Katsuyama
Yôko Koseki
Tamie Morisawa
Naoko Ohnaga
Rie Suzuki
Midori Iida
Momoko Okoshi
Mai Sugiyama

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

Visiting Scientists : **Mutô Masahiro**
Jafar Sharif

Part time : **Masayo Harada**

Administrative Staff : **Ryôko Moriizumi**

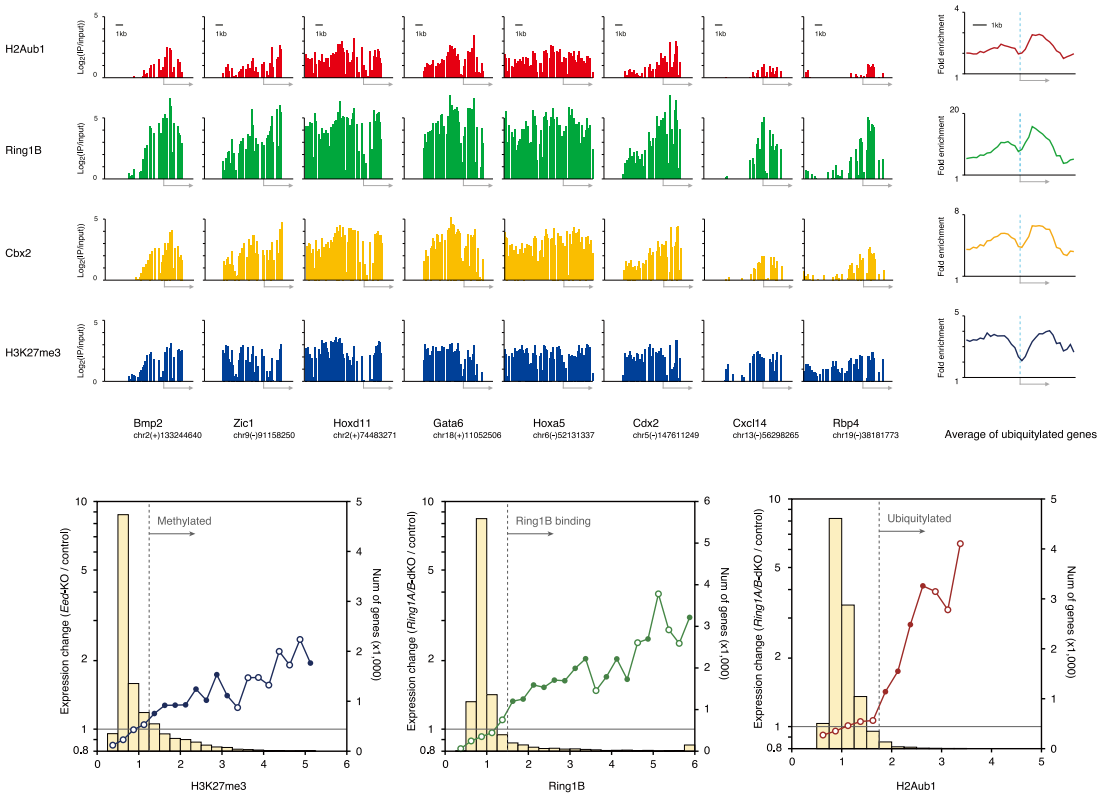
The Developmental Genetics Research Group fulfills a triple role within RCAI. A large portion of the manpower and financial resources of the group is devoted to the maintenance of a high-standard mouse facility at RCAI. Through the Animal Core Facility, the group is also responsible for the generation 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 Polycomb group (PcG) genes in development and signaling mechanisms mediated by fibroblast growth factor during differentiation. A third part of the group is extensively engaged in the establishment of induced pluripotent stem (iPS) cells from mature lymphocytes and the elucidation of molecular mechanisms that give rise heterogeneity of iPS cells. The ultimate goal of this study is to elucidate the therapeutic potential of lymphocyte-derived iPS cells.

Convergence of PRC2-dependent and –independent mechanisms mediates transcriptional repression by PRC1 to maintain ES cells

Polycomb group (PcG) protein complexes mediate transcriptional repression via epistatic engagement of at least two distinct complexes, PRC1 and PRC2, that have histone H2A ubiquitin ligase and histone H3 lysine 27 (H3K27) methyltransferase activity, respectively. Genome-wide analysis for deposition of mono-ubiquitinated H2A (H2Aub1) and its comparison to those of trimethylated H3K27 (H3K27me3) and Ring1B, a PRC1 component, identified at least three distinct layers of Polycomb-repressive domains. Recognition of H3K27me3 by chromodomains in PRC1 was required for recruitment of a functionally sufficient amount of PRC1 at target genes. This observation indicates that H3K27me3 is used as a platform for the hierarchical establishment of Polycomb repressive domains. We also found that genes encoding developmental transcription factors were significantly enriched in the fraction of genes co-occupied by H3K27me3, Ring1B and H2Aub1 (Triple positive genes; TP genes) and TP genes that include *Cdx2* and *Gata6* were significantly co-occupied by Oct3/4, Sox2, and/or Nanog. Together with the predominant de-repression of TP genes by developmental

Recent publications

1. Calés C, Román-Trufero M, Pavón L, Serrano I, Melgar T, Endoh M, Pérez C, Koseki H, Vidal M., Inactivation of the polycomb group protein Ring1B unveils an antiproliferative role in hematopoietic cell expansion and cooperation with tumorigenesis associated with Ink4a deletion. ***Mol Cell Biol.*** (2008) Feb;28(3):1018-28.
2. Puschendorf M, Terranova R, Boutsma E, Mao X, Isono K, Brykczynska U, Kolb C, Otte AP, Koseki H, Orkin SH, van Lohuizen M, Peters AH PRC1 and Suv39h specify parental asymmetry at constitutive heterochromatin in early mouse embryos. ***Nat Genet.*** (2008)Apr;40(4):411-20.



Figure

Local distribution of trimethylated H3 at K27 (H3K27me3), Ring1B and monoubiquitinated H2A at K119 (H2Aub1) as revealed by ChIP-chip analyses.

Distributions of H3K27me3, Ring1B and H2Aub1 at Cdx2, Gata6, Hoxa5, Olig2, Adcy7, Cxcl14, Guca1a and Nnat genes in undifferentiated ES cells are shown. Note the heterogeneity of the Polycomb repressive domain.

cues, we localized TP genes as central targets for Polycomb repression to maintain ES cell properties. TP genes were further identified as the most active domain for PRC1, but not PRC2, although repression at the TP genes requires both complexes. This functional gap between PRC1 and PRC2 at TP genes was ascribed to the intrinsic affinity of PRC1 to TP genes, an interpretation which was supported by the unique genomic signatures for TP genes. Taken together, recruitment of functionally sufficient amounts of PRC1 to the TP genes is mediated by synthetic interactions of PRC2-dependent and -independent mechanisms. We propose that PRC2 and PRC1 are juxtaposed at TP genes by means of their respective innate affinities, and that sensing of local H3K27me3 by PRC1 maximizes its local amounts at the target genes.

FGF9 monomer/dimer equilibrium regulates extracellular matrix affinity and tissue diffusion

The spontaneous dominant mouse mutant, Elbow-knee-synostosis (*Eks*), exhibits elbow and knee joint synosotsis, and premature fusion of cranial sutures. We identified a missense mutation in the F*gf9* gene that is responsible for the *Eks* mutation. Through investigation of the pathogenic mechanisms of joint and suture synostosis in *Eks* mice, we discovered a key molecular mechanism that regulates FGF9 signaling in developing tissues. We found that the *Eks* mutation prevents homodimerization of the FGF9 protein and that monomeric FGF9 binds to heparin with a lower affinity than dimeric FGF9. These biochemical defects result in increased diffusion of the mutant FGF9 protein (FGF9^{Eks}) through developing tissues, leading to ectopic FGF9 signaling and repression of joint and suture development. Based on these observations, we proposed a new mechanism in which the range of FGF9 signaling in developing tissues is limited by its ability to homodimerize and its affinity for extracellular matrix heparan sulfate proteoglycans.

3. Endoh M, Endo TA, Endoh T, Fujimura Y, Ohara O, Toyada T, Otte AP, 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.*** (2008)Apr;135(8):1513-24.

4. 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, D. & Koseki, H. FGF9 monomer-dimer equilibrium regulates extracellular matrix affinity and tissue diffusion. ***Nat Genet*** (2009) 41(43):289-298.

Research Unit for Lymphocyte Cloning

Unit Leader

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Bone marrow non-hematopoietic cells form a microenvironmental niche major for hemopoiesis. One of the most interesting yet mysterious cell types in this niche are the mesenchymal stem cells (MSC), which have been investigated for 30 years and yet no definitive set of surface markers to unequivocally define these cells has been identified. There is also considerable variability depending on the *in vitro* culture condition and age and strain of the donor. Meanwhile these cells are believed to be at the origin of hematopoietic niche formation and may be involved in immunodeficiency and other disorders that occur with aging. Thus, there have been many ideas for the application of these cells in regenerative medicine.

It is believed that to obtain healthy functional cells for cell therapy one can derive them from the patient's own somatic cells, converting them to a pluripotent state by either nuclear transfer (ntESC) -or by "master genes" transduction, -the induced pluripotent stem (iPS) cells. We have recently established several lines of ntES and iPS cells from the B6 strain of mice and are currently studying the ways to differentiate them into functional MSC. We have also begun to culture human iPS cells and plan to differentiate them into human MSC. These ntES and iPS derived MSC (ntMSC and iMSC) will be compared with bone marrow derived MSC isolated from donors of different ages (Fig. 1). Our long term goal is to obtain patient-specific rejuvenated MSC for cell therapy of immuno-aging.

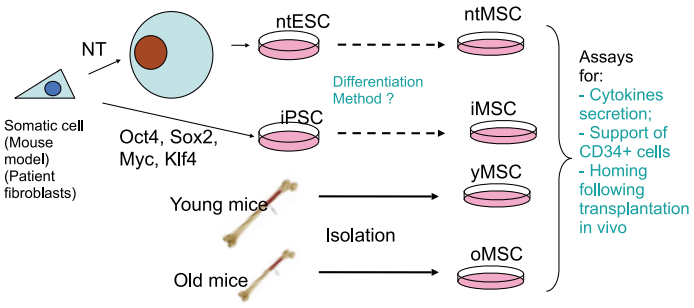

Figure 1 Scheme of the project for regeneration of the immunogenic niche of bone marrow using mesenchymal stem cells (MSC). A mouse model is under development but ultimately human cells would be used therapeutically.

Recent publications

1. A Rybouchkin, Y. Kato, Y Tsunoda Role of histone acetylation in reprogramming of somatic nucleus following nuclear transfer. ***Biol. Reprod.*** 2006, 74: 1083-1089.
2. Rybouchkin, A, Y. Kato, Y. Tsunoda. Enhanced reprogramming to facilitate production of somatic cell NT ES cell lines. ***Rejuvenation Research,*** 2005, N8, supple.1.

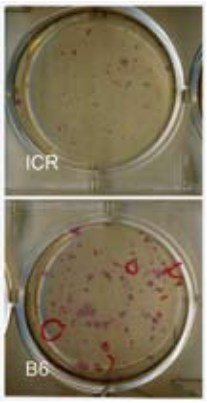

Figure 2 Alkaline phosphatase positive colonies obtained from ICR and B6 fibroblasts during induced pluripotent stem (iPS) cell production

Figure 3 Chimeras obtained after injection of B6 iPS cells (*black*) into blastocysts of BALB/c mice (*white*).

Mastering iPS derivation from mouse somatic cells.

The generation of iPS cells from mouse embryonic fibroblasts (MEFs) was reported recently by the Yamanaka group. However at that time only genetically modified MEFs from the 129/Ola strain were shown to be converted to iPS. We have produced iPS cells from unmodified MEFs of C57BL6 and ICR origin. We used retroviral transduction of MEFs with the 4 original reprogramming factors (*c-Myc, Klf4, Sox2 and Oct4*) as well as with only 3 reprogramming factors (*Klf4, Sox2 and Oct4*) without c-Myc or substitution of *c-Myc* by *Nanog*. All three combinations allowed us to produce ES-like colonies. Preliminary results show that the efficiency of iPS production is higher in C57BL6 compared to ICR mice as indicated by the number of AP-positive colonies obtained (Fig, 2). We selected several clones of both C57BL6 and ICR origin for primary characterization. The analysis of AP staining, SSEA1 and immunocytochemistry for c-Myc, Klf4, Sox2, Oct4 and known ES cell markers such as UTF1 and Esg1 revealed an expression pattern similar to ES cells, as did further characterization of 2 B6 iPS clones for the expression of pluripotency associated genes. Moreover, the cells showed high level of chimeras and chimerism after injection into blastocysts, again indicating pluripotency (Fig. 3). We are currently testing chimeras for germ line transmission properties, the most rigorous test of pluripotency.

Mastering culture of mouse MSC

It is generally accepted that it is rather difficult to isolate and culture mouse MSC. Moreover, there is still disagreement on the presence of particular surface markers and the best culture conditions and other variables. For standardization, we first obtained experience in isolation and differentiation of bone marrow BM-MSC from 129 and B6 strains of mice. We compared different culture media and serum sources, initial plating durations, and other factors. Our current optimized conditions allow us to obtain 33% of Sca-1$^+$CD44$^+$Flk1$^-$ cells after 3 weeks of culture. The cells were also CD34$^-$, CD106$^-$, CD45$^-$, CD11b$^-$, CD117$^-$. However, in contrast to some literature reports we were not able to expand cells for more then three passages. We next opted to try Mesencult medium and recovery of cells from the bones themselves, instead of bone marrow. This has given us more homogenous fibroblast-like cell populations which readily differentiated into adipocytes and osteoblasts. Still, long term passaging of the cells was rather difficult. Only when we adapted low oxygen culture conditions we were able to achieve robust growth. We have also found that 129 strain MSC proliferate much more efficiently than B6 MSC and were also recovered from bone at higher numbers.

Differentiation of mouse ntES and iPS cells into MSC *in vitro*

Precursors of MSC are identified by their migration from presomitic mesoderm to the bone marrow in the embryo and by expression of the PDGF-AA receptor (CD140a). Differentiation of mouse ES cells into MSC was recently reported for 129 mice. We applied this protocol to R1 (129 strain of mice) ES cells, transgenic B6 strain ESC (beta-actin-GFP) line, two B6-iPS lines and 4 B6-NKT-ntES cell lines. We found that after 9 days of differentiation, the CD140a$^+$ cell population was highest in 129 strain ES derived MSC (68%) and intermediate in all other B6 lines (38-58%) except for one B6-NKT-ntES line with only 13% of positive cells. We also found that B6 derived MSCs have a reduced proliferation rate (Fig. 4). Additionally after 14 days of culture R1 ES derived MSC still have 16% of CD140a$^+$CD44$^+$ cells while only 0.5-0.8% of these cells are present in B6 derived MSC lines. The observed differences should help us to identify better culture conditions for passaging differentiated cells.

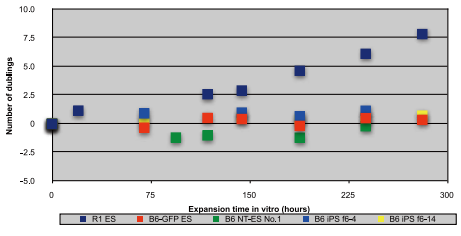

Figure 4 Difference in proliferation rate in presumptive MSC derived by differentiation of 129 and B6 strain ES, ntES and iPS.

Laboratory for Lymphocyte Development

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

Process of lineage commitment in hematopoiesis

The classical scheme of hematopoiesis proposes that the first branch point from the HSC produces a common myelo-erythroid progenitor (CMEP) and a common lymphoid progenitor (CLP) (Figure 1A). Based on our previous studies in fetal mice, however, we have proposed a different model, namely the “myeloid based model”, in which the first branch from HSC produces a CMEP and a common myelo-lymphoid progenitor (CMLP), which subsequently produces myeloid-B and myeloid-T progenitors (Figure 1B). In contrast to our studies in the fetus, studies of the bone marrow of adult mice have reported the existence of CLPs. In order to reconcile these disparate findings between fetus and adult, the concept has emerged that fetal and adult hematopoiesis differ, with the CLP stage existing only during adult hematopoiesis.

To critically address whether the classical or myeloid based models is operative during adult hematopoiesis, we set out to determine whether or not the CLP stage exists in the developmental pathway from the stem cell to T cells. We found that a substantial fraction of double negative 1 (DN1) cells, which have been shown to have almost completely shut-off B cell potential, can produce both macrophages and T cells when individually cultured with stromal cells (Wada et al, Nature, 2008). Derivation of macrophages from thymic T cell progenitors was further demonstrated *in vivo* in a physiological setting, i.e. after direct injection into the thymus and by tracing cell fate during steady state thymopoiesis in bone marrow chimeras.

Our findings strongly argue against the classical dichotomy model in which T cells are derived from CLP, and

Recent publications

1. 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)

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

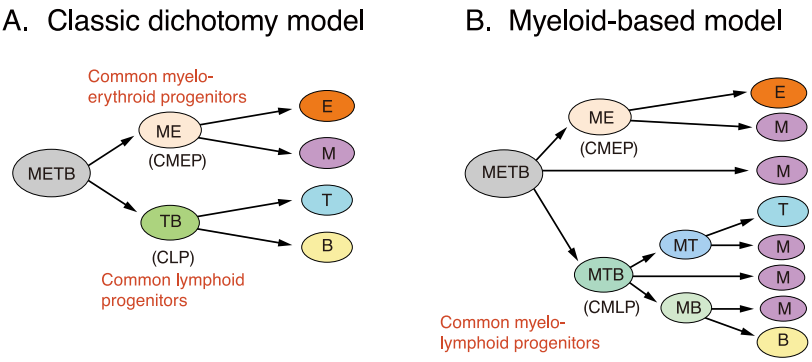

Figure 1 Models of lineage commitment during hematopoiesis.
A, Classic dichotomy model. Hematopoietic stem cells (HSC) diverge into CMEP and CLP. Note that CMEP are sometimes referred to as common myeloid progenitors (CMP). The findings reported by Weissman's group (Stanford) that CLP are present in adult BM have provided a support for this model.

B, Myeloid-based model. In this model, the first branch point generates CMEP and CMLP and the myeloid potential persists in the T and B cell branches even after these lineages have diverged. This model postulates that specification towards erythroid, T, and B cell lineages proceeds on the basis of a prototypical myeloid program (Kawamoto H., Trends in Immunol., 2006).

instead support the myeloid-based model for both adult and fetal hematopoiesis. The concepts based on this model will help clarify the developmental programs for production of hematopoietic cells, and may lead to a better understanding of the immune system and lymphoid leukemia.

In vitro expansion of hematopoietic progenitors

We are currently interested in approaches by which hematopoietic cells, including multipotent stem cells and functional immune cells, can be generated *in vitro*. We have recently succeeded in the *in vitro* expansion of murine multipotent hematopoietic progenitors (unpublished, but applied for patent). Although a variety of intrinsic and extrinsic factors have been tested for the development of such a method, it has been still difficult to amplify HSCs *ex vivo*. We have previously reported in collaboration with Dr. Murre (UCSD) that B cell development in the bone marrow of E2A-deficient mice is blocked at the pre-pro B cell stage, and that these pre-pro B cells exhibit multiple differentiation potentials both *in vitro* and *in vivo* (Ikawa et al. Immunity, 2004). Therefore, we reasoned that multipotent progenitors could be expanded if E2A activity could be suppressed at the early pre-pro B cell stage (figure 2A). Thus, we over-expressed ID3, one of the Id proteins which are natural inhibitors of E2A, in normal murine HSCs and cultured the transduced cells on stromal cells (Figure 2B). These cells expanded extensively and maintained multilineage differentiation potential, while retaining the pre-pro B cell phenotype (Figure 2C). Furthermore, these cells reconstituted multilineage hematopoietic cells in irradiated mice upon i.v. transfer. We call these cells iHSP (induced hematopoietic stem/progenitor) cells. Currently, we are trying to apply this method to human cells. These *in vitro* expanded hematopietic stem/progenitors have many potential uses, such as a source of stem cells in BM transplantation, and a source of progenitor cells to make mature blood cells for cell therapy.

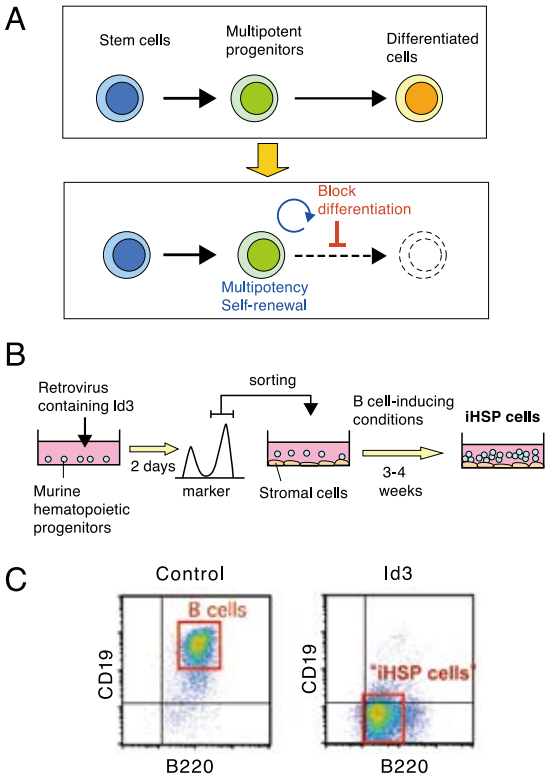

Figure 2 *In vitro* expansion of hematopoietic stem/progenitor cells

A. Rationale for induced expansion of progenitors. The failure of previous studies in expanding stem/progenitor cells is due to the fact that hematopoietic cells have intrinsic tendency to differentiate even in the presence of any cytokines. We reasoned that it would be important to suppress differentiation in order to induce self-renewal of progenitors.

B. We over-expressed one of Id3, a natural inhibitors of E2A, in normal murine HSCs, and cultured the transduced cells on TSt-4 stromal cells. These cells (iHSP cells) expanded extensively and maintained multilineage differentiation potential.

C. A flowcytometric profile of iHSP cells.

3. Ikawa, T., Kawamoto, H., Golodrath A.W., Murre C. E protein and Notch signaling cooperate to promote T cell lineage specification and commitment. ***J. Exp. Med.*** 15:1329-1342, (2006)

4. Kawamoto, H. Close developmental relationship between lymphoid and myeloid lineages. *Trends in* ***Immunology.*** 27: 169-175, (2006)

5. Masuda K., Kubagawa H., Ikawa T., Chen C.C., Kakugawa K., Hattori M., Kageyama R., Cooper M.D., Minato N., Katsura Y., Kawamoto H. Prethymic T-cell development defined by the expression of paired immunoglobulin-like receptors. ***EMBO J.*** 24, 4052-4060 (2005)

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Laboratory for Transcriptional Regulation

One of major questions in developmental biology is how the fate of progenitor cells differentiating into opposing lineages is determined. Even as we learn more about cell fate determination, other questions arise, namely how genetic programming after lineage specification functions to establish cell identity and then how cell identity, once established, is maintained in differentiated cells. Research in my laboratory is directed toward understanding (a) how progenitor cells sense external or intrinsic stimuli and turn on a genetic program for regulating lineage choice and (b) how a specific gene expression pattern is established during 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 transcriptional regulation of the lineage decision of $CD4^+CD8^+$ double-positive (DP) thymocytes differentiating into either $CD4^+$ helper- or $CD8^+$ cytotoxic-lineage T cells. Our previous studies had shown that Runx transcription factor complexes play an essential role in *Cd4* gene silencing by activating the intronic silencer in the *Cd4* gene through their direct binding. Subsequent studies during the past three years have characterized the roles of Runx complexes in the thymus and have revealed a critical regulatory pathway for cytotoxic T cell development. We identified a novel Runx-dependent transcriptional silencer in the *Ztb7b/ThPOK* gene, which encodes a central transcription factor for helper T cell development, ThPOK. We are expanding our findings to understand how expression of the *ThPOK* gene is regulated and how Runx complexes are involved in immune system development.

A transcriptional silencer regulating helper versus cytotoxic lineage choice

Two alternative T cell subsets, $CD4^+$ helper- and $CD8^+$ cytotoxic-lineage cells, are differentiated from common progenitor $CD4^+CD8^+$ DP thymocytes. Cells expressing class II-restricted TCR differentiate primarily into the helper lineage and cease to express CD8, whereas cells expressing class I-restricted TCR differentiate into the cytotoxic linage and silence CD4. However, molecular pathways in the cell nucleus that regulate cell fate determination of $CD4^+CD8^+$ DP thymocyte remain poorly understood. Current genetic approaches have shown that expression of the ThPOK transcription factor is both essential and sufficient to induce helper-lineage

Recent publications

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

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

fate in post-selection thymocytes regardless of MHC specificity of their TCRs. It is therefore pivotal to elucidate the mechanisms that regulate helper-lineage specific expression of the *ThPOK* gene. By using conditional inactivation of *Runx* gene family members, we found that loss of Runx complex function in $CD4^+CD8^+$ DP thymocytes results in de-repression of *ThPOK* and in redirected differentiation of MHC class I-specific thymocytes into $CD4^+CD8^-$ helper-like T cells. Furthermore we identified a novel transcriptional silencer within the *ThPOK* gene, termed the *ThPOK* silencer, whose activity requires binding of Runx complexes. Removal of the *ThPOK* silencer from the *ThPOK* gene resulted in loss of $CD8^+$ T cells, confirming its essential role for development of $CD8^+$ T cells. These results thus advanced our understanding that the developmental pathway toward the helper-lineage is actively repressed by Runx complex-dependent silencer activity (Fig. 1).

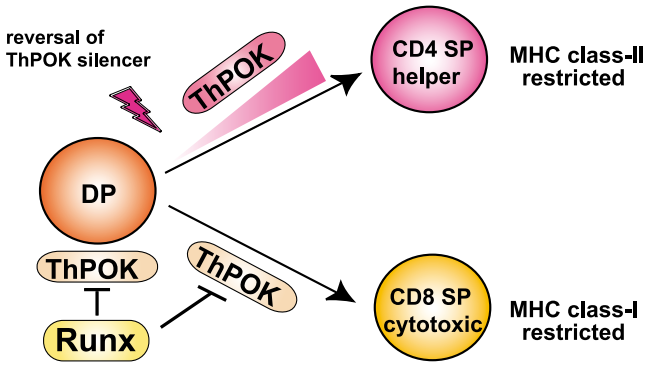


Figure 1 **Repression of ThPOK by the Runx-dependent *ThPOK* silencer.** The *ThPOK* gene actively repressed by the Runx-dependent *ThPOK* silencer in DP thymocytes and in thymocytes developing toward the CD8 lineage. This repression is essential for development of $CD8^+$ cytotoxic T cells. By contrast, development of $CD4^+$ helper T cell requires reversal of *ThPOK* silencer activity for induction of ThPOK expression.

Auto-regulatory loop for ThPOK upregulation by antagonism to distinct silencers

Following initial specification to a particular lineage, stable cell identity is established during the commitment process by eliminating differentiation potential toward other lineages. It has been shown that the intermediate $CD4^+CD8^{int}$ subset retains bi-potential for both helper- and cytotoxic-lineages. By using a GFP "knock-in" ThPOK allele, we detected cytotoxic potential in ThPOK expressing $CD4^+CD8^{int}$ cells. Furthermore, "knock-down" of ThPOK in helper-lineage specified cells by removal of the proximal enhancer causes *trans*-differentiation of those cells into the cytotoxic-lineage. Thus ThPOK acts at a later developmental stage as a commitment factor to erase differentiation potential to the cytotoxic-lineage. Furthermore, our genetic and molecular approaches indicate that direct antagonism by ThPOK of the *Cd4* and *ThPOK* silencers generates two regulatory loops, which are essential to amplify and stabilize ThPOK expression. These results revealed a novel auto-regulatory mechanism in which antagonistic function of a single transcription factor against distinct silencers is central.

Thus our studies have clarified a role of Runx complexes in transcription factor networks regulating lineage decision in the thymus. Further studies on the regulatory pathways that determine lineage specificity of the *ThPOK* silencer should shed light on how signals initiated by external stimuli are converted into genetic programs in the cell nucleus.

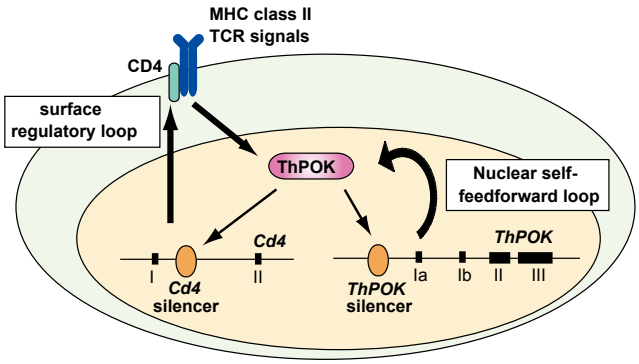
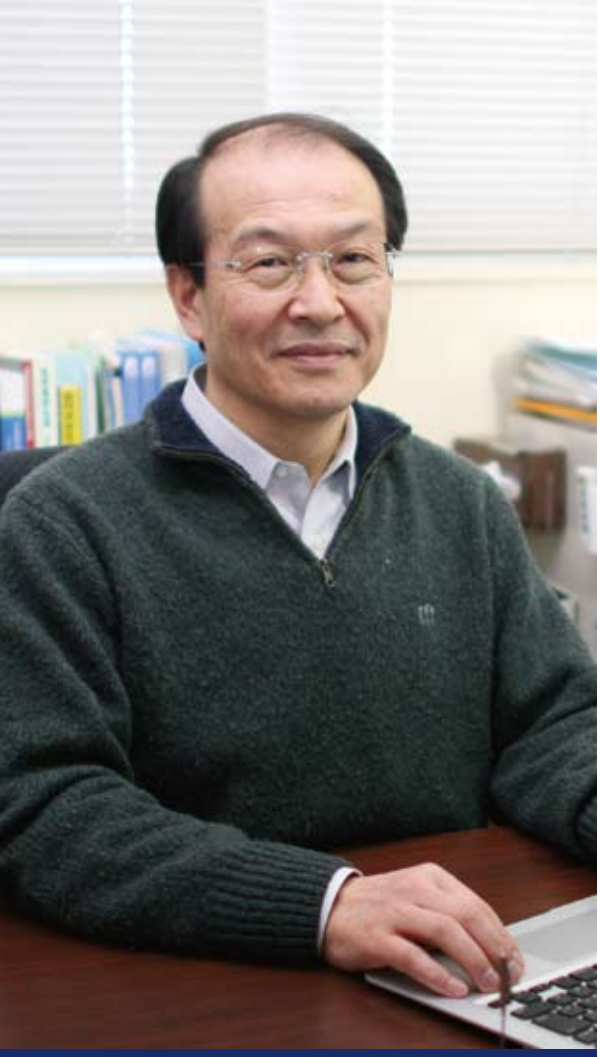


Figure 2 **Auto-regulatory loops for amplifying and stabilizing ThPOK expression.** After initial induction of ThPOK by TCR signal-mediated reversal of *ThPOK* silencer activity, ThPOK is targeted to the *Cd4* silencer and antagonizes its activity, thereby generating a surface regulatory loop that permits CD4 expression in class II-restricted cells. In parallel, ThPOK is targeted to the silencer at its own locus and antagonizes its activity, thus generating a nuclear self-feedforward loop that amplifies and stabilizes ThPOK expression

3. 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)
4. Maekawa Y., Minato Y., Ishifune C., Kurihara T., Kitamura A., Kojima H., Yagita H., Satake-Yanagimoto M., Saito T., Taniuchi I., Chiba S., Sone S., Yasutomo K. Notch 2 integrates signaling by the transcription factors RBP-J and CREB1 to promote T cell cytotoxicity. ***Nat. Immunol.*** 9, 1140-47 (2008)
5. Collins A., Littman D.R., and Taniuchi I. RUNX proteins in transcription factor networks that regulate T-cell lineage choice. ***Nat. Rev. Immunol.*** 9, 106-115 (2009)



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Yasutaka Wakabayashi (Leica)

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Mayuko Matsuda

The long-term goal of the Cell Signaling group is to determine the molecular mechanisms of activation and homeostasis of T cells in order to be able to modulate T cell activation/function in immunological disorders. Therefore, 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, the group has been using real-time imaging analysis, which has allowed us to gain novel insight into the dynamic regulation of the assembly of the TCR signalsome and the immunological synapse, as well as related downstream signaling pathways, upon antigen recognition. These downstream events include regulation of NF-κB activation and the cytoskeleton, as well as co-stimulation signals. The group also analyzes regulation at later phases of T cell activation for cell migration and establishment of peripheral antigen-specific effector functions. In addition to the TCR, the group has also been analyzing signal regulation through various ITAM-containing receptors on cells of the innate immune system, including macrophages, DCs, and mast cells, to understand mechanisms of regulation to generate functional diversity through multi-task receptor signaling.

Dynamic regulation of T cell activation and co-stimulation

We have studied the dynamic movement of signaling molecules in the process of the formation of the immunological synapse and T cell activation upon antigen recognition at the single-cell level. Using a novel single-molecule imaging technique developed by the RCAI Single Molecule Imaging Unit, we could visualize the behavior of single and clustered molecules during the dynamic process of T cell activation.

We visualized the dynamics of the TCR and associated kinase and adaptor molecules in the TCR signaling complex, and analyzed the entire process of synapse formation. This analysis provided the new insight that microclusters containing TCR, kinases and adaptors are generated upon antigen recognition and function as the structures responsible for the initial and sustained activation of T cells. Contrary to expectations from then-accepted models, the classical c-SMAC is not the site for signaling. The discovery of microclusters as the fundamental units of antigen recognition and activation lead to a major revision in current models on T cell activation, including the regulation of co-stimulation signals. Thus, the

Recent publications

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

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

dynamic regulation of the co-stimulation signals has been similarly analyzed. The major co-stimulation receptor CD28 was also found to be recruited to TCR microclusters at the initiation phase, but later it accumulated in the outer region of the cSMAC. CD28 recruits PKCθ to the microclusters for initial stimulation but to the outer region of the cSMAC at later timepoints, possibly for sustained co-stimulation. We observed the physical and functional association between CD28 and PKCθ. Thus, TCR signals and CD28-mediated co-stimulation signals are dynamically regulated by spatially distinct signalsomes and the c-SMAC contributes to activation signaling. We have been extending these analyses to other co-stimulatory receptors.

ITAM-mediated signaling in innate immunity

The group has analyzed activation signals through ITAM-containing adaptor molecules, such as CD3s and FcRγ, which are associated with receptors on T cells and myeloid cells, since ITAM-mediated signal transduction is a prototypic lymphoid-specific signaling mechanism. We found that a C-type lectin Mincle is a receptor associating with FcRγ through its charged amino acid within the transmembrane region. Mincle is predominantly expressed by macrophages and its expression is induced by various stimuli and by stress. We found that Mincle-expressing cells are activated by dead cells, which was blocked by a Mincle mAb. Subsequently, a component of small nuclear ribonucleoprotein (snRNP) SAP130 was isolated as a Mincle ligand. SAP130 was released from necrotic cells and stimulate macrophages to produce cytokines and chemokines. Whether Mincle is involved in inflammatory responses *in vivo* was analyzed by irradiation-induced thymocyte cell death. We found irradiation-induced neutrophil recruitment was blocked by a Mincle mAb. These results suggest that Mincle is a receptor that senses non-homeotic cell death as an emergency and induces inflammatory responses such as production of inflammatory cytokines/chemokines and recruitment of neutrophils.

We also investigated whether Mincle recognizes exogenous pathogens. Indeed we found that Mincle specifically recognizes a fungal species, *Malassezzia*, which is known to induce skin diseases and atopic dermatitis. Thus, Mincle may function as a dual recognition receptor for both damaged self and pathogenic non-self as emergencies of the body.

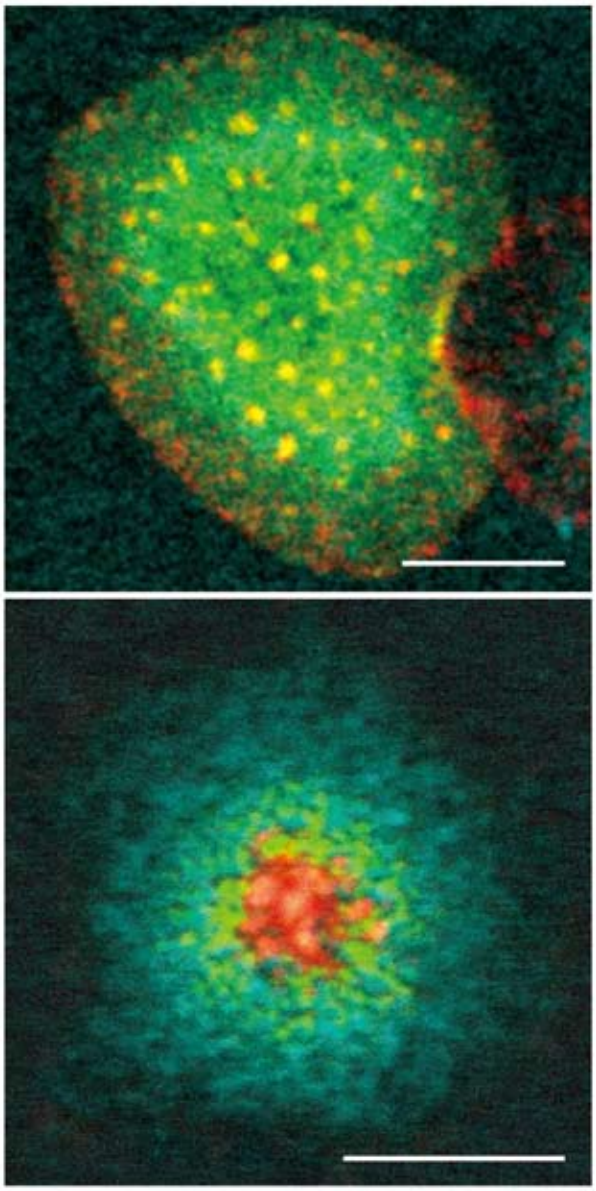

Figure **Imaging of T cell co-stimulation : spatial temporal regulation of CD28-mediated co-stimulation signals in T cells**. In the presence of co-stimulation signals induced by the interaction between CD28 and its ligand CD80/86, CD28-PKCθ is initially colocalized within TCR-microclusters (*upper panel*) and later accumulates into the outer region of cSMAC in an unique annular form (*lower panel*) to contribute to sustained T cell activation.

3. Hara H., Ishihara C., Takeuchi A., Imanishi T., Xue L., Morris SW., Inui M., Takai T., Shibuya A., Saijo S., Iwakura Y., Ohno N., Koseki H., Yoshida H., Penninger JM.,+ Saito T. The adaptor protein CARD9 is essential for activation of myeloid cells through ITAM-associated and Toll-like receptors. ***Nat. Immunol.*** 8, 619-629 (2007)
4. Suzuki N., Suzuki S., Millar D.G., Unno M., Hara H., Calzascia T., Yamasaki S., Yokosuka T., Chen N-J., Elford A. R., Suzuki J-I., Takeuchi A., Mirtsos C., Bouchard D., Ohashi P. S., Yeh W-C., Saito T. A critical role for the innate immune signaling molecule IRAK-4 in T cell activation. ***Science*** 311, 1927-1932 (2006)
5. Yamasaki S., Ishikawa E., Sakuma M., Ogata K., Sakata-Sogawa K., Hiroshima M., Wiest D.L., Tokunaga M., Saito T. Mechanistic basis of pre-T cell receptor-mediated autonomous signaling critical for thymocyte development. ***Nat Immunol.*** 7, 67-75 (2006)

Research Unit for Single Molecule Imaging

Unit Leader

Kumiko Sakata-Sogawa, Ph.D.

Technical Staff : **Yoshiyuki Sasaki**

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

Single molecule microscopy

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

Recent publications

1. 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)
2. Tokunaga, M., Imamoto, N., Sakata-Sogawa, K.: Highly inclined thin illumination enables clear single-molecule imaging in cells. ***Nat Methods.*** 5, 159-161 (2008)

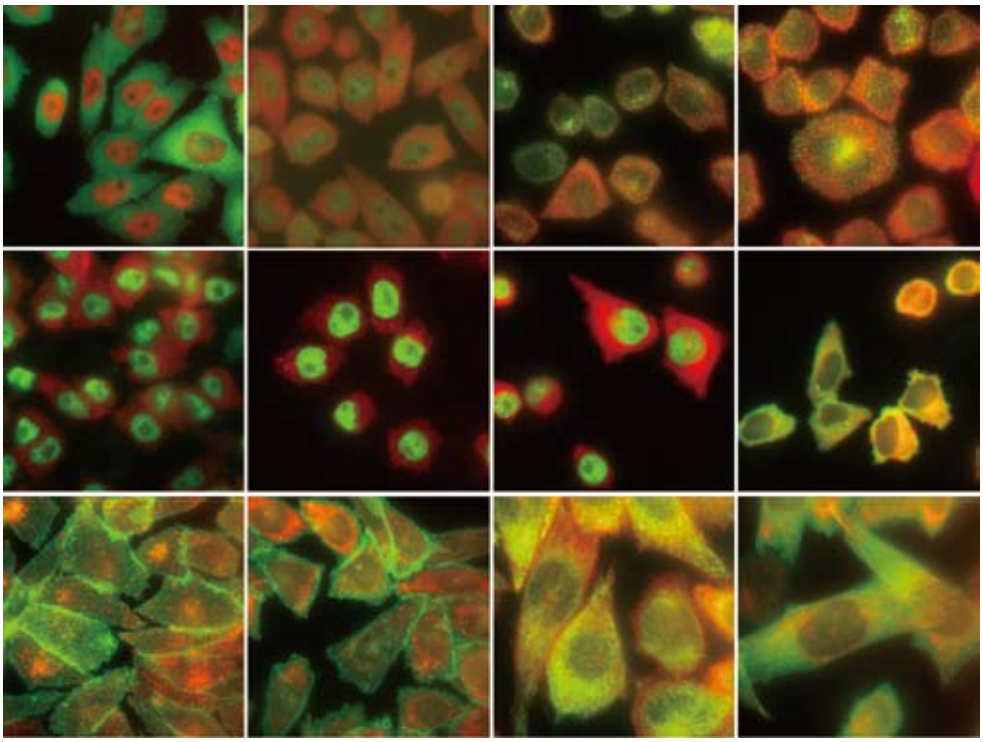

Figure 1 Dual color imaging for protein interactions in CHO cell lines. EGFP-and mCherry fusion proteins of two different signaling molecules or transcription factors are stably expressed at low and homogenous level.

face to the interior of the cell. To this end, we have developed various microscopy techniques. For example, the computer-controlled z-scanning system enabled us to build a 3D image to study distribution of Nuclear Pore Complexes on the nuclear envelope. For single cell/ single molecule imaging and quantification, several research collaborations including external collaborations are ongoing.

Dual color imaging of cell signaling

Dual color single molecule imaging is required for quantitative analysis of the interactions of signaling proteins. To this end, we have established methods to obtain cell lines expressing two fluorescence tagged proteins at homogenous and low level. Figure 1 shows examples of dual color expressing CHO cell lines. We have been able to control expression levels by selecting suitable promoter sequences. We also imaged the interaction between microtubule plus-end-binding protein EB1 and the ER membrane resident Ca^{2+} sensor protein STIM1 (Fig. 2). Both fusion proteins were stably expressed and observed in CHO cells. Real time imaging showed STIM1 tracking at growing microtubule ends and thus its involvement in remodeling of the ER. (Ref : Grigoriev, I. et al. *Curr.Biol.* 19, 177-182, 2008)

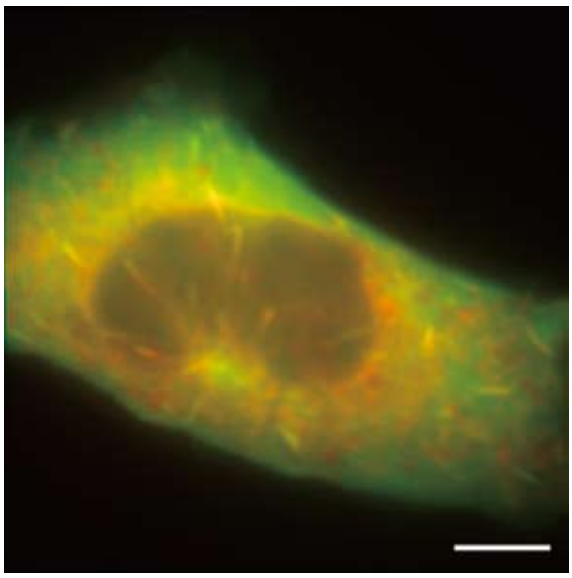

Figure 2 Imaging the interaction of STIM1 and EB1 by HILO microscopy. Scale bar: 5 µm.

3. Yamasaki, S., Sakata-Sogawa, K., Hasegawa, A., Suzuki, T., Kabu, K., Sato, E., Kurosaki, T., Yamashita, S., Tokunaga, M., Nishida, K., Hirano, T.: Zinc is a novel second messenger. ***J. Cell Biol. 177***, 637-645 (2007)
4. Miletic A. V., Sakata-Sogawa K., Hiroshima M., Hamann M. J., Gomez T. S., Ota N., Kloeppel T., Kanagawa O., Tokunaga M., Billadeau D. D., Swat W. Vav1 Acidic Region Tyrosine 174 Is Required for the Formation of T Cell Receptor-induced Microclusters and Is Essential in T Cell Development and Activation. ***J Biol Chem.*** **281**, 38257-38265 (2006)
5. Yamasaki S., Ishikawa, E., Sakuma, M., Ogata, K., Sakata-Sogawa K., Hiroshima, M., Wiest, D. L., Tokunaga M., Saito, T. Mechanistic basis of pre-T cell receptor-mediated autonomous signaling critical for thymocyte development. ***Nature Immunol.*** **7**, 67-75 (2006)

Laboratory for Lymphocyte Differentiation

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Signals propagated through the pre B cell (preBCR) and B cell (BCR) receptors are crucial for the development of B lymphocytes and their subsequent antigen-triggered differentiation into memory B cells and antibody secreting plasma cells. The outcomes of these signaling events, for example proliferation, differentiation, or apoptosis, are dependent on the developmental stage of the cell and the quality of the signal. The latter is dictated by multiple factors including which co-receptors, e.g. CD19, are engaged during preBCR/BCR signaling and which downstream effector molecules are recruited to the signaling complexes. Our laboratory has focused on understanding the molecular composition of these signaling complexes and the mechanisms of signaling pathway crosstalk that lead to crucial cell fate decisions during B lymphocyte differentiation. We have also applied insights gained from our studies of B cells to another important immune regulatory cell, the T cell.

Function of Erk kinases in B lymphocyte development

B cell maturation can be divided into a series of developmental stages that are characterized by the differential expression of a variety of marker proteins and changes in the rearrangement status of the immunoglobulin heavy (Igh) and light (Igl) chain genes. *Igh* gene rearrangement is initiated during pro-B cell stage, and the resulting product in association with λ5 and VpreB forms a surface-expressed pre-B cell receptor (pre-BCR). Correct assembly of the pre-BCR triggers a signaling cascade that induces proliferation, down-regulation of the pre-BCR complex, and differentiation into small pre-B cells. Subsequently, productive gene rearrangement at the *Igl* loci takes place, leading to synthesis of IgM on the surface of immature B cells. Although certain aspects of proximal pre-BCR signaling have been studied, the intermediate signal transducers and the distal transcriptional modulators are poorly characterized. We found that deletion of both Erk1 and Erk2 kinases was associated with defective pre-BCR-mediated cell expansion as well as a block in the transition of pro-B to pre-B cells. Phosphorylation of transcription factors Elk1 and CREB is mediated by Erk, and a dominant-negative mutation in the Erk-mediated phosphorylation sites of Elk1 or CREB suppresses pre-BCR-mediated cell expansion as well as expression of genes including *Myc*, which is involved in cell-cycle progression. Together, our

Recent publications

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

2. Yasuda, T., Sanjo, H., Pagès, G., Kawano, Y., Karasuyama, H., Pouysségur, J., Ogata, M., and Kurosaki, T. Erk kinases link pre-B cell receptor signaling to transcriptional events required for early B cell expansion. ***Immunity*** 28, 499-508 (2008)

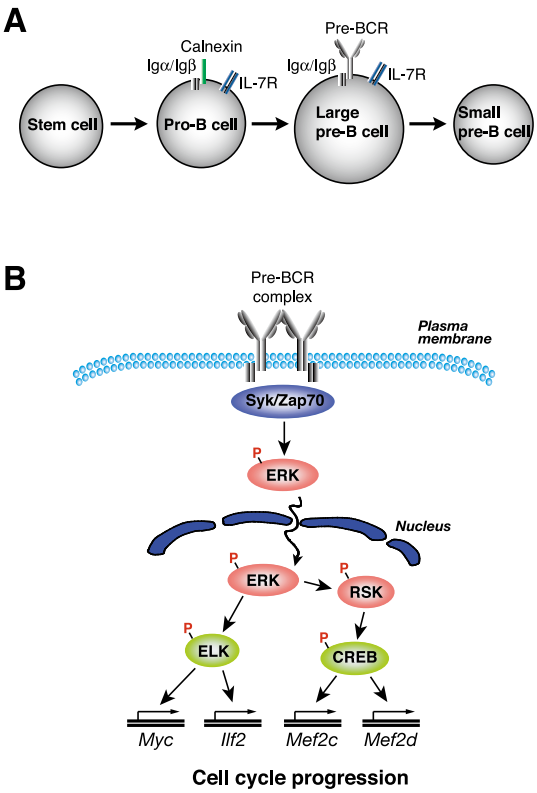
results identify a crucial role for Erk kinases in regulating B cell development by initiating transcriptional regulatory networks and thereby pre-BCR-mediated cell expansion.

Function of PLCγ2 in T-dependent humoral immune responses

Humoral memory is characterized by recall immune responses, which are more rapid than the primary response, and by the production of higher serum titers of antigen-specific antibodies, mostly of the IgG isotype. The prevailing view is that antigen-specific B cells are maintained as a pool of memory B cells after clonal expansion during the primary immune responses. Most memory B cells have been thought to originate from the germinal center (GC) reaction. In the GC, the combined processes of somatic hypermutation and selection based on the affinity of the BCR for the antigen, are responsible for the generation of high-affinity antibody variants which ultimately differentiate into long-lived plasma cells or long-lived memory B cells. In the GC reaction, *de novo* generated antigen-specific memory B cells are thought to acquire intrinsically different traits from their naïve predecessors, accounting for more rapid and heightened secondary responses. Thus, understanding the mechanisms by which memory B cells are generated and are maintained as well as intrinsic functional differences existing between naïve and memory B cells are of fundamental interest to reveal the basis of immunological memory. To ask whether the establishment and maintenance of memory B cells require BCR signaling, we have focused on the function of PLCγ2, as this enzyme has been established as an important signaling component of the BCR signaling pathway. We show that PLCγ2 is required for the efficient generation and maintenance of memory B cells, probably through the delivery of a pro-survival signal.

Involvement of MEKK3 in antigen receptor-mediated NF-κB activation

Some MAP3Ks are known to control the response of innate immune cells to Toll-like receptor (TLR) stimulation and to inflammatory cytokines by regulating IκB kinase (IKK) and JNK activity. For instance, TAK1, MEKK2, and MEKK3 have been reported to activate IKK and JNK following TNFα, IL-1, LPS, and TRL8 stimulation. In contrast to such an established role of MEKK2 and MEKK3 in the innate immune responses, their roles in adaptive immunity are less clear and somewhat controversial. We show that deletion of MEKK3 in a T cell-specific manner results in reduced development of thymocytes. Furthermore, following T cell receptor (TCR) stimulation, MEKK3 was found to regulate IKK, leading to NF-κB activation. Together, our results provide genetic evidence that MEKK3 plays a crucial role in adaptive as well as innate immunity.



Figure

A. At the end of the pro-B cell stage of B cell development, expression of a signaling-competent pre-BCR on transitional pre-B cells represents a key checkpoint. Constitutive signaling by the pre-BCR promotes cell survival, terminates VDJ recombination at the *Igh* locus, induces large dividing pre-B cells, and synergizes with IL-7 to drive clonal expansion. After several rounds of cell division, the cells further differentiate to the small quiescent pre-B cell stage.

B. A proposed model that pre-BCR mediates cell expansion. The pre-BCR complex transduces a signal for activation of ERK1 and ERK2 through Syk and Zap70 tyrosine kinases. Then, activated ERK translocates to the nucleus and phosphorylates transcription factors such as ELK and CREB. The phosphorylated ELK preferentially activates promoters of the *Myc* and *Ilf2* genes, and the phosphorylated CREB activates promoters of the *Mef2c* and *Mef2d* genes. ERK activates the RSK serine/threonine kinase, thereby indirectly phosphorylating CREB.

3. Weber, M., Treanor, B., Depoil, D., Shinohara, H., Harwood, N.E., Hikida, M., Kurosaki, T., and Batista, F.D. Phospholipase C-γ2 and Vav cooperate within signaling microclusters to propagate B cell spreading in response to membrane-bound antigen. ***J. Exp. Med.*** 205, 853-868 (2008)

4. Kurosaki, T. Paradox of B cell-targeted therapies. ***J. Clin. Invest.*** 118, 3260-3263 (2008)

5. Yasuda, T., and Kurosaki, T. Regulation of lymphocyte fate by Ras/ERK signals. ***Cell Cycle*** 7, 3634-3640 (2008)

Unit Leader
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Yoshikazu Ando

Administrative staff : **Mari Kurosaki**

Research Unit for Immunodynamics

This research unit was established in April 2008, and its focus is to understand the mechanisms regulating dynamic cellular interactions in the tissues that shape immune responses. For this purpose we use real time imaging, in particular multi-photon microscopy, to analyze *in vivo* cellular migration and interactions. Multi-photon microscopy, which was introduced only recently to 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 (Figure). Utilizing this technique, along with other methods, we are currently working on two projects. The first one is to understand the regulation of B cell – helper T cell interactions that determine the fate of B cells in the antibody response. Antigen-engaged B cells undergo selection and multidirectional differentiation to achieve both immediate and long-term humoral immunity. T cell help plays a pivotal role in most of these processes, but it is not known how it differentially promotes development of the germinal center, antibody-forming cells, and memory B cells (Figure). We tackle this problem by tracking individual B cell interactions with helper T cells and their subsequent differentiation. This lab also studies the immunomodulatory function of cancer cells that metastasize to the lymph node. We aim to understand mechanisms of infiltration of cancer cells into the tumor-draining lymph node, and to reveal mechanisms of their interactions with lymph node cells leading to suppression of anti-cancer immune responses initiated in the lymph node.

Physiological function and regulation of synapse formation between germinal center B cells and helper T cells

Successful humoral immune responses require appropriate proliferation, selection, and differentiation of antigen-engaged B cells, events that are regulated by a number of other cells in the secondary lymphoid organs. The goal of this research is to understand the mechanisms underlying cellular interactions that control the fate of antigen-engaged B cells during antibody responses. Our previous studies suggest that regulation of synapse formation between antigen-engaged B cells and helper T cells plays an important role in cell selection during the germinal center reaction. Therefore, it is important to elucidate what regulates this synapse for-

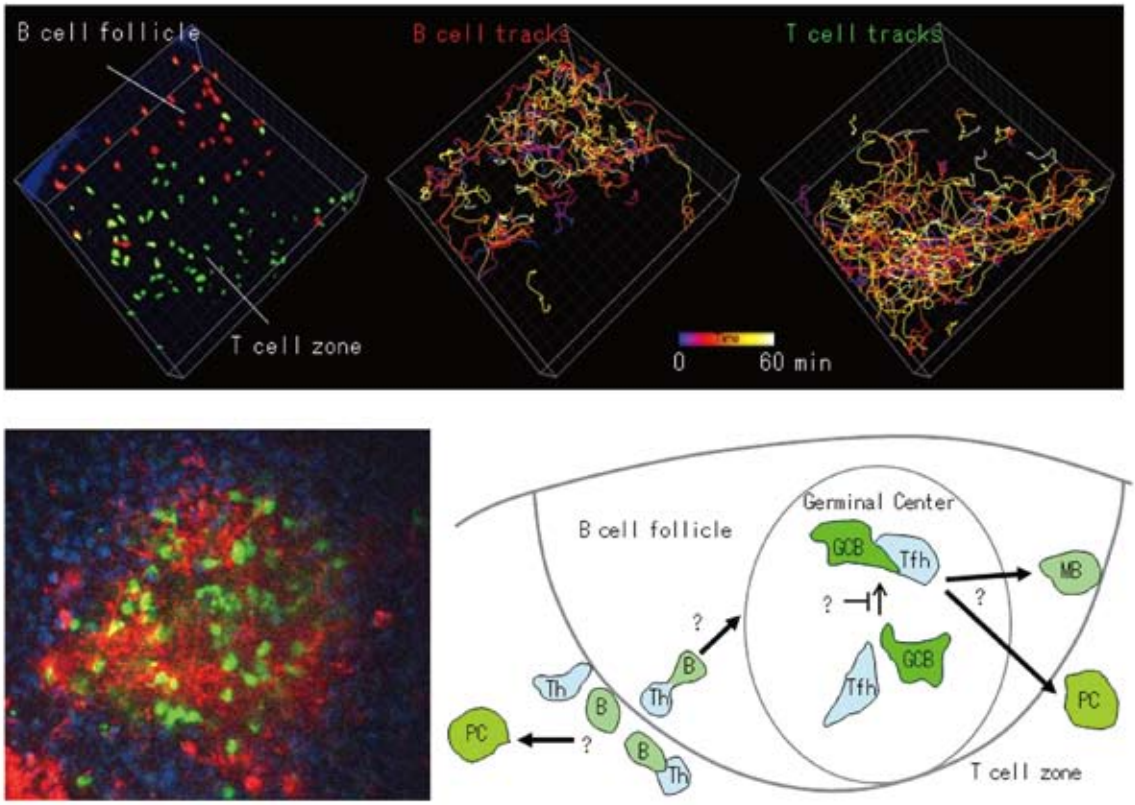

Figure **Real time multi-photon imaging of lymphoid organs to study the mechanism of B cell differentiation in the antibody response.**
Migration of B and T cells in the lymph node were tracked in a 300 (x) x 300 (y) x 100 (z) μm imaging stack for 60 min (top). Multi-photon imaging of the germinal center (bottom left, 290 (x) x 240 (y) μm, 24 μm z-projection, green: germinal center B cells, red: follicular dendritic cells, blue: mantle zone B cells). B cell –T cell interactions and B cell differentiation within the secondary lymphoid organs (bottom right, B: antigen-engaged B cell, Th: helper T cell, PC: plasma cell, GCB: germinal center B cell, MB: memory B cell).

mation in the germinal center, what this synapse formation outputs in regard to the B cell fate, and what molecular signals at the synapse are involved in the fate commitment. We are particularly interested in whether affinity-based competition for the antigen is involved in the regulation of synapse formation, and whether synapse formation with helper T cells promotes terminal differentiation of GC B cells.

Mechanisms of melanoma infiltration and immunomodulation in the metastatic lymph node

Migration of cancer cells in metastatic sites has been scarcely characterized. By *in vivo* two-photon microscopy, we have visualized cancer cell migration in the lymph node using the melanoma metastasis model. In addition to forming secondary tumors in the subcapsular sinus, melanoma cells vigorously infiltrated into the lymph node parenchyma, and showed distinct migratory behavior in the different compartments of early metastatic lymph nodes. The molecular mechanisms of their migration in the lymph node and their interactions with lymph node immune cells and stromal cells are currently being analyzed.

Unit Leader
Makio Tokunaga, Ph.D.

Research Scientists : **Akihiro Fukagawa**

Technical Staff : **Kazumi Shinkura**

Research Unit for Molecular Systems Immunology

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

Single Molecule Imaging and Molecular Quantification in Cells

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

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

To evaluate the HILO microscopy technique, we reconstructed three-dimensional images of the nuclear pore complexes (NPCs) in cells from z-scanned serial images (Fig. 1b). Clear point-like images of NPCs were obtained without the need for deconvolution to remove out-of-focus haze. The fluorescence intensity of the point images of NPCs corresponded well to the theory of image formation. Further, there was much lower photobleaching than in conventional confo-

Recent publications

1. Fukagawa, A., Hiroshima, M., Sakane, I., Tokunaga, M.: Direct Observation of Multiple and Stochastic Transition States by a Feedback-controlled Single-molecule Force Measurement. ***Anal. Sci.*** **25** (1), 5-7 (2009).
2. Tokunaga, M., Imamoto, N., Sakata-Sogawa, K.: Highly inclined thin illumination enables clear single-molecule imaging in cells. ***Nature Methods*** **5** (2), 159-161 (2008).

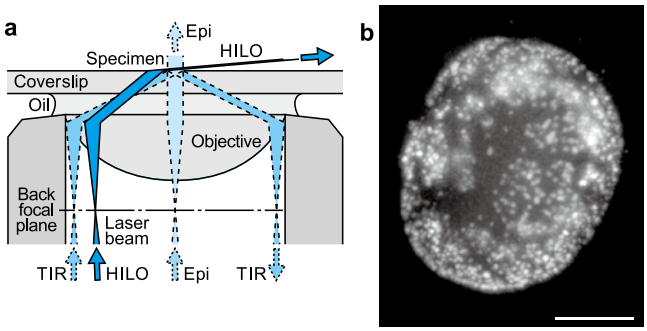

Figure 1 Molecular imaging enables one to visualize and quantify molecular dynamics, interactions, and kinetics in cells for molecular systems biology. (a) HILO microscopy for molecular imaging in cells. Illumination by a highly-inclined and thin beam increases image intensity and decreases background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

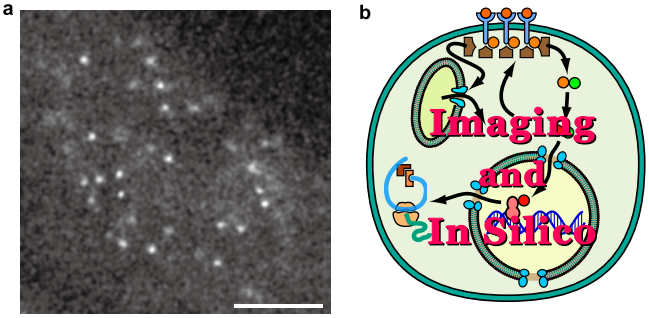

Figure 2 The combination of single molecule quantification and "in silico" modeling opens new approaches for developing molecular systems biology. (a) Single molecule images of the Sp1 transcription factor in a living cell. Molecular dynamics and interactions can be quantified by image analysis using these single molecule images. Bar, 5 μm. (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.

cal microscopy because of the lower intensity and non-focused nature of illumination.

We examined the thickness of the illumination beam and obtained 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 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 theoretical predictions. In contrast, the background intensity is substantially decreased by illumination inclination. As the background is composed of out-of-focus images, the decrease is explained by the reduction in 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. As a result, HILO illumination microscopy significantly increased the signal:background ratio up to 7.6-fold.

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

"*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 experimental results of single-molecule analysis and the molecular kinetic features in cells.

We are now expanding our 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 different parameters have a huge number of degrees of freedom.

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

3. 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).

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

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

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Kazunori Kadokura
Tamotsu Kato
Masumi Shinbori
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Administrative Staff : **Yuuki Yamada**

The mucosal epithelium that lines the inner surfaces of the body, especially within the intestine, is exposed to various antigens including food-borne macromolecules and microorganisms, and efficient uptake of these antigens is vital for maintaining appropriate immune responses. 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 itself contains a specialized subset of epithelial cells, the M cells, which are thought to play a pivotal role in immune surveillance by delivering luminal microorganisms to the underlying lymphoid cells via transcytosis. Despite their significance, the study of M cells has remained in its infancy until recently, mainly because the low frequency of M cells and the lack of specific surface markers have made it difficult to purify the M cells for molecular/biochemical analyses. Accordingly, one of the primary aims of our laboratory is to understand the mechanisms that underlie the differentiation and function of FAE and M cells. Our research team is also investigating the interaction of commensal microbiota with the intestinal epithelium and its influence on the mucosal and systemic immune systems. 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 GP-2 as Peyer's patch M cell-specific molecule

Although M cells are believed to play an important role in mucosal immunity, the molecular mechanisms of M-cell function and differentiation remain unclear. A recent study reported that cells with M-cell-like phenotype also exist in villous epithelium, although the trait shared by, or distinguishing, the Peyer's Patch M cells and villous M-like cells has not been identified. Our transcriptomic analysis has identified several M-cell-specific genes. One of them, glycoprotein-2 (GP-2) was originally reported to be abundantly expressed in pancreatic acinar cells and secreted in the pancreatic fluid, although its physiological role remains elusive. We found, in collaboration with Dr. Kiyono's group in the University of Tokyo, that GP-2 was exclusively expressed by Peyer's patch M cells, but not by villous M cells. Another molecule, myris-

Recent publications

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

2. Terahara, K.*, Yoshida, M.*, Igarashi, O., Nochi, T., Pontes, G. S., Hase, K., Ohno, H., Kurokawa, S., Mejima, M., Takayama, N., Yuki, Y., Lowe, A. W., Kiyono, H. Comprehensive gene expression profiling of Peyer's patch M cells, villous M-like cells, and intestinal epithelial cells. ***J. Immunol.*** 180, 7840-7846, (2008) (*First two authors contributed equally to this work.)

toylated alanine-rich C kinase substrate (MARCKS)-like protein was also expressed by PP M cells but not villous M-like cells. These data suggest that Peyer's patch M cells and villous M cells may be functionally distinct cell populations, although they share some morphological features.

Autoimmune gastritis with activation-induced cytidine deaminase deficiency

Activation-induced cytidine deaminase (AID) expressed by germinal center B cells is a central regulator of somatic hypermutation (SHM) and class switch recombination (CSR). Humans with AID mutations develop not only the autosomal recessive form of hyper-IgM syndrome (HIGM2) associated with B cell hyperplasia, but also autoimmune disorders by unknown mechanisms. We reported that AID^{-}/$^{-}$mice spontaneously develop tertiary lymphoid organs (TLOs) in non-lymphoid tissues including the stomach at around 6 months of age. At a later stage, AID^{-}/$^{-}$mice develop a severe gastritis characterized by loss of gastric glands and epithelial hyperplasia. The disease development was not attenuated even under germ-free (GF) conditions. Gastric autoantigen -specific serum IgM was elevated in AID^{-}/$^{-}$mice, and the serum levels correlated with the gastritis pathological score. Adoptive transfer experiments suggest that autoimmune CD4^{+} T cells mediate gastritis development as terminal effector cells. These results suggest that abnormal B-cell expansion due to AID deficiency can drive B-cell autoimmunity, and in turn promote TLO formation, which ultimately leads to the propagation of organ-specific autoimmune effector CD4^{+} T cells. Thus, AID plays an important role in the containment of autoimmune diseases by negative regulation of autoreactive B cells.

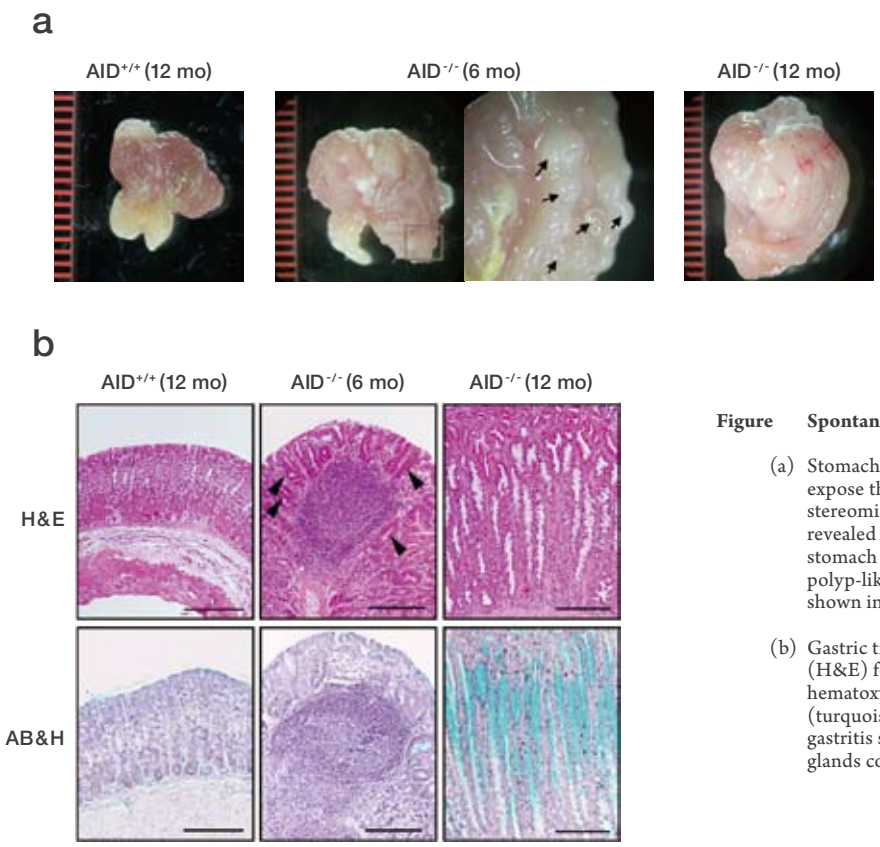

Figure **Spontaneous gastritis in AID^{-}/$^{-}$mice**

(a) Stomachs of AID^{+}/$^{+}$ and AID^{-}/$^{-}$ mice were cut longitudinally to expose the gastric mucosa. Inside-out tissues were examined by stereomicroscopy. High magnification view of mucosal surface revealed the presence of a number of polyps in the AID^{-}/$^{-}$ mouse stomach (arrows). Histological examination revealed that the polyp-like lesions resulted from ectopic lymphoid follicles as shown in (b). A representative sample in each group is shown

(b) Gastric tissue sections were stained with hematoxylin-eosin (H&E) for histological examination, or with Alcian blue-hematoxylin (AB&H) for detection of mucin-producing cells (turquoise). AID^{-}/$^{-}$ stomachs displayed characteristics of gastritis such as epithelial hyperplasia and destruction of gastric glands coupled with an increase in mucus-producing cells.

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

4. Kawano K.*, Ebisawa M.*, Hase K., Fukuda S., Hijikata H., Kawano S., Date Y., Tsuneda S., Itoh K., Ohno H. Psg18 is specifically expressed in follicle-associated epithelium. ***Cell Struct. Funct.*** 32, 115-126 (2007) (*First two authors contributed equally to this work.)

5. Hase K., Murakami T., Takatsu H., Shimaoka T., Iimura M., Hamura K., Kawano K., Ohshima S., Chihara R., Itoh K., Yonehara S., Ohno H. The membrane-bound chemokine CXCL16 expressed on follicle-associated epithelium and M cells mediates lympho-epithelial interaction in GALT. ***J. Immunol.*** 176, 43-51 (2006)

Laboratory for Mucosal Immunity

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* until March, 31th
**until November, 30th

Our research goal is to understand the cellular and molecular basis of gut immune regulation, a front line of body defense, and to develop its application for health and diseases of the immune system.

The gastrointestinal tract is a site of extraordinarily complex and dynamic host-bacterial interactions which, under constant antigenic pressure evolved highly sophisticated and efficient defensive mechanisms. Essentially two distinct patterns of responses are exhibited by the gut immune system: local immunity involving IgA production and the development of oral tolerance. Thus, developing strategies for manipulating the gut immune system toward either positive immunity or tolerance appears extremely attractive when aiming at protecting the host from invasive pathogens as well as at preventing and/or modulating systemic pathology.

We aim to understand commensal host-bacterial relationships in the gut, with their multiple feed-back and feed-forward controls involving both bacteria and immune cells, and to characterize cellular and molecular interactions responsible for tolerance or immunity in the intestinal mucosa.

T cell-independent immunoglobulin A generation in the gut

Tsuji M, Suzuki K, Kitamura H, Maruya M, Kinoshita K, Ivanov II, Itoh K, Littman DR, Fagarasan S. Requirement for lymphoid tissue-inducer cells in isolated follicle formation and T cell-independent immunoglobulin A generation in the gut. *Immunity,* 29, 261-271, 2009

Immunoglobulin A (IgA) is generated in the gut by both T cell-dependent and T cell-independent processes. However, the sites and the mechanisms for T-independent IgA synthesis remain elusive. We found that isolated lymphoid follicles (ILFs) are sites where induction of activation-induced cytidine deaminase (AID) and IgA class switching of B cells take place in the absence of T cells. The formation of ILFs is regulated by multiple, complex interactions between lymphoid tissue inducer cells expressing the nuclear receptor RORγt (RORγt$^+$LTi cells) and stromal cells (SCs). Activation of SCs by RORγt$^+$LTi cells through the lymphotoxin (LT)-β receptor (LTβR) and simultaneously by bacteria through TLRs induces the recruitment of dendritic cells (DCs) and B cells, and formation of ILFs (Fig. 1). Our findings provide insight into the crosstalk between bacteria, RORγt$^+$LTi cells, SCs, DCs and B cells required for ILF formation and establish a critical role

Recent publications

1. Tsuji M, Komatsu N, Kawamoto S, Suzuki K, Kanagawa O, Honjo T, Hori S, Fagarasan S. Preferential generation of follicular B helper T (T_{FH}) cells from Foxp3$^+$ T cells in gut Peyer's patches. ***Science***, in press.
2. Tsuji M, Suzuki K, Kitamura H, Maruya M, Kinoshita K, Ivaylo II, Itoh K, Littman DR, Fagarasan S. Requirement for Lymphoid tissue inducer cells in isolated follicle formation and T cell-independent immunoglobulin A generation in the gut. ***Immunity.*** 2008, 29, 261-271.

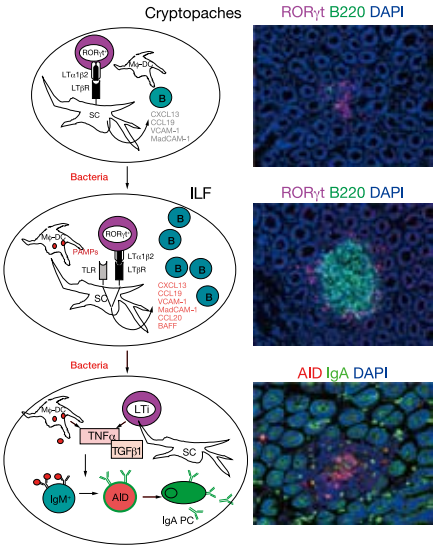

Figure 1 **Proposed model for CP and ILF formation and generation of IgA in ILFs in the absence of T cells.** The formation of CPs requires RORγt$^+$LTi cells and their interaction with SCs through LTβR. This interaction leads to activation of SCs and production of adhesion molecules and chemokines, which are involved mainly in the recruitment of Mϕ-DCs. CPs receive signals from gut bacteria, most likely through the Mϕ-DCs. Concomitant signals from RORγt$^+$LTi cells through LTβR and bacteria through TLRs further augment the activation of SCs, leading to recruitment of B cells and organization of ILF. The recruited B cells are then activated by antigen- presenting DCs or by polyclonal stimulation by the captured microbes. Activated B cells up-regulate AID and undergo class switching to IgA, even in the absence of T cells. TNFα secreted by both Lin-RORγt$^+$ cells as well as activated Mϕ-DCs induces activation of TGFβ1 (likely through production of matrix metalloproteinases), leading to preferential class switching of B cells from IgM to IgA in the ILFs. Sections from the small intestine depicting CP, ILF and generation of IgA are also shown.

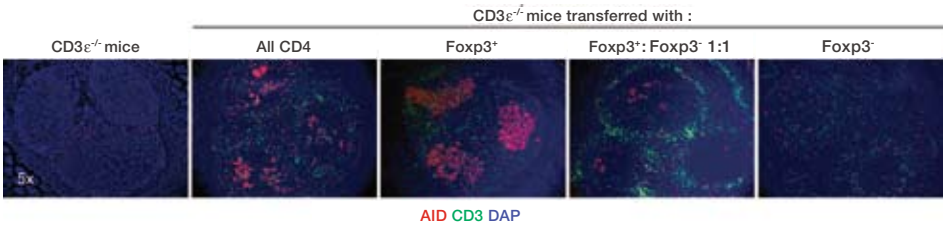

Figure 2 Immunofluorescent microscopy analyses of AID, DAPI and CD3 expression in horizontal sections of the PPs from CD3ε$^{-/-}$ mice before and four weeks after reconstitution with the indicated T cell populations.

of ILFs in T cell-independent IgA synthesis in gut.

T-dependent generation of IgA responses in the gut

Tsuji M, Komatsu N, Kawamoto S, Suzuki K, Kanagawa O, Honjo T, Hori S, Fagarasan S. Preferential generation of follicular B helper T (T_{FH}) cells from Foxp3$^+$ T cells in gut Peyer's patches. *Science*, 323 (5920): 1488-1492, 2009.

It is well known that the formation of germinal centers (GCs) requires the help of T cells. However, the origin of the helper T cells has remained unknown. We investigated the origin of these follicular B helper T cells (T_{FH}) in Peyer's patches (PPs) by examining the contribution of Foxp3$^+$ T cells obtained from Foxp3^{EGFP} reporter mice. We demonstrate that suppressive Foxp3$^+$ T cells can differentiate into T_{FH} cells in mouse PPs. Furthermore, the induction of GCs by Foxp3$^+$ T cells and generation of IgA-producing cells in the gut lamina propria was more effective than that by unfractionated CD4$^+$ T cells or a 1:1 mixture of Foxp3$^+$ and Foxp3$^-$ T cells (Fig. 2). The conversion of Foxp3$^+$ T cells into T_{FH} cells requires loss of Foxp3 expression and subsequent interaction with B cells (summarized in Fig. 3). These studies have implications for how the suppression of inflammatory reactions and induction of IgA synthesis occur in the gut.

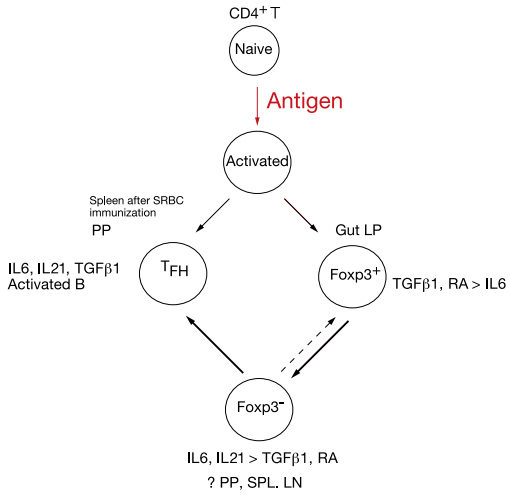

Figure 3 **Diagram of Foxp3$^+$ and T_{FH} cell differentiation. Activation of naïve CD4$^+$ T cells by antigens through TCR in the presence of TGF-β1 leads to induction of Foxp3.** In a microenvironment with abundant TGF-β1 and retinoic acid (RA) (i.e. in the LP of the small intestine), Foxp3$^+$ T cells maintain Foxp3 expression. Even in the presence of large amounts of IL-6 produced by LP DCs and stromal cells, the expression of Foxp3 is maintained because of the RA-induced down-regulation of the IL-6Rα. By contrast, in a microenvironment with abundant IL-6 and less TGF-β1, the Foxp3$^+$ T cells lose the Foxp3 expression and become Foxp3$^-$ T cells. Some of the Foxp3$^-$ T cells derived from Foxp3$^+$ T cells may reacquire Foxp3 expression, while others differentiate into T_{FH} cells in the presence of IL-6 and/or IL-21 and activated B cells (i.e in the PPs). T_{FH} cells can also be generated directly upon antigen activation under the influence of IL-6, IL-21 and interactions with B cells.

3. Fagarasan S. Evolution, development, mechanism and function of IgA in the gut. ***Curr.Opin. Immunol.***,2008,April (20), 170-177.

4. Ha SA, Tsuji M, Suzuki K, Meek B, Yasuda N, Kaisho T, Fagarasan S. Regulation of B1 cell migration by signals through Toll-like receptors. ***J Exp Med.*** 2006 Oct 30;203(11):2541-50

5. Suzuki K, Meek B, Doi Y, Honjo T, Fagarasan S.: Two distinctive pathways for recruitment of naive and primed IgM+ B cells to the gut lamina propria ***Proc Natl Acad Sci U S A.*** 2005 Feb 15;102 (7):2482-6.

Laboratory for Immune Diversity

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Germinal center (GC) B cells represent a unique cell population that is induced during an immune response against foreign antigens. These rapidly dividing cells undergo dynamic genetic alterations including somatic hypermutation (SHM) and class switch recombination of the immunoglobulin (Ig) genes and can ultimately differentiate into antibody-producing plasma cells or memory B cells. Dysregulation of this terminal differentiation pathway can lead to immunodeficiency, autoimmune diseases and B cell malignancies. The goal of our research is to clarify the mechanism of Ig gene SHM and to understand the molecular basis of B cell activation and terminal differentiation.

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

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

Mechanism of A:T mutations in the GC B cells

To understand why AID-triggered U:G lesions lead to mutations at non-damaged A:T pairs, we have compared

Recent publications

1. Ukai, A., Ishimaru, K., Ouchida, R., Mori, H., Kano, C., Moritan, T. and Wang, J.-Y. Induction of A:T mutations is dependent on cellular environment but independent of mutation frequency and target gene location. ***J. Immunol.*** 181: 7835-7842 (2008).
2. Masuda, K., Ouchida, R., Yokoi, M., Hanaoka, F., Azuma, T. and Wang, J.-Y. DNA polymerase η is a limiting factor for A:T mutations in Ig genes and contributes to the antibody affinity maturation. ***Eur. J. Immunol.*** 38: 2796-2805 (2008).

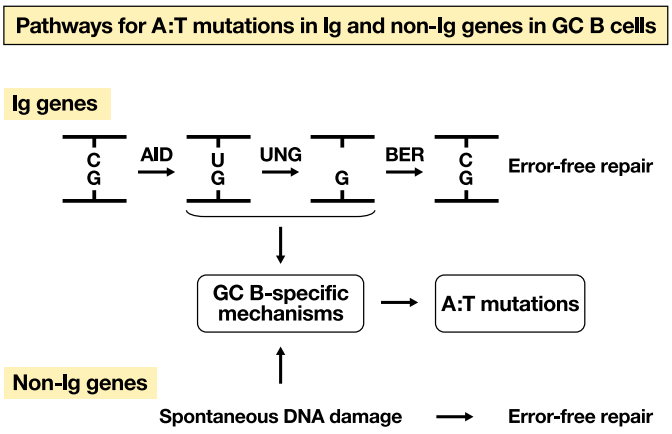


Figure 1 **GC B cells have an intrisic property to generate A:T mutations in Ig and non-Ig genes.** In Ig genes, AID deaminates cytosine (C) to uracil (U) and generates a U:G lesion. Under normal situations, this U:G lesion is correctly repaired by the error-free base excision repair (BER) pathway. During Ig gene SHM, however, the U:G lesion can be resolved by a GC B-specific error-prone repair pathway, leading to mutations at non-damaged A:T base pairs. Using a lacZ-trangenic system, we found that GC B cells also efficiently generate A:T mutations in non-Ig genes in an AID-independent but POLH-dependent manner, likely utilizing a similar GC B-specific mechanism.

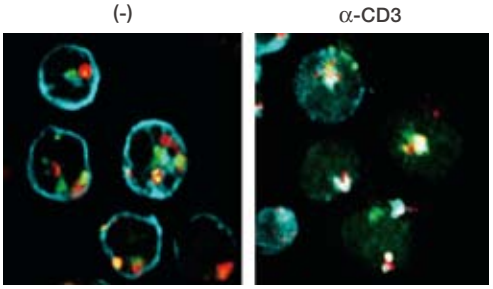


Figure 2 **LAPTM5 downmodulates cell surface TCR expression by promoting CD3ζ degradation.** Left, CD3ζ (cyan) is localized on the plasma membrane whereas LAPTM5 (green) and the lysosome-associated protein LAMP1 (red) are in the lysosomes in T cells before stimulation. Right, after antigen stimulation, CD3ζ is translocated to the lysosomal compartment where it co-localizes with LAPTM5 and LAMP1 and is degraded.

mutation patterns in non-Ig genes in the GC B cells with those in other cells/tissues. We utilized a sensitive lacZ-transgenic (lacZ-Tg) system in which genome mutations can be detected in an unbiased way since there is no selective pressure to obscure them. We found that approximately half of the mutations in the lacZ gene occurred at A:T pairs in GC B cells, which was in striking contrast to naïve B, non-GC B, as well as other non-lymphoid cells where mutations occurred predominantly at C:G pairs. Importantly, the increased A:T mutations in GC B cells were also observed in mice lacking AID, indicating that the induction of A:T mutations is a process independent of AID-mediated Ig gene SHM. These results suggest that GC B cells have an intrinsic propensity to mutate A:T pairs in both Ig and non-Ig genes (Figure 1). Using the GC B-like Ramos cells, we further demonstrated that the ability to induce A:T mutations is dependent on the cellular environment but independent of mutation frequency and target gene location. Experiments are now in progress to address the following three non-exclusive hypotheses for the high proportion of A:T mutations in GC B cells. 1) Rapid cell division of GC B cells interrupts the normal repair process and causes error-prone DNA synthesis. 2) GC B cells express a specific factor(s) that function to recruit POLH, a low-fidelity DNA polymerase essential for the induction of A:T mutations, instead of other high-fidelity polymerases during repair of DNA lesions. 3) POLH, mismatch repair (MMR) components, or AID itself undergo specific modifications in GC B cells, resulting in an atypical repair process not observed in other cell types.

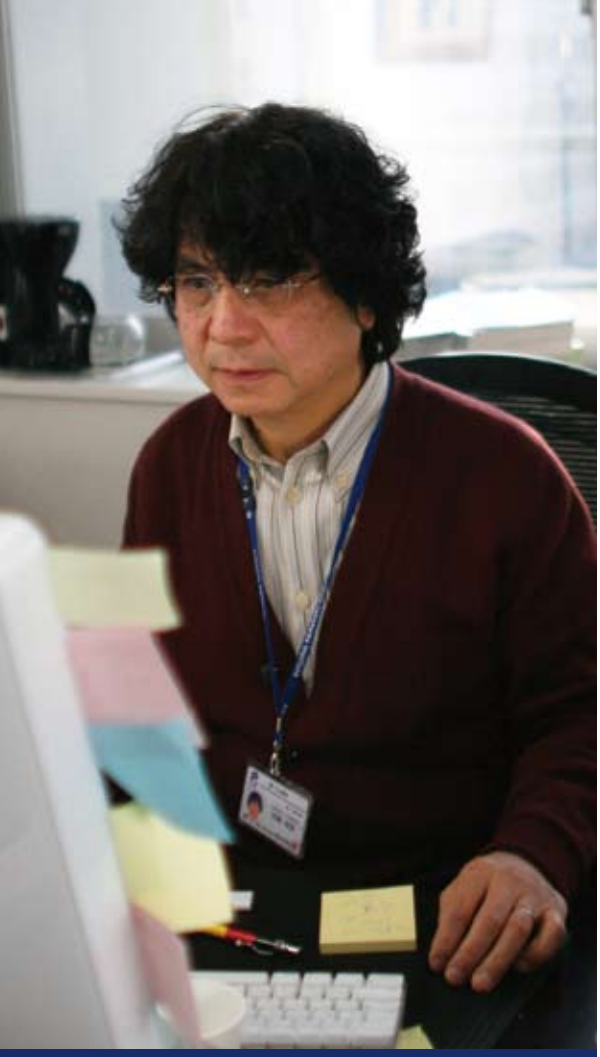
Identification of a lysosomal protein that negatively regulates cell surface expression of T and B cell antigen receptors

LAPTM5 is a lysosomal protein specifically expressed in hematopoietic cells and transiently down-modulated during B and T cell activation. We found that LAPTM5 deficiency resulted in elevated TCR levels on both $CD4^+CD8^+$ thymocytes and spleen T cells after CD3 stimulation, as well as enhanced T cell responses *in vitro* and *in vivo*. Further analysis revealed that LAPTM5 downmodulated cell surface TCR expression by interacting with the invariant signal transducing CD3ζ chain, promoting its degradation in the lysosomal compartment (Figure 2). These results identify the first lysosomal protein important for CD3ζ degradation and illustrate a novel mechanism to control surface TCR levels and T cell activation. More recently, we found that LAPTM5 also downmodulated the cell surface expression of the pre-BCR and BCR. In future studies, we aim to clarify the mechanism of LAPTM5-mediated BCR downmodulation and to identify additional immune-specific receptors that are targeted by LAPTM5. We are also generating B cell-specific LAPTM5-deficient mice to explore its physiological role during B cell activation and terminal differentiation.

3. Ehrhardt, G., Hijikata, A., Kitamura, H., Ohara, O., Wang, J.-Y. and Cooper, M. D. Distinctive gene expression profiles of memory B cell subpopulations. ***J. Exp. Med.*** 205: 1807-1817 (2008).

4. Ouchida, R., Yamasaki, S., Hikida, M., Masuda, K., Kawamura, K., Wada, A., Mochizuki, S., Tagawa, M.,Sakamoto, A., Hatano, M., Tokuhisa, T., Koseki, H., Saito, T., Kurosaki, T. and Wang, J.-Y. A Lysosomal protein negatively regulates surface T cell antigen receptor expression by promoting CD3ζ degradation. ***Immunity*** 29: 33-43 (2008).

5. Ouchida, R., Ukai, A., Mori, H., Kawamura, K., Dolle, M.E.T., Tagawa, M., Sakamoto, A., Tokuhisa, T., Yokosuka, T., Saito, T., Yokoi, M., Hanaoka, F., Vijg, J. and Wang, J.-Y. Genetic analysis reveals an intrinsic property of the germinal center B cells to generate A:T mutations. ***DNA Repair*** 7: 1392-1398 (2008).



Laboratory for Immunological Memory

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Immunological memory is the hallmark of adaptive immunity and is characterized by the prompt and effective response to antigen upon re-challenge, even a long time after the initial stimulation; memory provides a highly selective mechanism to ensure quick protection upon re-exposure to recurrent pathogens. Therefore, an important question is how immunological memory is maintained for such long periods of time in the absence of repeated antigenic stimulation. In addition, we have little knowledge for the differences between memory and naïve B cells, for example the longevity of memory B cells and their prompt differentiation into plasma cells that secrete large amounts of antibodies upon antigen re-exposure. Another important question is how and when memory B cells develop after initial antigen exposure.

Our goals are to answer these questions by utilizing a murine system, which is superior to other systems to characterize molecular mechanisms using genetic approaches and analyze functional activity.

Memory B cell development and maintenance

It has been widely accepted that mutated, high-affinity memory B cells originate from the GC response, in which Bcl-6 plays a critical role in allowing GC B cells to undergo a high rate of proliferation. However, it remains obscure whether Bcl-6 is required for development or maintenance of GC B cells, or both. We have previously observed that the generation of IgG1$^+$ GC and memory B cells with hypermutated V_H genes was abrogated in Bcl6$^{-/-}$ mice, whereas the generation of non-mutated IgG1$^+$ memory B cells was unaffected. However, it remained unclear whether this phenotype was solely due to the lack of Bcl-6 in the B cell itself, or was influenced by aberrant cytokine production in these KO mice.

In order to evaluate the role of Bcl-6 in memory/GC B cell development more precisely than with the original model, we have established conditional Bcl6KO mice and analyzed the IgG response to NP. The results suggest that Bcl6 deletion in the B cell itself diminished the generation of GC B cells, suggesting that Bcl-6 expression in B cells is essential for GC development. However, Bcl6 deletion did not affect the development of antigen specific IgG1$^+$ B cells with memory phenotype. Although such memory phenotype B cells did not accumulate V_H gene mutations, their gene expression profiles

Recent publications

1. Hori, J., Wang, M., Miyashita, M., Tanemoto, K., Takahashi, H., Takemori, T., Okumura, K., Yagita, H. and Azuma, M. B7-H1-induced apoptosis as a mechanism of immune privilege of corneal allografts. ***J. Immunol.*** 177:5928-5935, 2006.

2. Nagawa, F., Kishishita, N., Shimizu, K., Hirose, S., Miyoshi, M., Nezu, J., Nishimura, T., Nishizumi, H., Takeuchi, M., Miyajima, A., Hashimoto, S., Takahashi, Y.,Toshitada Takemori, T. Otsuka, A. -J. and Sakano, H. The agnathan lamprey diversifies antigen receptor genes by shifting junctions during gene rearrangement. ***Nature Immunol.*** 8:206-13, 2007

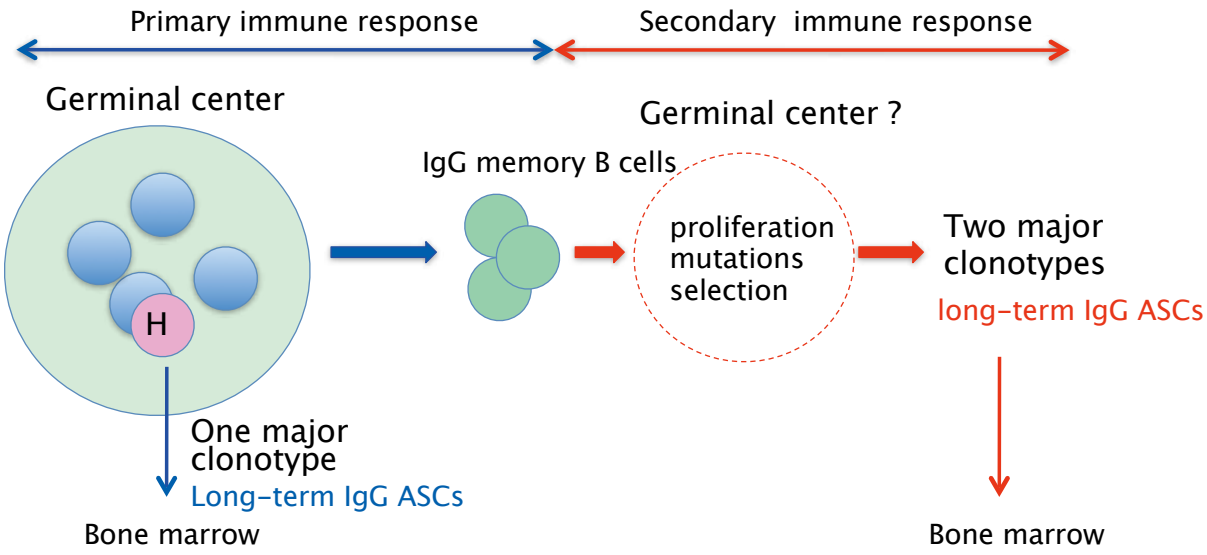


Figure **The antibody repertoire established by IgG memory B cells in the secondary response is distinct from that generated by B cells in the primary response.**
In the primary response, GC B cells generate high-affinity plasma cells with a nucleotide substitution indicative of affinity maturation. In the secondary response, a limited number of memory B cells expand under selection by antigen, resulting in the establishment of populations with two distinct clonotypes.

were comparable to those of wild type memory B cells. These results raise the possibility that IgG1$^+$ memory B cells develop in the absence of GC formation and that IgG1 class switching can occur by both GC-dependent or independent mechanisms.

The IgG1$^+$ memory B cell compartment is initially dominated by non-mutated cells, but mutated cells gradually accumulate and eventually predominate. We are currently attempting to determine if non-mutated memory B cells are replaced by mutated B cells of GC origin or if the non-mutated memory B cells enter into the GC reaction as the immune response progresses.

Population dynamics of memory B cell terminal differentiation.

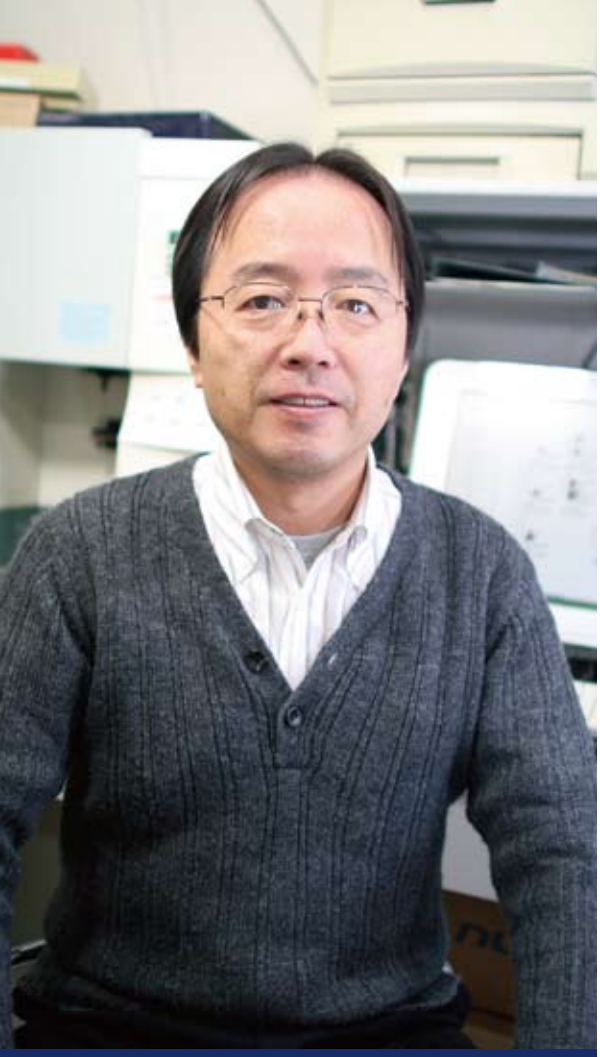
Previous analysis, based on hybridoma studies of the responses elicited in primed, intact animals with a low dose boost of soluble antigen, suggested that antibody-secreting cells (ASCs) in the secondary response were largely mutated with an accumulation of nucleotide transition indicative of affinity maturation. To investigate the population dynamics of memory B cells terminal differentiation, we transferred NP-specific/IgG1$^+$ memory B cells purified from immunized mice into carrier-primed AID KO mice (kindly provided by Dr. Honjo) and monitored the IgG1 response upon secondary challenge. This system provides a unique opportunity to monitor the secondary response independently of pre-existing long-lived plasma cells or the responses of naïve B cells recruited into the GC reaction during the secondary response.

The results showed that IgG1 memory B cells produced large amounts of high-affinity anti-NP IgG1 antibodies over a long period after secondary challenge. Antigen-specific ASCs originated from memory cells that were initially localized in the spleen and homed to the BM later in the response, where they were sustained over a long period.

V_H gene sequence analysis suggested that a limited number of memory B cells expand and diversify in the secondary response, accompanied by somatic mutations to some extent, and that they are finally selected as two major populations with characteristic amino acid substitutions. Thus the secondary memory B cells provide a unique repertoire, distinct from the primary response. Curiously, the IgG secondary response was barely detectable in naïve AID KO mice cotransferred with memory B cells and carrier primed helper T cells, suggesting the idea that a prompt secondary response may require antigen-specific circulating antibodies or pre-established GCs, or both. Alternatively, the secondary response requires very potent T-cell help. Further analysis is needed to clarify this intriguing issue.

3. In competence of neutrophils to invasive group A streptococcus is attributed to induction of plural virulence factors by dysfunction of a regulator. Ato, M., Ikebe, T., Kwabata, H., Takemori, T and Watanabe, H. ***Plos One***, 3(10):e3455, 2008

4. PLC-γ2 is essential for formation and maintenance of memory B cells. Hikida, M., Casola, S., Takahashi, N., Kaji, T., Takemori, T, Rajewsky, K. and Kurosaki, T. ***J. Exp. Med.*** in press, 2009.



Laboratory for Host Defense

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Administrative Staff : **Sachiko Haraguchi**

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

Roles of IKKα in dendritic cell subsets

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

Plasmacytoid DC (PDC) are a unique DC subset expressing nucleic acid sensors, TLR7 and TLR9. PDC are characterized by their ability to produce large amounts of type I IFN, especially IFN-α, in response to TLR7/9. TLR7 and TLR9 are quite similar in their amino acid structures and can activate similar signaling pathways, which depend on the 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 clarified that

Recent publications

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

2. D. Kinoshita, F. Hirota, T. Kaisho, M. Kasai, K. Izumi, Y. Bando, Y. Mouri, A. Matsushima, S. Niki, H. Han, K. Oshikawa, N. Kuroda, M. Maegawa, M. Irahara, K. Takeda, S. Akira, M. Matsumoto. Essential role of IκB kinase α in thymic organogenesis required for the establishment of self-tolerance. ***J. Immunol.*** 176, 3995-4002 (2006).

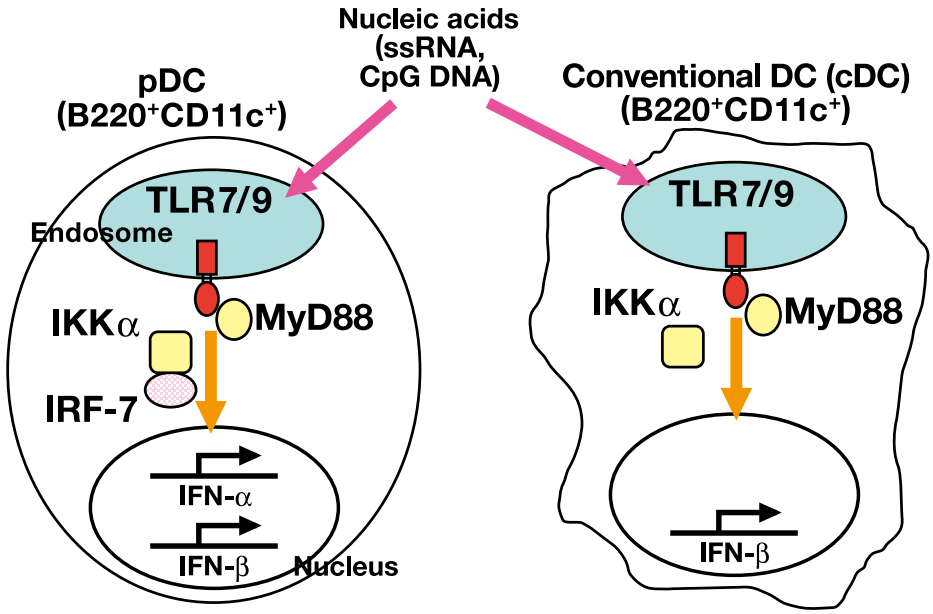

Figure **Critical roles of IKKα in TLR7/9-induced type I IFN gene induction.** In mice, both pDC and cDC express TLR7/9 and can produce IFN-β in response to TLR7/9 signaling, although cDC do not produce IFN-α. IKKα is required for TLR7/9-mediated type I IFN induction in both pDC and cDC.

a serine threonine kinase, IκB kinase-α (IKKα), is critically involved in this type I IFN induction pathway in PDC. IKKα-deficient PDC showed severe defects in IFN-α and IFN-β production, but retained the ability to produce proinflammatory cytokines in response to TLR7/9 agonists. IKKα is ubiquitously expressed, not just in PDC, therefore, a PDC-specific molecule should be a target for IKKα. In this context, we have focused on IRF-7, which is constitutively expressed at high levels in PDC. IKKα associated with and phosphorylated IRF-7. Furthermore, IKKα deficiency resulted in impaired IRF-7 activation in PDC. These results demonstrate that IKKα is critically involved in IRF-7 activation by a direct interaction. It is notable that conventional DC (cDC) can also produce IFN-β, although not IFN-α. This IFN-β production is regulated differently from that in PDC. Analysis of IKKα-deficient cDC revealed that IKKα was also required for the IFN-β production from TLR7/9-stimulated cDC. Thus, IKKα plays critical roles in TLR7/9-dependent type I IFN production regardless of the DC subset and should be a potential target not only for manipulating antiviral immunity but also for treating autoimmune diseases in which IFN-α production is elevated. In addition to the IKKα-IRF-7 axis, other PDC-specific molecules are also likely to be required for PDC function. We are further analyzing the molecular mechanisms regulating pDC functions.

Critical roles of TLR3 signaling

Dendritic cells are quite heterogeneous and cDC can be further divided into three subsets based on their expression of CD4 and CD8. These DC subsets have specific functions and responses to immune adjuvants including nucleic acids, therefore it is critical to understand the molecular mechanisms by which such DC subset-specific functions are regulated. Splenic cDCs consist of $CD4^-CD8^+$, $CD4^-CD8^-$, and $CD4^+CD8^-$ subsets. We are focusing on $CD4^-CD8^+$ cDC, because this subset is the only DC subset that can respond to the signals through the endosomal dsRNA sensor, TLR3. Cytosolic proteins of the RIG-I like receptor (RLR) family can also sense dsRNA, but RLRs do not function in $CD4^-CD8^+$ cDCs. Furthermore, notably, TLR3 is not expressed in other DC subsets including pDC.

We have analyzed the immune adjuvant effects of a double-stranded RNA (dsRNA), poly(A:U), and clarified that it is a ligand for TLR3 and TLR7, but not for RLRs. This is in contrast to the fact that a widely used dsRNA, poly(I:C), is a ligand for TLR3 and MDA5, an RLR family member. By using poly(A:U), we have clarified that TLR3 mediates the signal for generating CD8 T cell responses *in vivo*, i.e. crossprentation. Crosspresentation is a critical function for effective immune responses against tumors or viral infections.

We have also found that TLR3-mediated effects are restricted on one *in vitro* DC subset, which can be defined amongst Flt3L-induced BM DCs as the $CD24^{high}$ cDC. Because this DC subset corresponds to the $CD4^-CD8^+$ cDC in the spleen, it seems very likely that poly(A:U) targets TLR3 in splenic $CD4^-CD8^+$ cDC. We further plan to analyze how the function of this DC subset is regulated by TLR3 signaling.

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

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

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

Laboratory for Infectious Immunity

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Ubiquitination of proteins is an important post-translational modification that plays important roles in proteasome-mediated protein degradation as well as in gene transcription, membrane trafficking, and signal transduction. This modification is achieved through the concerted action of three enzymes: ubiquitin-activating enzyme E1 (E1), ubiquitin-conjugating enzyme E2 (E2), and ubiquitin-protein ligase E3 (E3). E1 activates ubiquitin in an ATP-dependent reaction, and the activated ubiquitin is subsequently transferred to a substrate through the interaction of E2 and E3. E3 determines the substrate specificity for ubiquitination through its binding properties.

We have recently described a new family of E3s named Modulator of Immune Recognition (MIR). The original members of this family were first identified ~ 2000 as viral proteins that were thought to be involved in immune-evasion. Subsequently, it was discovered that mammals also possess related molecules named MARCH (membrane-associated RING-CH). Therefore, the MIR family consists of viral MIR family members and eukaryotic MARCH family members. Interestingly, several MIR family members target MHC molecules and have been proposed to interfere with antigen presentation. Discovery of these MIR family members is leading us to explore the possible existence of novel regulatory mechanism for antigen presentation *in vivo*.

Among MARCH family members, MARCH-I has been identified as a physiological E3 by genetic manipulation of its expression levels. Forced expression of MARCH-I induces down-regulation of MHC class II on antigen-presenting cells (APCs) through ubiquitination of a lysine residue at position 225 in the cytoplasmic domain of the β chain, whereas ablation of the *MARCH-I* gene causes accumulation of MHC class II molecules on APCs. Given that MARCH-I is expressed mainly in APCs located in secondary lymphoid tissues (e.g. spleen and lymph node), these observations suggest that MARCH-I is the master E3 for MHC class II *in vivo*. Indeed, deletion of MARCH-I results in the complete loss of ubiquitinated MHC class II, at least in splenic B cells. Thus, in the steady state, expression of MHC class II on the cell surface is regulated by ubiquitination, primarily through MARCH-I (Figure).

Recent publications

1. Young, L.J., Wilson, N.S., Schnorrer, P., Proietto, A., ten Broeke, T., Matsuki, Y., Mount, A.M., Belz, G.T., O'Keeffe, M., Ohmura-Hoshino, M., Ishido, S., Stoorvogel, W., Heath, W.R., Shortman, K., Villadangos, J.A. 2008. Differential MHC class II synthesis and ubiquitination confers distinct antigen-presenting properties on conventional and plasmacytoid dendritic cells. ***Nat Immunol.*** 9(11):1244

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

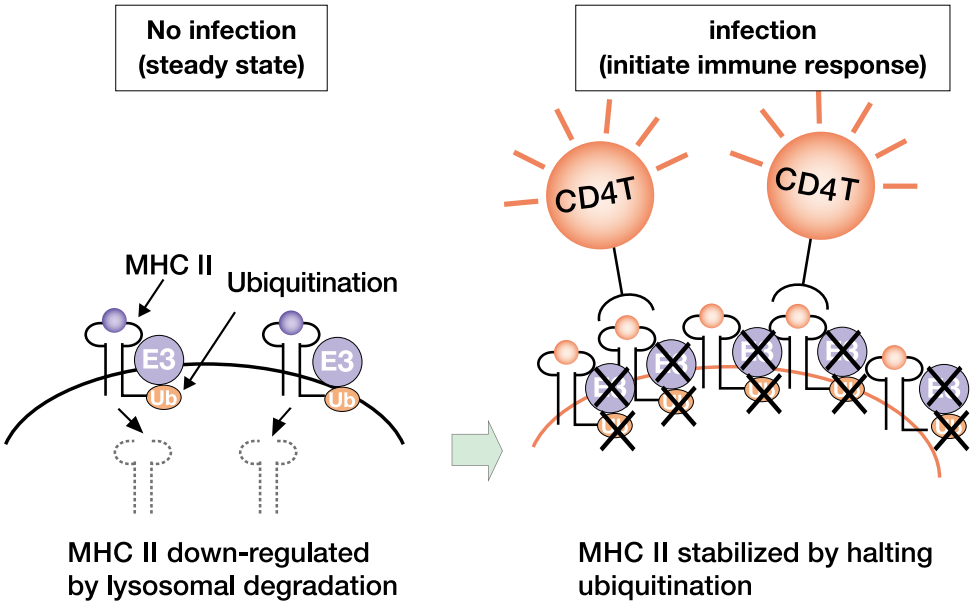


Figure **Possible contribution of MARCH-I to immune responses.**
In the steady state, MARCH-I (shown as "E3") is constitutively expressed in APCs and regulates the expression of MHC II through ubiquitination-mediated lysosomal degradation (*left panel*). Once APCs are activated by infection, MARCH-I expression or its enzymatic activity is down-regulated, and pathogen-derived peptides are presented by MHC II stabilized due to loss of ubiquitination (*right panel*).

How does MHC class II ubiquitination contribute to antigen-presentation? To address this question, we have examined where and when MHC class II is ubiquitinated during the antigen-presentation process. We demonstrated that peptide loaded MHC II is selectively ubiquitinated in immature dendritic cells (DCs), however, MHC II ubiquitination was effectively inhibited by DC maturation signals, such as TLR-signaling. These findings suggest that down-regulation of MHC II ubiquitination is important for effective antigen-presentation, as loss of MHC II ubiquitination is thought stabilize peptide-bound MHC II on the surface of DCs (Figure). Consistent with this hypothesis, we could show that MARCH-I-deficient B cells have enhanced antigen-presentation capacity to CD4 T cells.

How is MHC II ubiquitination inhibited by DC maturation signals? We found that MARCH-I mRNA expression is significantly inhibited by maturation stimuli in splenic conventional DCs. This may partially explain the mechanism, but Stoorvogel et al. have found that loss of MHC II ubiquitination precedes down-regulation of MARCH-I transcription, which seems to imply a post-transcriptional regulatory component of MARCH-I expression during DC maturation. One candidate could be ubiquitination of MARCH-I itself. However, our current studies have shown no differences between the RINGv mutant and wild type of MARCH-I in terms of protein stability. Another interesting possibility is the contribution of a de-ubiqutinating enzyme to this process.

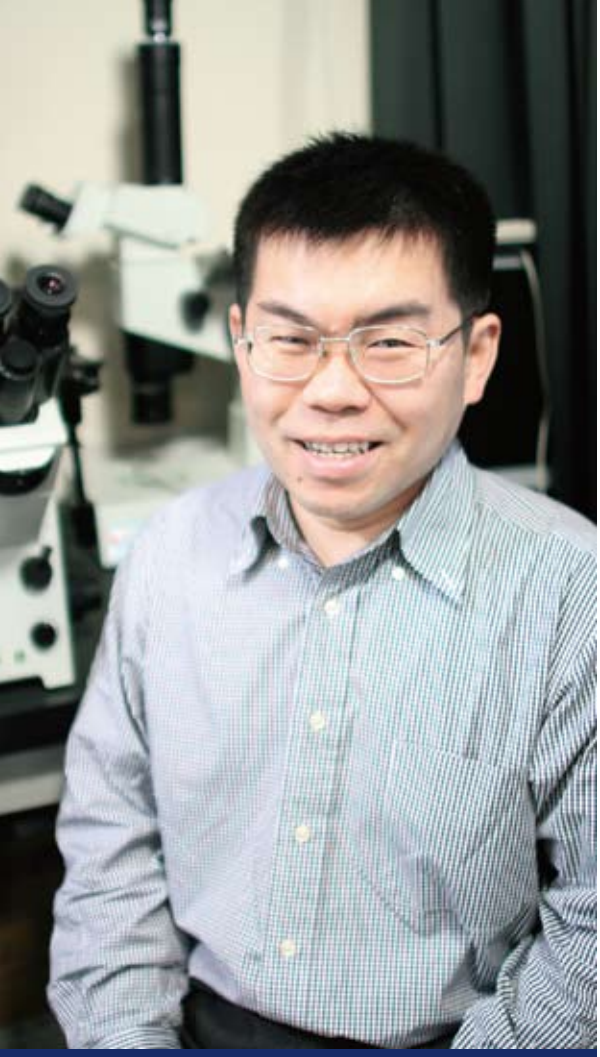
Maturation-regulated ubiquitination is not a general phenomenon in all types of DCs. Several subpopulations of DCs are currently recognized, and the two major populations in spleen are the plasmacytoid (pDCs) and conventional (cDCs) dendritic cells. Analysis of these cells has shown that MHC class II ubiquitination in cDCs, but not pDCs, is reduced by maturation stimuli. This different modes of regulation of MHC II ubiquitination have been explained partially by differential regulation of MARCH-I in the two DC populations. MARCH-I mRNA expression was not efficiently downregulated in activated pDC. Indeed, the level of MHC II expression in MARCH-I deficient pDC was higher than that in wild type pDC even in the activated state. These findings highlight MARCH-I as a determinant of DC functions among DC subsets.

3. Matsuki, Y., M. Ohmura-Hoshino, E. Goto, M. Aoki, M. Mito-Yoshida, M. Uematsu, T. Hasegawa, H. Koseki, O. Ohara, M. Nakayama, K. Toyooka, K. Matsuoka, H. Hotta, A. Yamamoto, and S. Ishido. 2007. Novel regulation of MHC class II function in B cells. ***Embo J*** 26:846.

4. Ohmura-Hoshino, M., Y. Matsuki, M. Aoki, E. Goto, M. Mito, M. Uematsu, T. Kakiuchi, H. Hotta, and S. Ishido. 2006. Inhibition of MHC class II expression and immune responses by c-MIR. ***J Immunol*** 177:341.

5. Ohmura-Hoshino, M., E. Goto, Y. Matsuki, M. Aoki, M. Mito, M. Uematsu, H. Hotta, and S. Ishido. 2006. A novel family of membrane-bound E3 ubiquitin ligases. ***J Biochem (Tokyo)*** 140:147.

6. Yonashiro, R., S. Ishido, S. Kyo, T. Fukuda, E. Goto, Y. Matsuki, M. Ohmura-Hoshino, K. Sada, H. Hotta, H. Yamamura, R. Inatome, and S. Yanagi. 2006. A novel mitochondrial ubiquitin ligase plays a critical role in mitochondrial dynamics. ***Embo J*** 25:3618.



Laboratory for Innate Cellular Immunity

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The maintenance of self-tolerance is critical so that an appropriate immune response to foreign organisms can be elicited, while at the same time preventing recognition of normal body tissues. Self-tolerance within the T cell compartment is maintained in both central and peripheral lymphoid compartments. Self-antigens are presented by antigen presenting cells in the thymus, and T cells overly reactive to them can be eliminated by apoptosis. As a result, most self-reactive T cells do not appear in peripheral tissues; however, a small population of such cells still escapes thymic negative selection. 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 leads to deletion or anergy of any self-reactive T cells, thereby providing a safeguard mechanism to maintain T cell tolerance.

Phagocytes engulf apoptotic cells upon recognizing molecules on the surface of the corpses. One known “eat me” molecule is phosphatidylserine (PS). PS is normally located on the inner leaflet of the plasma membrane bilayer of living cells, but when the cells undergo apoptosis, it is translocated to the outer leaflet. Several cell surface receptors and soluble proteins have been identified as PS-binding proteins involved in phagocytosis of apoptotic cells. Inhibition or absence of these functions *in vivo* causes failure of apoptotic cell clearance and subsequent autoimmunity. Findings of this sort clearly indicate that apoptotic cell clearance plays an important role in maintenance of self-tolerance.

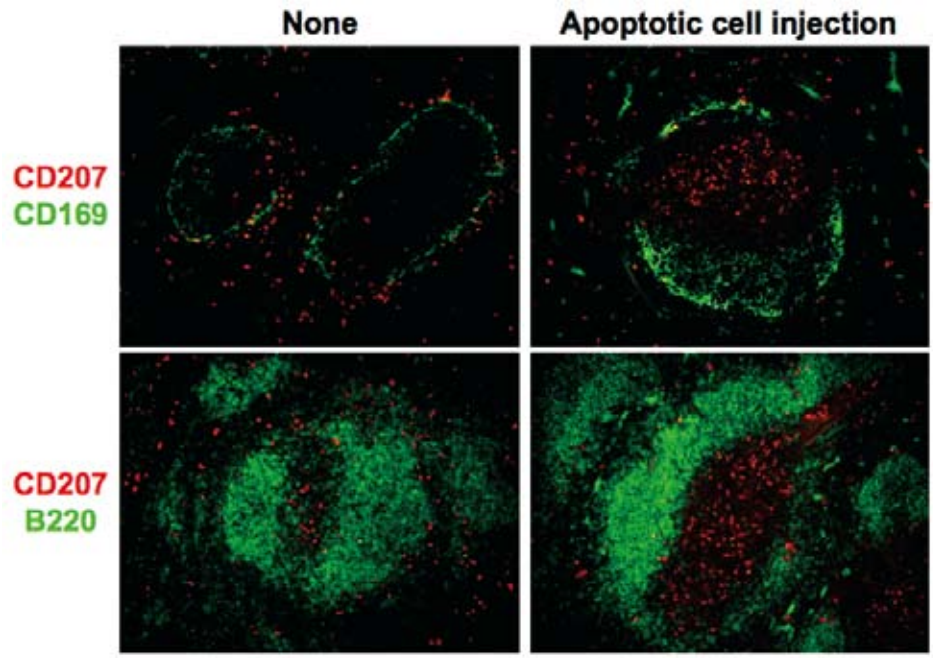
The Laboratory for Innate Cellular Immunity is investigating the molecular mechanisms for recognition and phagocytosis of dying cells, and the pathological relevance of impaired phagocytosis to inflammatory disorders including autoimmune diseases.

Novel subset of CD8α^+ dendritic cells responsible for tolerance to cell-associated antigens

We have previously established an experimental model for tolerance induction to cell-associated antigens using experimental autoimmune encephalomyelitis (EAE), a mouse model of human multiple sclerosis. Immunization with myelin oligodendrocyte glycoprotein (MOG) peptide induces EAE, but we found that injection of apoptotic cells expressing the

Recent publications

1. Tanaka M., Miyake Y., and Asano K. Maintenance of self-tolerance by apoptotic cell clearance. ***Front. Biosci.*** 13, 6043-6049 (2008)
2. Miyake Y., Asano K., Kaise, H., Uemura, M., Nakayama, M. and Tanaka, M. Critical role of macrophages in the marginal zone in the suppression of immune responses to apoptotic cell-associated antigens. ***J. Clin. Invest.*** 117, 2268-2278 (2007)



Figure

Migration of CD8α$^+$, CD103$^+$, CD207$^+$ DCs into the T cell zone after apoptotic cell phagocytosis.

Apoptotic W3 cells were injected intravenously into mice. Spleens were obtained 12 h after injection (right panels), and cryosections were stained with CD207 and CD169 (upper panels), or CD207 and B220 (lower panels).

MOG fragment suppressed disease development. Intravenously injected apoptotic cells initially accumulate in the marginal zone (MZ) of spleen. These apoptotic cells are phagocytosed not only by MZ macrophages located but also by splenic DCs. Among the DC subsets in spleen, the CD8α$^+$ subset predominates in the phagocytosis of injected apoptotic cells and in presenting cell-associated antigens to T cells, suggesting that they are responsible for the tolerance induction. However, as CD8α$^+$ DCs are believed to be predominantly localized in the T cell zone, it remained to be determined how these DCs could phagocytose blood-borne apoptotic cells, which accumulate in the MZ. Here, we identified a subpopulation of CD8α$^+$ DCs responsible for tolerance induction to cell-associated antigens. Among splenic CD8α$^+$ DCs, the CD103$^+$/CD207$^+$ subset preferentially localized to the MZ, where it dominantly phagocytosed blood-borne apoptotic cells. After phagocytosis of these cells, the DCs migrated into the T cell zone for cross-presentation of cell-associated antigens. Transient ablation of this DC subset resulted in a failure of tolerance induction to cell-associated antigens, indicating its essential role for tolerance induction by apoptotic cell clearance.

Roles of marginal zone macrophages in immune responses

Because of its unique position at the interface between the lymphoid compartment of the spleen and the scavenging red pulp compartment, the marginal zone (MZ) is well equipped to constantly screen the blood for foreign particles and organisms, as well as aberrant molecular debris and dying cells. The MZ contains two types of macrophages, marginal metallophilic macrophages (MMM) and marginal zone macrophages (MZM). MMM are localized at the inner border of the MZ close to the white pulp, whereas MZM can be found at the outer rim, near the red pulp. To determine the role of splenic MZ macrophages, we established transgenic mice in which these cells could be transiently depleted by DT administration (CD169-DTR mice). Using these mice, we have previously found that macrophages in the MZ are indispensable for tolerance induction to cell-associated antigens. We are now attempting to understand the roles of these macrophages in various immune reactions using CD169-DTR mice. In addition, as sinus macrophages in lymph nodes can also be transiently deleted in CD169-DTR mice, we are analyzing the roles of these macrophages in immune responses to dying cells in peripheral tissues.

3. Tanaka, M., and Miyake Y. Apoptotic Cell Clearance and Autoimmune Disorder. ***Curr. Med. Chem.*** 14, 2892-2897 (2007)

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

5. Nakaya M., Tanaka M., Okabe Y., Hanayama R., and Nagata S. Opposite effects of Rho family GTPases on engulfment of apoptotic cells by macrophages. ***J. Biol. Chem.*** 281, 8836-8842 (2006)

Unit Leader
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Technical Staff : **Emiri Haga**

Administrative Staff : **Sachiko Haraguchi**

Research Unit for Inflammatory Regulation

Our immune system is comprised of two distinct but interacting components, innate and adaptive immunity. Innate immune cells, such as macrophages and dendritic cells, detect invading microbial pathogens by their sensors (e.g. Toll-like receptors [TLR]). Signal through TLR leads to the activation of the transcription factor NF-κB, which enters the nucleus and induces the expression of a series of inflammation-related genes, including those encoding proinflammatory cytokines, such as interleukin-6 (IL-6) and IL-12. The resultant inflammatory responses then direct differentiation of T-helper (Th) lymphocytes into distinct subsets, such as Th1, Th2 and Th17, to fight against particular pathogens. On the other hand, inflammatory responses must be terminated at an appropriate time, otherwise an excess of these responses can damage normal tissue and may cause autoimmune or allergic diseases. Our research goal is to identify key regulators of inflammation-related signal transduction pathways, and to clarify the molecular mechanisms for regulating inflammatory responses. These studies should contribute to the development of new therapeutic tools to control the exaggerated inflammation seen in certain human diseases. Our research now focuses on the role of PDLIM2 (PDZ and LIM-domain protein-2) in the negative regulation of inflammatory responses.

PDLIM2 is a negative regulation for STAT4-mediated Th1 responses

PDLIM2, also known as SLIM (STAT-interacting protein), was originally isolated as a factor that interacts with the transcription factor STAT4, which is essential for IL-12-mediated Th1 cell differentiation. We demonstrated that PDLIM2 is a nuclear ubiquitin E3 ligase that acts on STAT4 to cause its ubiquitination and subsequent proteasome-mediated degradation (Tanaka, T. et al, *Immunity*, 22, 729-736, 2005). STAT4 is tyrosine phosphorylated upon IL-12 stimulation and translocated into the nucleus to induce target gene expression. PDLIM2 interacts with phosphorylated STAT4 in the nucleus. Overexpression of PDLIM2 leads to impaired STAT4 activity due to reduced STAT4 protein levels, while SLIM-deficiency results in increased STAT4 expression and enhanced IFNγ production by Th1 cells. This study also demonstrated for the first time that a LIM domain-containing protein could function as an ubiquitin E3 ligase.

Recent publications

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

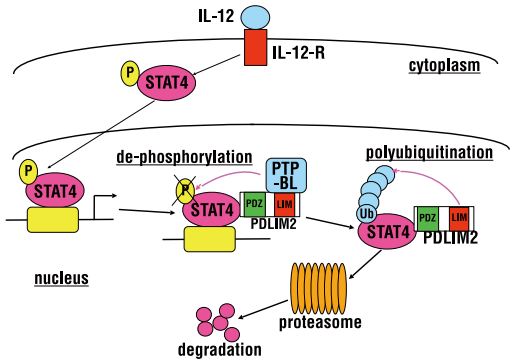

Figure 1 **PDLIM2, a nuclear ubiquitin E3 ligase, negatively regulates STAT4-mediated Th1 responses.** PDLIM2 binds to STAT4 and promotes its ubiquitin/proteasome-dependent degradation, thus downregulating STAT4 activity. PTPBL also binds to STAT4 and promotes its dephosphorylation. PDLIM2 functions as an adaptor that bridges STAT4 and PTPBL, facilitating STAT4 dephosphorylation.

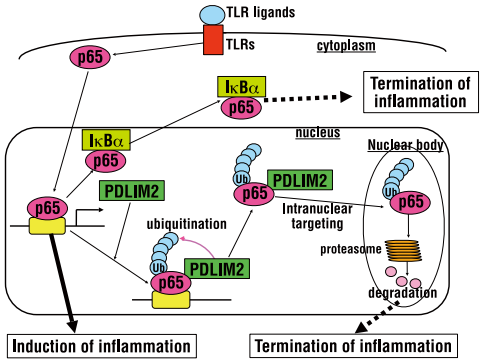

Figure 2 **PDLIM2 terminates NF-κB-mediated inflammatory responses.** PDLIM2 binds to the p65 subunit of NF-κB and promotes its polyubiquitination in the nucleus. PDLIM2 then targets p65 into discrete intranuclear compartments, called PML nuclear bodies, where polyubiquitinated p65 is ultimately degraded by the proteasome.

In addition, PDLIM2 also enhances dephosphorylation of activated STAT4, thereby downregulating STAT4 activity. However, PDLIM2 does not have any recognizable phosphatase domains, indicating the likely involvement of PDLIM2-associated tyrosine phosphatases. We have isolated another STAT4-interacting molecule, PTP-BL (Protein tyrosine phopsphatase Basophil like) (Nakahira et al, *Immunity* 26, 163-176, 2007). PTP-BL dephosphorylates STAT4, resulting in attenuation of STAT4-mediated gene activation, while PTP-BL deficiency leads to increased and prolonged activation of STAT4 and Th1 cell differentiation. Interestingly, PDLIM2 associated with both STAT4 and PTP-BL and enhanced STAT4 dephosphorylation synergistically with PTPBL, indicating that PDLIM2 functions as an adaptor that bridges STAT4 and PTPBL (our unpublished data).

PDLIM2 terminates NF-κB-mediated inflammatory responses

NF-κB is a key transcription factor for the activation of innate immune cells (Kaisho and Tanaka, *Tr. Immunol.*, 29, 329-336, 2008). However, excessive and prolonged activation of NF-κB can cause massive damage to the host and result in human diseases, such as asthma and arthritis, suggesting that NF-κB activation must be tightly regulated. One well established mechanism of NF-κB suppression is the export of nuclear NF-κB by IκBα. IκBα is resynthesized in an NF-κB-dependent manner, enters the nucleus, and transports nuclear NF-κB back out to the cytoplasm, thereby downregulating NF-κB-mediated transcription. Recent studies have disclosed another mechanism to shut down NF-κB activation by degrading nuclear NF-κB through the ubiquitin/proteasome-dependent pathway. However, the ubiquitin E3 ligase that interacts with and polyubiquitinates nuclear NF-κB was not identified. Moreover, the fate of polyubiquitinated NF-κB in the nucleus was unclear. We have demonstrated that PDLIM2 negatively regulates NF-κB activity, acting as a nuclear ubiquitin E3 ligase targeting the p65 subunit of NF-κB (Tanaka et al, *Nat. Immunol.* 8, 584-591, 2007). PDLIM2 binds to p65 and promotes p65 polyubiquitination through its LIM domain. In addition, PDLIM2 targeted p65 to discrete intranuclear compartments, called PML nuclear bodies. These are nuclear proteolytic centers where proteasomal components are concentrated. Polyubiquitinated p65 is ultimately degraded by the proteasome in these compartments. A PDLIM2 mutant lacking the PDZ domain fails to target p65 to nuclear bodies and the PDZ domain of PDLIM2 can bind to α-actinin, an actin binding protein, suggesting that PDLIM2 mediates intranuclear trafficking of p65 in an α-actinin/actin-dependent manner through its PDZ domain. PDLIM2 deficiency results in increased amounts of nuclear p65, defective p65 ubiquitination, and augmented production of proinflammatory cytokines in response to TLR ligands, such as LPS and CpG-DNA. These findings delineate a novel pathway by which PDLIM2 terminates NF-κB activation through intranuclear sequestration and subsequent degradation.

We are currently investigating how the activity of PDLIM2 itself is regulated. We have found that heat shock protein 70 (HSP70) binds to PDLIM2 and augments its ability to degrade NF-κB p65.

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

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

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

Research Unit for Therapeutic Model

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Administrative Staff : **Mariko Iwaki**

The goal of our laboratory is to develop immunotherapeutic models for cancer. We have been focusing on the role of *in vivo* DC maturation as a link between innate and adaptive immunity. In particular, we have studied the biological interactions between dendritic cells (DCs) and NKT cells. NKT cells can be stimulated with the glycolipid, alpha-galactosylceramide (α-GalCer) loaded on APCs. NKT cells have unique immunoregulatory features that include the ability to rapidly produce large quantities of cytokines. We have p reviously demonstrated that when α-GalCer-loaded DCs, rather than free α-GalCer, were administered to mice, NKT cells were generated and they produced IFN-γ for an extended period. We also found that, once activated, NKT cells play a role in inducing antigen-specific T cell immunity via *in vivo* DC maturation. Recently, we have developed an immunotherapeutic strategy using α-GalCer-loaded tumor cells for enhancing both innate (NKT and NK cells) as well as adaptive immunity (T cells). We further have been elucidating the mechanism of DCs in detail and continue to analyze memory T cells.

Tumor cells loaded with α-GalCer lead to innate and adaptive immune resistance to tumor implantation in mice

We have attempted to generate an approach for inducing adaptive immunity based on the adjuvant effect of α-GalCer and using *in vivo* DC maturation, which we have found to be more effective than *ex vivo* manipulation of DCs. CD1d expressing antigen presenting cells (APCs) loaded with α-GalCer are known to be active APCs for the stimulation of innate NKT and NK cell responses *in vivo*. In this study, we evaluated the capacity of non-DCs to present α-GalCer *in vitro* and *in vivo*, particularly α-GalCer-loaded tumor cells (tumor/Gal). Even though the tumor cells did not express CD40, CD80 or CD86 costimulatory molecules, the intravenous injection of α-GalCer-loaded tumor cells (tumor/Gal) resulted in IFN-γ secretion by NKT and NK cells. These innate responses to tumor/Gal, including the induction of IL-12p70, were comparable to or better than DC/Gal. B16 melanoma cells that were stably transduced to express higher levels of CD1d (CD1dhi-B16/Gal) showed an apparent increased capacity relative to wild type B16 cells to present α-GalCer *in vivo*.

Recent publications

1. Shimizu K, Fujii S. Adjuvant role of in situ dendritic cells (DCs) in linking innate and adaptive immunity. ***Front Biosci.*** 13, 6193-201 (2008)
2. Fujii S, Shimizu K, Hemmi H, Steinman RM. Innate Vα14+ naturatl killer T cells mature dendritic cells, leading to strong adaptive immunity. ***Immunol Rev.*** 220, 183-98 (2007)

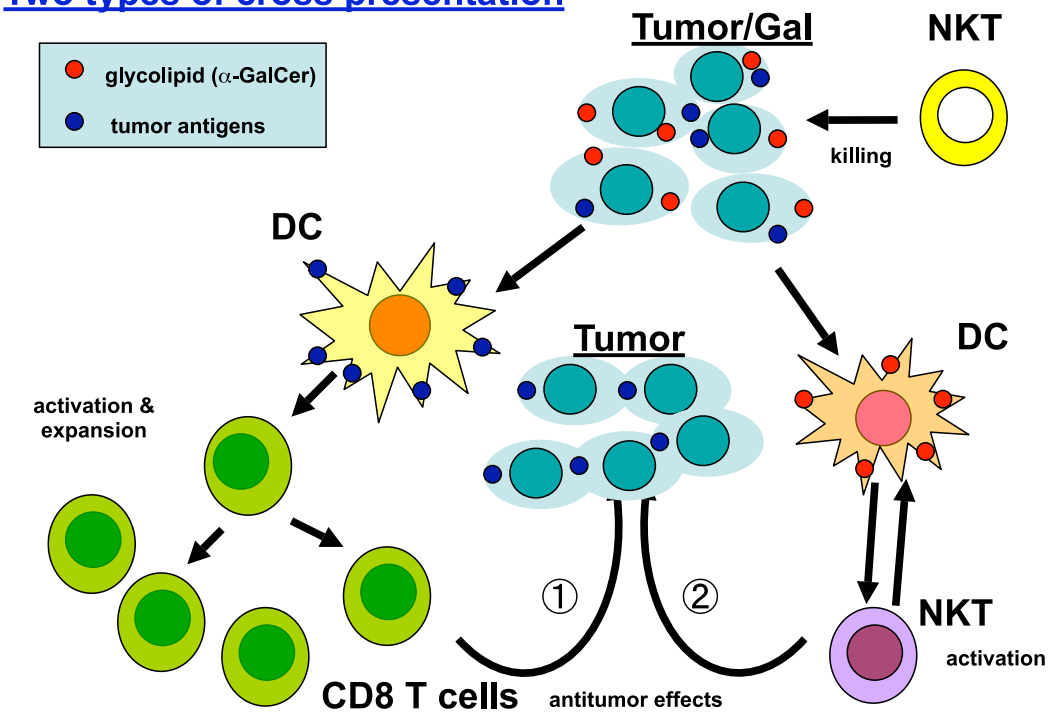

Figure **Two types of cross-presentation by DCs.**

Activated NKT cells are responsible for DC maturation in tumor/Gal immunized mice. $CD11c^+$ DCs and $CD11c^-$ non-DCs isolated from tumor/Gal immunized mice were subsequently cocultured with liver mononuclear cells (an enriched source of primary NKT cells) from naïve C57BL/6 mice. Only $CD11c^+$DCs were able to stimulate liver mononuclear cells to secrete IFN-γ. However, liver mononuclear cells from $J\alpha18^{-/-}$ mice lacking NKT cells did not respond to the DCs. In this study, DCs were found to cross present glycolipid from phagocytosed tumor/Gal to NKT cells in a CD1d-dependent manner. Thus, DCs can cross present both glycolipid to NKT cells (①) and tumor antigen to T cells (②) following i.v. injection of tumor/Gal.

In addition, mice given tumor/Gal intravenously became resistant to subcutaneous challenge with tumor cells in a CD4 and CD8 T cell dependent manner. Taken together, these results indicate that tumor cells loaded with α-GalCer are effective APCs for driving innate and adaptive immune responses, which are able to resist the establishment of metastases *in vivo*.

Analyses of critical role of host DCs in the adaptive immune response to tumor cells loaded with α-GalCer

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

3. Shimizu K, Kurosawa Y, Taniguchi M, Steinman RM, Fujii S. Cross-presentation of glycolipids from tumor cells loaded with α-galactosylceramide leads to potent and long-lived T cell mediated immunity via dendritic cells. ***J. Exp. Med.*** 204, 2641-53 (2007)
4. Shimizu K, Goto A, Fukui M, Taniguchi M, Fujii S. Tumor cells loaded with α-galactosylceramide induced innate NKT and NK cell-dependent resistance to tumor implantation in mice. ***J Immunol.*** 178, 2641-53 (2007)
5. Shimizu K, Hidaka M, Kadowaki N, Makita N, Konishi N, Fujimoto K, Uchiyama T, Kawano F, Taniguchi M, Fujii S. Evaluation of the function of human invariant NKT cells from cancer patients using α-alactosylceramide-loaded murine dendritic cells. ***J Immunol.*** 177, 3484-92 (2006).

Laboratory for Autoimmune Regulation

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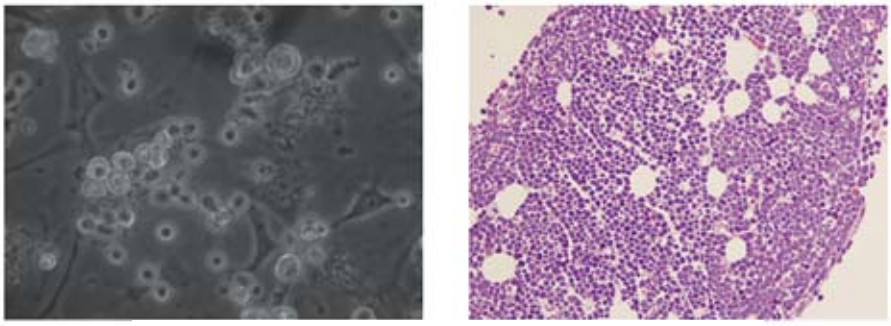
In my laboratory, we are developing new technologies for the analysis of immune responses *in vivo* and applying these technologies for the understanding of the autoimmune process. We have established, at present, four new *in vivo* imaging technologies: 3D visualization of target organ and cells, monitoring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

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

Monitoring Molecular Movement using Diffracted X-ray tracking (DXT) has been undertaken in collaboration with Drs. Kozono (Tokyo Science Univ.) and Sasaki (Spring8). DTX can detect sub-micro second level movements of proteins in solution and has been shown to be a useful tool to visualize molecular movement in physiological conditions. We used this technology to monitor the movement of the MHC/peptide complex and found that the movement of the peptide in the groove is relatively constant among peptides with different affinity to the MHC. However, movement of MHC differs significantly depending on the peptide in the groove. This is the first demonstration that peptides bound to the MHC influence the structural stability of the MHC/peptide complex. Moreover, analysis of the relationship between T cell recogni-

Recent publications

1. Tsuji M., Komatsu N., Kawamoto S., Suzuki K., Kanagawa O., Honjo H., Hori S. and Fagarasan S. Preferential generation of follicular B helper T (Tfh) cells from Foxp3+ T cells in gut Peyer's patches. ***Science*** (in press)
2. Wakayama S., Hikichi T., Ohta H., Mizutani E., Iwaki T., Kanagawa O., and Wakayama T. Production of healthy cloned mice from bodies frozen for 16 years. ***PNAS***, 2008 105; 17318



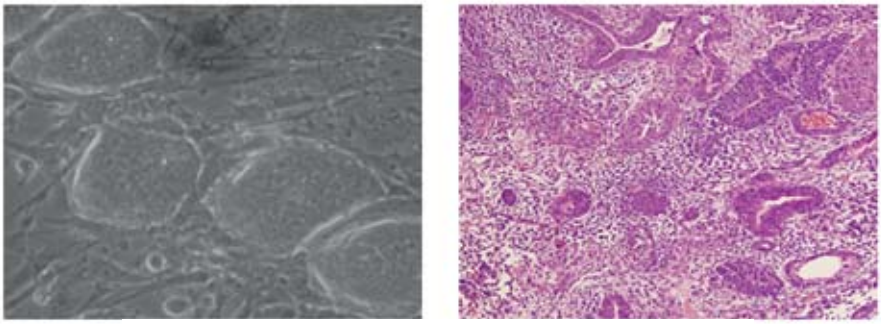


Figure **Comparison of the original B lymphoma and the lymphoma-derived ES cells**
In vitro cultured cells (*left panel*) and *in vivo* tumor/teratoma formation (*right panel*)

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

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

3. Tomura M., Yoshida N., Tanaka J., Karasawa S., Miwa Y., Miyawaki A., and Kanagawa O. Monitoring cellular movement *in vivo* with photoconvertible fluorescence protein "KAEDE' transgenic mice. ***PNAS*** 2008 105; 10871

4. Mori Y., Kato T., Kodaka T., Kanagawa EM., and Kanagawa O., Protection of IFN-g signaling–deficient NOD mice from diabetes by cyclephosphamide. ***Int. Immunol.*** 2008 20; 1231

5. Takase T., Kanagawa EM., and Kanagawa O., Age-dependent T cell receptor revision mediated by interaction between αβTCR and self-antigens. ***The Journal of Immunology*** 2007 179: 2163-9.

Research Unit for Immune Homeostasis

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A small subpopulation of T lymphocytes known as regulatory T cells (T_{reg}) play a central role in establishing and maintaining immunological self-tolerance. This concept has been well illustrated by previous findings by us and others that the development and function of T_{reg} is controlled by the transcription factor Foxp3 and that defective generation of functional T_{reg} underlies the catastrophic autoimmune pathology that develops in Foxp3-mutant *scurfy* mice and human patients with the IPEX syndrome. The identification of Foxp3 as a central regulator of T_{reg} differentiation and function has provided a key to a number of outstanding unresolved questions concerning these cells: their role in tolerance and immune regulation, their physiology including their origins, the mechanisms controlling their development and function, and their antigen specificity. Resolving these issues is the goal of this laboratory.

Heterogeneity of natural Foxp3$^+$ T cells: A committed regulatory T cell lineage and a minor subpopulation retaining developmental plasticity

Natural T_{reg} represent a dedicated lineage of T lymphocytes committed to suppressive functions, and expression of the transcription factor Foxp3 is thought to specifically identify this lineage. Recent studies have, however, challenged this prevailing notion and suggested that T_{reg} may be a plastic population with the capacity to convert into effector T helper cells (T_h). To reconcile these two apparently contradictory views, we examined the stability of Foxp3 expression and T_{reg} phenotype in natural Foxp3$^+$ T cells isolated from un-manipulated Foxp3-reporter mice. Our results showed that, whereas the majority of natural CD4$^+$Foxp3$^+$ T cells maintain stable Foxp3 expression after adoptive transfer to lymphopenic or lymphoreplete recipients, a minor fraction enriched within the CD25$^-$ subset do not. Some of those Foxp3$^-$ T cells adopt effector T_h functions, whereas some retain "memory" of previous Foxp3 expression and reacquire Foxp3 upon activation. This minority "unstable" population exhibits flexible responses to cytokine signals, relying on TGF-β to maintain Foxp3 expression, and responding to others by differentiating into effector T_h *in vitro*. In contrast, CD4$^+$Foxp3$^+$CD25^{high} T cells are resistant to effector T_h conversion even after many rounds of cell division. These results demonstrate that natural Foxp3$^+$ T cells are a heterogeneous

Recent publications

1. Tsuji, M.*, Komatsu, N.*, Kawamoto, S.* (*equal contribution), Suzuki, K., Kanagawa, O., Honjo, T., Hori, S.†, and 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)
2. Komatsu, N., Mariotti-Ferrandiz, M.E., Wang, Y., Malissen, B., Waldmann, H., and Hori, S. Heterogeneity of natural Foxp3$^+$ T cells: A committed regulatory T cell lineage and an uncommitted minor population retaining plasticity. ***PNAS***, 106: 1903-1908 (2009)

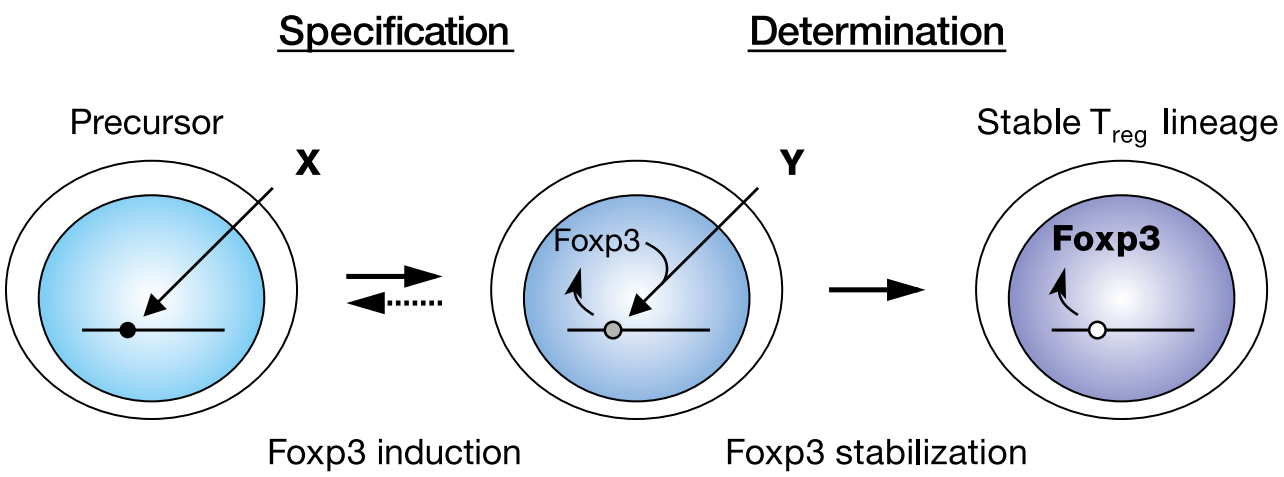

Figure **A model for the T_{reg} lineage commitment.** T_{reg} precursor cells receive signals (denoted as "X") that initiate Foxp3 transcription. These signals and the induced Foxp3 protein are not sufficient to reinforce and sustain Foxp3 transcription and thus this Foxp3 expression may be transient and reversible. For the T_{reg} lineage determination, these transitory cells must receive other signals (denoted as "Y") that cooperate with Foxp3 to ensure stable and high levels of Foxp3 expression.

population consisting of a committed T_{reg} lineage and an uncommitted subpopulation retaining developmental plasticity, and provide evidence that the T_{reg} lineage is determined by a higher order regulation operating upstream of, and in cooperation with, Foxp3 (Figure).

Based on these findings, we have investigated the role of "unstable" Foxp3$^+$ T cells in antibody responses, in collaboration with Dr. Fagarasan's laboratory (Lab for Mucosal Immnity). Our results showed that, compared to Foxp3$^-$ "conventional" CD4 T cells, Foxp3$^+$ T cells preferentially differentiate into follicular B helper T (T_{FH}) cells that promote IgA production in the Peyer's patches when transferred into T cell-deficient mice. The conversion of Foxp3$^+$ T cells into T_{FH} cells requires Foxp3 down-regulation and interaction with B cells. These new findings provide insight into how the suppression of inflammatory responses and induction of IgA synthesis occur in the gut.

Delving into the Foxp3-dependent molecular program of T_{reg} function: Impact of IPEX mutations on T_{reg} differentiation and function

In order to understand the molecular mechanisms by which Foxp3 controls T_{reg} differentiation and function, we have been addressing how Foxp3 mutations identified in IPEX patients impact on T_{reg} development and function. We first conducted retroviral gene transductions studies and showed that all the IPEX mutations which we examined are amorphic or hypomorphic in that T cells expressing these mutants fail to exert full suppressive activity *in vitro* and *in vivo*. Interestingly, one of the mutations fails to affect the expression of T_{reg} phenotypic markers, suggesting that the patients carrying this particular mutation develop IPEX syndrome due to impaired T_{reg} effector function rather than defective T_{reg} development. To test this possibility *in vivo*, we recently established mice in which this particular IPEX mutation is knocked into the endogenous *foxp3* locus. Our analyses revealed that the knock-in animals develop an autoimmune disease similar to, albeit less severe than, *scurfy* disease, indicating that this mutation causes the IPEX syndrome in patients harboring it. Furthermore, T cells expressing this mutant Foxp3 do develop in the thymus and periphery, and display a transcriptional profile which closely resembles that of WT Foxp3-expressing T cells except for a small number of genes. We are now investigating whether any of these genes contributes to T_{reg} function.

One of the key questions regarding T_{reg} cell biology is to understand how T_{reg} cells suppress immune responses. By learning from experiments in nature, i.e. naturally occurring *Foxp3* gene mutations identified in IPEX patients, we hope to understand the molecular mechanisms by which Foxp3 controls the T_{reg} suppressive function.

3. Mori, Y., Kato, T., Kodaka, T., Kanagawa E.M., Hori, S., and Kanagawa, O. Protection of IFN- γ signaling deficient NOD mice from diabetes by cyclophosphamide. ***International Immunology*** 20: 1231-1237 (2008)
4. Hori, S. Rethinking the molecular definition of regulatory T cells. ***European Journal of Immunology*** 38: 928-930 (2008)
5. Komatsu, N. and Hori, S. Full restoration of peripheral Foxp3$^+$ regulatory T cell pool by radioresistant host cells in scurfy bone marrow chimeras. ***PNAS*** 104: 8959-8964 (2007)

Laboratory for Immuno-Chaperones

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

Mechanisms that regulate production of T cell epitopes by the proteasome, however, are still poorly understood. One of our research goals is to identify the mechanisms of proteasome-mediated epitope production from endogenous proteins, especially focusing on the role of a regulatory particle, proteasome activator PA28, and hsp90-mediated 26S proteasome biogenesis.

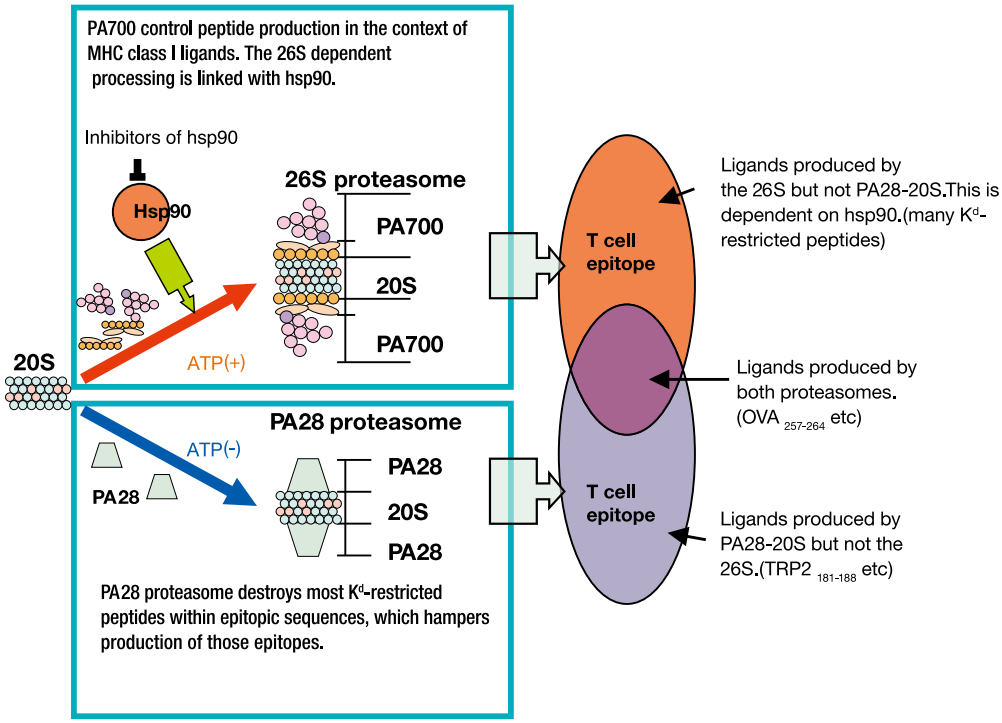
The role of PA28 in MHC I antigen processing.

IFN-γ stimulation increases the occurrence of football- (homo PA28-20S) and hybrid-shaped (PA28-20S-PA700) proteasomes: The former is a complex where PA28 is attached to both ends of the central 20S proteasome while the latter comprises the 20S proteasome flanked by PA28 on one end and PA700 (alias 19S Cap) on the other, functioning as an ATP-dependent protease, similar to the 26S proteasomes, which have PA700 on both ends. The fundamental role of PA28 is still largely unknown. One mechanism by which PA28 is postulated to stimulate the proteasome is called the gate-opening theory. Here, the usually closed α-ring of the 20S is thought to immediately open up after association with PA28, which enables translocation of substrates into the core catalytic 20S and production of antigenic peptides. PA700 is also implicated as a chaperone complex that passes through substrates into the core 20S. Based on that perspective, both PA28 and PA700 may open the α-ring of the 20S to facilitate substrate loading and peptide generation. It is not known whether this gate-opening is the only mechanism for these regulatory particles but, if so, the peptide repertoire produced either by PA28 or PA700 dependent

Recent publications

1. Yamano T, Mizukami S, Murata S, Chiba T, Tanaka K and Udono H. Hsp90 mediated assembly of the 26S proteasome is involved in MHC class I antigen processing. ***J. Biol. Chem.*** 283, 28060-28065, 2008.

2. 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.Immunology*** 181, 1655-1664, 2008.



Figure

There are two distinct pathways for MHC class I antigen processing. One is dependent on PA 28 that associates and stimulates the 20S proteasome in an ATP independent manner and produces T cell epitopes. The other is dependent on hsp90 that stimulates assembly of the 26S proteasome in an ATP dependent manner, by which it contributes to production of T cell epitopes. These mechanisms give rise to three different T cell epitope repertoires that are dependent on either PA28 or hsp90 or both.

mechanisms should be the same and depend on the activity of the 20S proteasome complex itself.

We demonstrated an allele-specific role of PA28 in antigen processing. Retrovirus transduced overexpression of PA28α decreased expression of K^d (D^d), while it increased K^b and L^d on the cell surface. By contrast, overexpression of PA28αΔC5, a mutant with a deletion of its five C-terminal residues and capable of attenuating the activity of endogenous PA28, produced the opposite effect on expression of these MHC class I molecules. Knockdown of both PA28α and β by siRNA profoundly augmented expression of K^d and D^d but not of L^d on the cell surface. Moreover, we found that the PA28-associated proteasome preferentially digested within epitopic sequences of K^d, although correct C-terminal flanking sequences were removed, which in turn hampered production of K^d ligands. Therefore, PA28 negatively influences processing of K^d (D^d) ligands, thereby, down-regulating antigen presentation by those MHC class I molecules. It also efficiently produces K^b (L^d) epitopes, leading to up-regulation of these MHC molecules.

The role of hsp90 in MHC I antigen processing.

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

We demonstrated that hsp90 is essential in assembly of the 26S proteasome and, as a result, is involved in epitope production. Addition of recombinant hsp90α to cell lysates enhanced chymotrypsin-like activity of the 26S proteasome in an ATP dependent manner. We pulled down histidine-tagged hsp90α-and PA28α-induced, newly assembled 26S proteasomes from the cell extracts for *in vitro* epitope production assays, and found that these structures are sensitive to geldanamycin, an hsp90 inhibitor. We found that a cleaved epitope unique to the proteasome was pulled down by both hsp90α and PA28α, while two different epitopes were identified in the hsp90α and PA28α pulldowns, respectively. Processing of these respective peptides *in vivo* was faithfully enhanced by the protein combinations used for the proteasome pulldowns. Inhibition of hsp90 *in vivo* by geldanamycin partly disrupted the 26S proteasome structure, consistent with downregulated MHC class I expression. Thus, hsp90 facilitates MHC class I antigen processing through epitope production in a complex of the 26S proteasome.

3 Mizukami S, Kajiwara C, Hiroshi Ishikawa H, Katayama I, Yui K, and Udono H. Both $CD4^+$ and $CD8^+$ T cell epitopes fused to heat shock cognate protein 70 (hsc70) can function to eradicate tumors. ***Cancer Science*** vol.99,1008-1015, 2008.

4. Susumu S, Nagata Y, Ito S, Matsuo M, Valmori D, Yui K, Udono H, Kanematsu T. Cross-presentation of NY-ESO-1 cytotoxic T lymphocyte epitope fused to human heat shock cognate protein 70 by dendritic cells. ***Cancer Science*** Vol.99 107-112. 2008.

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

6. Honma,K., Udono,H., Kohno, T., Yamamoto,K., Ogawa, A., Takemori,T., Kumatori,A., Suzuki,S., Matsuyama,T and Yui, K. Interferon regulatory factor-4 negatively regulates the production of proinflammatory cytokines by macrophages in response to LPS. ***Proc. Natl Acad. Sci. USA.*** Vol.102, 16001-16006, 2005.

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CD1d-restricted natural killer T (*i*NKT) cells express both a single invariant Vα14 antigen receptor, and are therefore also known as Vα14^{+} NKT or Vα14 iNKT cells, and NK receptors such as NK1.1. Because of this unique combination of receptors, the *i*NKT cells occupy a distinctive intermediary position between innate and acquired immunity. Furthermore, because of their ability to quickly release large amounts of cytokines, such as interferon (IFN)-γ and IL-4, and their apparent self-reactivity, *i*NKT cells have been hypothesized to play important roles in the initiation and regulation of many types of immune responses. The multifunctional *i*NKT cells have also been implicated in various autoimmune diseases such as rheumatoid arthritis, SLE, type I diabetes, systemic sclerosis, and in allergic diseases such as asthma. In the case of organ transplantation, the control of *i*NKT cells is required for the maintenance of allograft tolerance. Our group studies the regulation of immune functions and responses, both beneficial and harmful, mediated by *i*NKT cells. We hope to contribute to the development of new strategies for human immune therapy.

A novel subset of mouse NKT cells bearing the IL-17 receptor B responds to IL-25 and contributes to airway hyperreactivity.

Airway hyperreactivity (AHR) is known to be associated with Th2 cytokines including IL-4, IL-5, and IL-13, which regulate effector cell functions. Indeed, over-expression of these Th2 cytokines results in the development of AHR. However, efforts to ameliorate experimental asthma with antibodies against Th2 cytokines have generally proven unsuccessful. Among these, only IL-13 seems to be a key cytokine responsible for goblet cell hyperplasia, airway remodeling, and AHR, because inhibition of IL-13 activity, but not that of other Th2 cytokines, by a blocking antibody suppresses both AHR and airway inflammation.

IL-25 (also known as IL-17E) is a member of the structurally related IL-17 cytokine family that has recently been reported to be produced by activated Th2 cells and mast cells, resulting in enhancement of AHR. Administration of a blocking antibody against IL-25 or genetic ablation of the *Il25* gene eliminates Th2 responses. Conversely, systemic expression of either human or mouse IL-25, or administration of recombinant IL-25, induces Th2-type immune responses, including increased serum IgE levels, blood eosinophilia, and pathologic

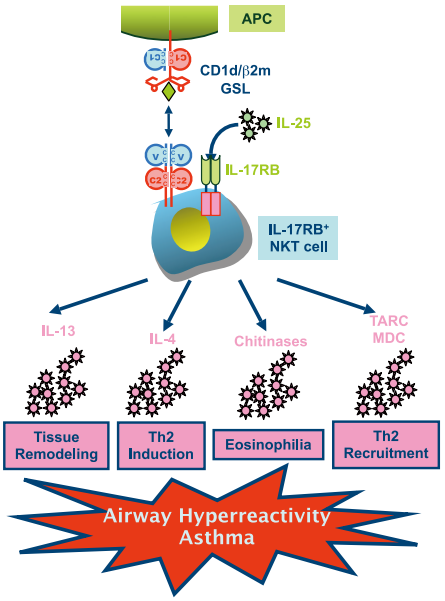
Recent publications

1. Terashima A., Watarai H., Inoue S., Sekine E., Nakagawa R., Hase K., Iwamura C., Nakajima H., Nakayama T., Taniguchi M. A novel subset of mouse NKT cells bearing the IL-17 receptor B responds to IL-25 and contributes to airway hyperreactivity. ***J. Exp. Med.*** 205, 2727-2733 (2008).
2. Nyambayar D., Watarai H., Sakata S., Taniguchi M. Identification of CD4-CD8- double-negative natural killer T cell precursors in the thymus. ***PLoS ONE*** 3, e3688 (2008).

changes in the lung and other tissues. These findings clearly demonstrate a pivotal role of IL-25 as a mediator of Th2 responses, suggesting that IL-25 lies upstream of the classical Th2 cytokine responses.

Natural killer T (NKT) cells, characterized by the expression of an invariant antigen receptor encoded by a *Vα14Jα18* in mice or *Vα24Jα18* in humans, are also involved in the development of asthma, because NKT cell-deficient Jα18$^{-/-}$ mice fail to develop antigen-induced AHR. Th2 cells are not essential for NKT cell-mediated AHR development, because activation of NKT cells induces AHR in the absence of CD4^{+} T cells in *MHC class* II-deficient mice. These findings suggest that NKT cells are directly involved in the development of AHR independent of Th2 responses under some conditions.

Here, we identified a novel subset of NKT cells that expresses the IL-17 receptor B (IL-17RB) for IL-25 and is essential for the induction of AHR. IL-17RB is preferentially expressed on a fraction of CD4^{+} NKT cells, but not on other splenic leukocyte populations tested. IL-17RB^{+} NKT cells produce predominantly IL-13 and Th2 chemokines upon stimulation with IL-25 *in vitro*. IL-17RB^{+} NKT cells were detected in the lung, and mice depleted of IL-17RB^{+} NKT cells by IL-17RB specific monoclonal antibodies and NKT cell-deficient Jα18$^{-/-}$ mice failed to develop IL-25-dependent AHR. Moreover, cell transfer of IL-17RB^{+} but not IL-17RB^{-} NKT cells into Jα18$^{-/-}$ mice successfully reconstituted AHR induction. These results strongly suggest that IL-17RB^{+} NKT cells play a crucial role in the pathogenesis of asthma. Our findings also clearly demonstrated that IL-17RB^{+} NKT cells are the target of IL-25 in the development of AHR or asthma. The efficacy with which IL-17RB antibodies prevent AHR and reduce Th2-cytokine-induced inflammation *in vivo* suggests that IL-17RB is an ideal therapeutic target for asthma.



Figure

Mechanisms in the Induction of IL-25 dependent AHR

IL17RB+ iNKT cells activated by Cd1d/GSL (glycosphingolipid) bearing APC in the presence of IL25 secrete Th2 cytokines and chemokines, eg. TARC and MDC, and other inflammatory mediators that ultimately induce AHR and asthma.

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

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

Although triggering of toll-like receptors (TLRs) activates PDCs to produce type I IFNs, optimal production does not depend solely on TLRs, but requires TLR-mediated secondary events. Since type I IFN-α/β receptor (IFNAR)-deficient PDCs fail to produce type I IFNs in response to TLR agonists, IFNAR-signaling provides these TLR-mediated secondary events in a positive feedback loop. Constitutive expression of interferon regulatory factor (IRF)-7 in PDCs is also thought to account for the augmentation of type I IFN production. However, IRF-7 is expressed in various cell types, and the level of IRF-7 expression in these cells does not correlate with the amount of type I IFN produced, indicating that some other signaling events are involved in type I IFN production following TLR triggering.

We identified a novel cell surface molecule, PDC-TREM, a member of the triggering receptor expressed on myeloid cells (TREM) family, which is preferentially expressed on TLR-stimulated PDCs. Surface expression of PDC-TREM requires not only TLR- but also IFNAR-signaling. PDC-TREM$^{-/-}$-derived PDCs had a dramatically reduced TLR-dependent IFN-α response. In terms of signaling events, PDC-TREM tends to activate PDC via a BCR-like signalosome composed of Phospholipase C (PLC) γ2, the CARMA1/MALT1/Bcl-10 complex, IkappaB kinase-alpha (IKK-α), phosphatidylinositol 3 kinase (PI3K), and mammalian target of rapamycin (mTOR), in the late phase of TLR-mediated activation. Therefore, TLR- and IFNAR-signaling induce expression of PDC-TREM, and PDC-TREM signaling then mediates augmented production of type I IFNs.

3. Watarai H., Sekine E., Inoue S., Nakagawa R., Kaisho T., Taniguchi M. PDC-TREM, a plasmacytoid dendritic cell-specific receptor, is responsible for augmented production of type I interferon. ***Proc. Natl. Acad. Sci. U S A.*** 105, 2993-2998 (2008).

4. Watarai H., Nakagawa R., Omori-Miyake M., Nyambayar D., Taniguchi M. Methods for detection, isolation, and culture of mouse and human invariant NKT cells. ***Nat. Protoc.*** 3, 70-78 (2008).

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Laboratory for Dendritic Cell Immunobiology

Dendritic cells (DCs) are specialized antigen (Ag)-presenting cells (APCs) that play a dual role in inducing adaptive immune responses and in maintaining self-tolerance. DCs consist of heterogeneous subsets, including conventional DCs (cDCs) and plasmacytoid DCs (pDCs), distinguishable by surface and intracellular phenotypic markers, immunological function, and anatomic distribution. Immature DCs (iDCs) serve as sentinels, recognizing the presence of invading pathogens through various pattern-recognition receptors (PRRs), and become mature DCs (mDCs) with upregulated expression of major histocompatibility complex (MHC) and costimulatory molecules under inflammatory conditions. The mDCs are also able to move via the afferent lymphatics into the T-cell area of secondary lymphoid tissues. There they select rare Ag-specific naïve T cells and induce their activation and differentiation into effector cells, thereby initiating primary immune responses. Accumulating indirect evidence suggests that iDCs are involved in the induction of peripheral tolerance under steady state conditions *in vivo*. On the other hand, the modification of iDCs by certain immunosuppressive molecules generates tolerogenic DCs, which not only show a reduced T-cell stimulatory capacity but also induce anergic T cells and regulatory T cells (T_{regs}). We have previously identified modified DCs that have a greater capacity than previously characterized tolerogenic DCs to induce anergic T cells as well as T_{regs}. We have designated this unique DC subset regulatory DCs (DC_{regs}). Our goal is: (1) to clarify the molecular mechanisms underlying the T-cell immunoregulatory function of DC_{regs},: (2) to develop immunotherapy with DC_{regs} for immunopathogenic diseases, and (3) to characterize the specific DC subsets involved in immune regulation.

Crucial role of CD200R3 in the induction of peripheral tolerance by regulatory dendritic cells

We have previously reported that *in vitro*-generated DC_{regs}, which have moderately high levels of MHC class II but limited expression of costimulatory molecules, could induce anergic $CD4^+$T cells and $CD4^+CD25^+Foxp3^+T_{regs}$ from $CD4^+CD25^-Foxp3^-$T cells, and had a greater capacity to protect mice from immunopathogenesis than the classical tolerogenic DCs. Although the tolerogenicity of DC_{regs} generated *in vitro* may involve the characteristic MHC class II and

Recent publications

1. 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., and Sato K. Naturally occurring regulatory dendritic cells regulate murine cutaneous chronic graft-versus-host disease. ***Blood,*** *in press*, 2009.
2. Fujita S., Yamashita N., Ishii Y., Sato Y., Sato K., Eizumi K., Fukaya T., Nozawa R., Takamoto Y., Yamashita N., Taniguchi M., Sato K. Regulatory dendritic cells protect against allergic airway inflammation in a murine athmatic model. ***J. Allergy Clin. Immunol.*** 121, 95-104 (2008)

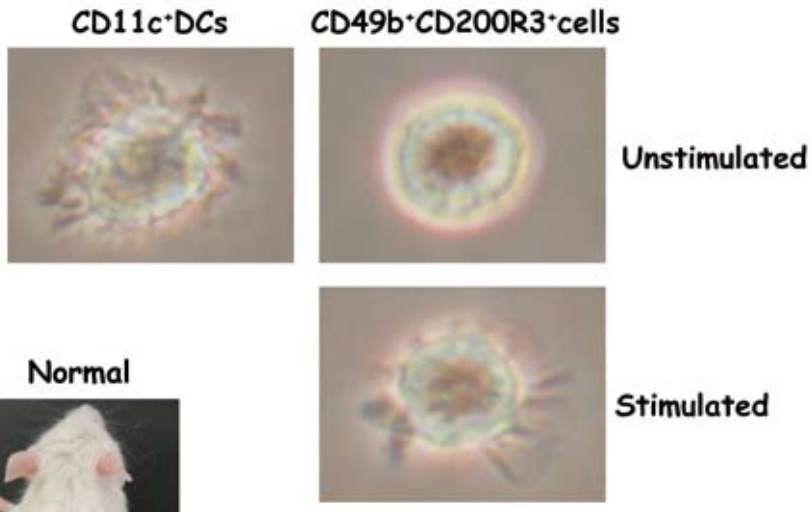

Figure 1 Morphology of CD11c$^+$DCs and CD49b$^+$CD200R3$^+$DC$_{regs}$ was examined by phase contrast microscopy (x 400) following stimulation with or without CpG ODN for 24 h.

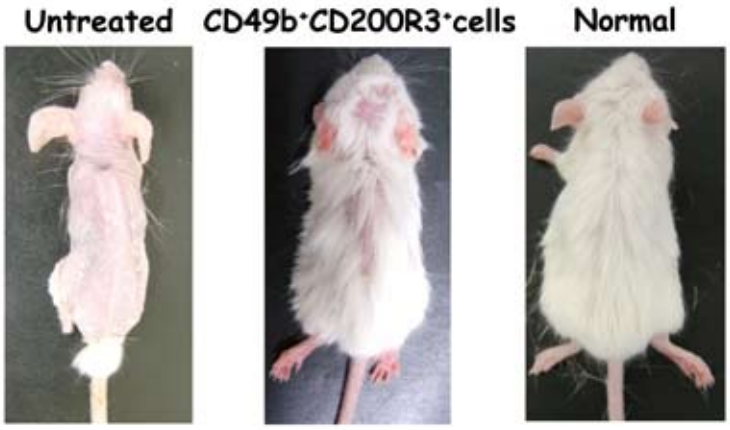

Figure 2 Regulation of cutaneous cGVHD by CD49b$^+$CD200R3$^+$ DCregs. Appearance of cutaneous cGVHD in recipient BALB/c mice on day 45 after transplantation with T-cell-depleted BM and CD4$^+$CD25$^-$T cells obtained from B10.D2 mice.

costimulatory molecule phenotype, the precise molecular mechanism underlying their unique function is largely unknown, primarily owing to the lack of specific functional molecules. In the present study, we attempted to identify such molecules. DC$_{regs}$ generated *in vitro* exclusively expressed the CD200 receptor 3 (CD200R3), and the defective activation of Ag-specific CD4$^+$T cells by *in vitro*-generated DC$_{regs}$ was abrogated by anti-CD200R3 mAb. A soluble CD200R3ext-huIgFc fusion protein displayed the suppressive and the tolerogenic effects on the Ag-specific CD4$^+$T-cell response. Both IL-10 and TGF-β1 induced the expression of CD200R3 on splenic CD11c$^+$DCs, although this treatment had no effect on the expression of MHC and costimulatory molecules, and CD11c$^+$CD200R3$^+$DCs showed a lower ability to stimulate Ag-specific CD4$^+$T cells than the CD11c$^+$CD200R3$^-$DCs. The ectopic expression of CD200R3 by retroviral transfection converted iDCs generated *in vitro* into tolerogenic DCs, although there was no change in the expression of MHC and costimulatory molecules. In conclusion, our results suggest that CD200R3 plays a pivotal role in the unique function of *in vitro*-generated DC$_{regs}$, and that CD200R3 expressed on CD11c$^+$DCs mediates the control of peripheral immune suppression in tolerogenic settings.

Naturally occurring regulatory dendritic cells regulate cutaneous chronic graft-versus-host disease

Chronic graft-versus-host disease (cGVHD) is a limiting factor in allogeneic bone marrow transplantation (alloBMT) for the treatment of leukemia and other malignancies. Relative to the process that initiates and promotes cGVHD, its regulation is poorly understood. In this study, we examined the role of naturally occurring DC$_{regs}$ in a murine MHC-compatible and multiple minor histocompatibility Ag (miHAg)-incompatible model of cGVHD in alloBMT. CD49$^+$CD200R3$^+$cells showed similar phenotype and function to *in vitro*-generated DC$_{regs}$, which formally distinguishes them from other leukocytes, suggesting that CD49$^+$CD200R3$^+$cells are the natural counterpart of *in vitro*-generated DC$_{regs}$. Treatment of the recipient mice following alloBMT with the recipient-type CD49$^+$CD200R3$^+$cells as well as with *in vitro*-generated DC$_{regs}$ protected against cGVHD, and the protection was associated with the generation of Ag-specific anergic CD4$^+$T cells as well as CD4$^+$CD25$^+$Foxp3$^+$T$_{regs}$ from donor-derived alloreactive $^-$T cells. In addition, the depletion of CD49$^+$CD200R3$^+$cells before alloBMT enhanced the progression of cGVHD. In conclusion, CD49$^+$CD200R3$^+$cells act as naturally occurring DC$_{regs}$ to control the pathogenesis of cGVHD following alloBMT, an effect mediated through the control of the transplanted alloreactive CD4$^+$T cells.

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

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

5. Matsumura Y., Kobayashi T., Ichiyama K., Yoshida R., Hashimoto M., Takimoto T., Tanaka K., Chinen T., Shichita T., Wyss-Coray T., Sato K., Yoshimura A. Selective expansion of Foxp3-positive regulatory T cells and immunosuppression by SOCS3-deficient dendritic cells. ***J. Immunol.*** 179, 2170-2179 (2007)

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

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Laboratory for Cytokine Signaling

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

Mast Cell Biology and molecular mechanisms of mast cell functions

We have investigated the roles of mast cells in allergy and the molecular mechanisms of mast cell activation. We have dissected the degranulation process of mast cells and are now able to define several distinct phases in the process. First, FcεRI stimulation triggers microtubule polymerization and granule translocation to the plasma membrane in a calcium-independent manner. Second, the granules fuse with the plasma membrane in a well-characterized calcium-dependent manner. Furthermore, we showed that the Fyn / Gab2/RhoA signaling pathway plays a critical role in the calcium-independent microtubule-dependent pathway (Nishida et al., *J Cell Biol.* 2005). FcεRI-induced granule translocation to the plasma membrane was found to be dependent on zinc (Zn). A Zn-dependent process is also involved in cytokine production. Remarkably, *in vivo* treatment with a Zn chelator inhibited the PCA reaction (Kabu et al., *J Immunology*. 2006). We recently found that a Zn transporter, Znt5/Slc30a5 is required for FcεR-mediated cytokine production, but not degranulation. As a result, Znt5$^{-/-}$ mice had defects in mast cell-mediated delayed-type allergic reactions but not in the immediate-type reaction. Znt5 was required for FcεRI-induced translocation of PKC to the plasma

Recent publications

1. Fukada, T*., N. Civic*, T. Furuichi*, S. Shimoda, K. Mishima, H. Higashiyama, Y. Idaira, Y. Asada, H. Kitamura, S. Yamasaki, S. Hojyo, M. Nakayama, O. Ohara, H. Koseki, H.G. dos Santos, L. Bonafe, R. Ha-Vinh, A. Zankl, S. Unger, M.E. Kraenzlin, J.S. Beckmann, I. Saito, C. Rivolta, S. Ikegawa, A. Superti-Furga, and T. Hirano. (*equal contribution). The Zinc Transporter SLC39A13/ZIP13 is Required for Connective Tissue Development; Its Involvement in BMP/TGF-β Signaling Pathways. ***PLoS ONE*** 3 (11), e3642, (2008)

membrane and the nuclear translocation of NF-kappa B. Thus, Znt5 is selectively required for mast cell-mediated delayed-type allergic responses, and is a novel player in PKC signaling (Figure 1, Nishida et al., under revision).

Role of Zn and its transporters in immune and connective tissues

We found that the Zn transporter Slc39a6/Zip6/Liv1 is a STAT3 target gene and showed that it has a role in cell migration during early zebrafish development. (Yamashita et al., *Nature*. 2004). We also found that LPS-induced maturation of DCs is partly mediated through lowering the intracellular concentration of free Zn by down regulating Zn transporters, including Slc39a6 (Kitamura et al., *Nature Immunology*. 2006). These observations suggest that intracellular Zn is involved in MHC class II cell surface expression through regulating endocytosis and membrane trafficking. In order to understand the role and function of Zn transporters *in vivo*, we have generated mice deficient in the Slc39/Zip family of zinc transporters. Recently, we found that Slc39a13/Zip13 deficient mice show changes in connective tissues reminiscent of the human disease, Ehlers-Danlos syndrome (EDS). The *Slc39a13*-KO mice have defects in the maturation of osteoblasts, chondrocytes, odontoblasts, and fibroblasts. Impairment of bone morphogenic protein (BMP) and TGF-beta signaling was observed in the corresponding tissues and cells. Homozygosity for a *SLC39A13* loss of function mutation was identified in sibs affected with a unique variant of EDS that recapitulates the phenotype observed in *Slc39a13*-KO mice. Hence, our results have revealed a crucial role of SLC39A13 in connective tissue development at least in part due to its involvement in BMP/TGF-β signaling pathways (Figure 2, Fukada et al, *PLoS ONE*, 2008). Together these results support the idea that Zn transporters have roles not only in maintaining Zn homeostasis, but also for mediating intracellular signaling events. We propose the term “Late Zn signaling” for this type of Zn signaling, in contrast to “Early Zn signaling”. The former is dependent on changes in the transcription of Zn transporter genes, whereas the latter is not (Hirano et al, *Adv. Immunol.*, 2008).

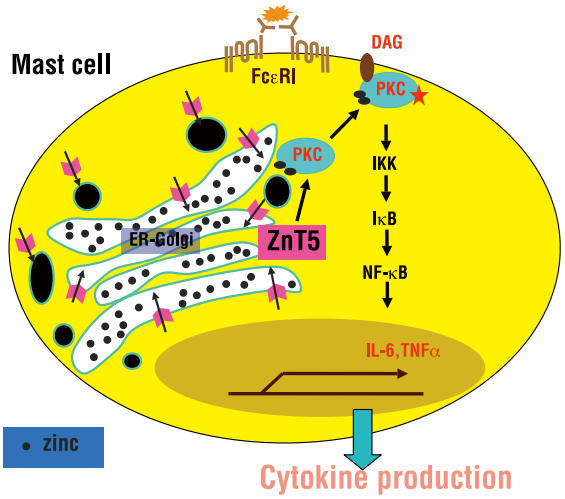


Figure 1 **Role of the zinc transporter, Znt5/Slc30a5 in mast cell-mediated allergic reaction**
Znt5 is selectively required for the mast cell-mediated delayed-type allergic response, and is a novel player in PKC signaling.

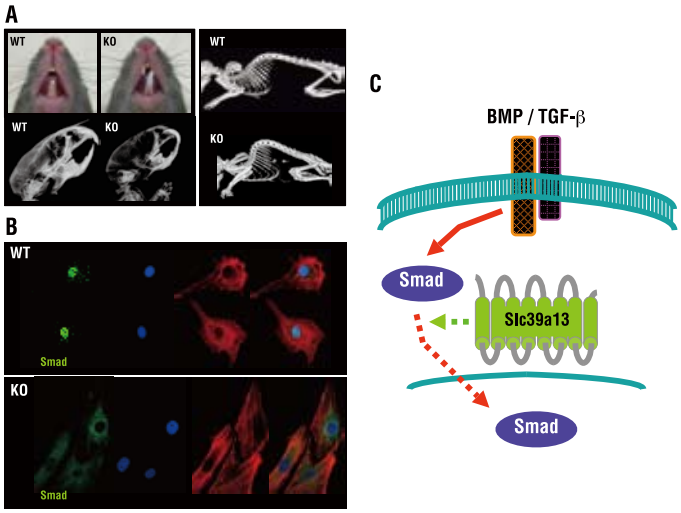


Figure 2 **Significant role of the zinc transporter Slc39a13/Zip13 in connective tissue development**

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

B. Nuclear localization of Smad proteins mediated by BMP4 stimulation is impaired in *Slc39a13*-KO osteoblasts.

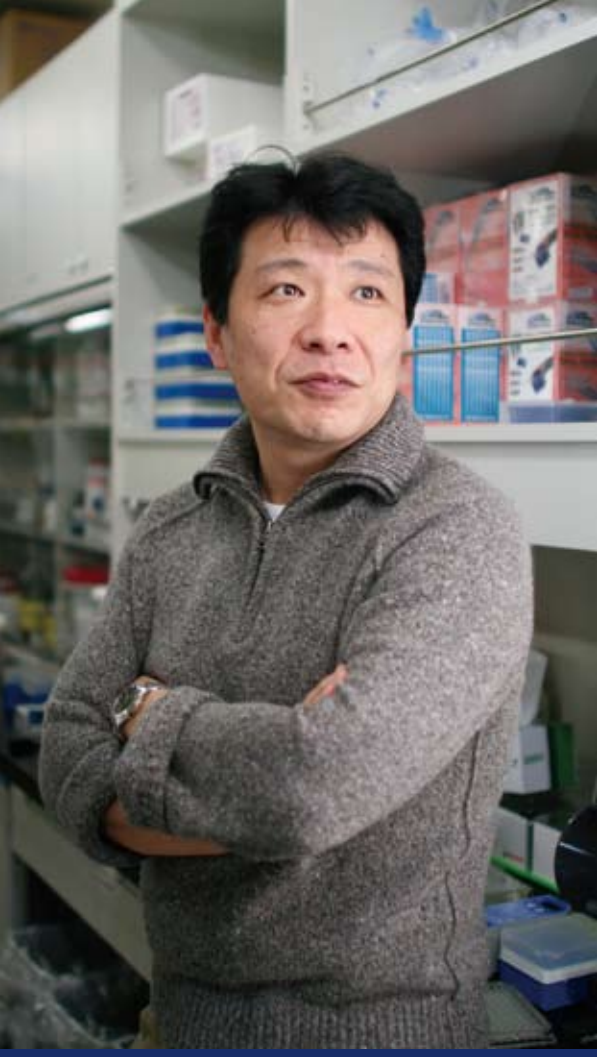
C. Involvement of Slc39a13/Zip13 in BMP/TGF-beta signaling pathways. Slc39a13 protein, which is expressed in osteoblasts, odontoblasts, chondrocytes, and fibroblasts, controls nuclear translocation of Smad transcription factors.

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

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

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

5. Kitamura, H., H. Morikawa., H. Kamon., M. Iguchi., S. Hojyo., T. Fukada., S. Yamashita., T. Kaisho., S. Akira., M. Murakami., T. Hirano. Toll-like receptor-mediated regulation of zinc homeostasis influences dendritic cell function. ***Nature Immunol.*** 7, 971-977 (2006)



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

Alternative regulation of IL-13 expression in a novel T cell subset, Th13

During the last two decades, it has become dogma that the Th1 and Th2 cytokine profiles are strictly conserved and immutable during T cell differentiation. Among the signature Th2 cytokine genes, *Il13* is thought to be controlled coordinately with *Il4* by common transcriptional mechanisms, although rare IL-13-only expressing cells have been found among canonical Th2 lineage cells in mouse and human. However, we found significant IL-13 expression in the absence of IL-4 under chronic Th1 stimulation conditions. These IL-13 producing T cells that express undetectable levels of IL-4, IL-5, and GATA3 were designated as Th13 cells. Knockdown and ectopic expression experiments demonstrated that enhanced expression of a putative clock-controlled gene, *E4bp4*, was critical for IL-13 production by Th13 cells. E4BP4 protein specifically accessed the *Il13* distal promoter, which was transcriptionaly permissive even under acute Th1 conditions. Th13 cells were generated following Th1 and Th2 biased immunization protocols in mice and were detected at elevated levels in bronchial fluid of asthmatic human patients. Consistent with a pathogenic role in airway hypersensitivity, the administration of Th13 cells in a mouse asthma model enhanced airway responses. Therefore, the

Recent publications

1. Kano, S., Sato, K., Morishita, Y., Vollstedt, S., Kim, S., Taki, S., Honda, K., **Kubo, M.**, & Taniguchi, T.; Regulation of Th1 vs. Th17 differentiation: Selective contribution of thetranscription factor IRF1 to the IFN-γ-IL-12 axis of signaling networks in $CD4^+$ T cells. ***Nature Immunology*** *9*, 34 - 41, 2008
2. Myburgh, E., Horsnell, W., Cutler, A.J., Arendse, B., **Kubo, M.**, Brombacher, F., Murine IL-4 is able to signal via chimeric human IL-4Rα/mouse γ-chain receptor, ***Mol. Immunol.*** 45(5):1327-1336. 2008

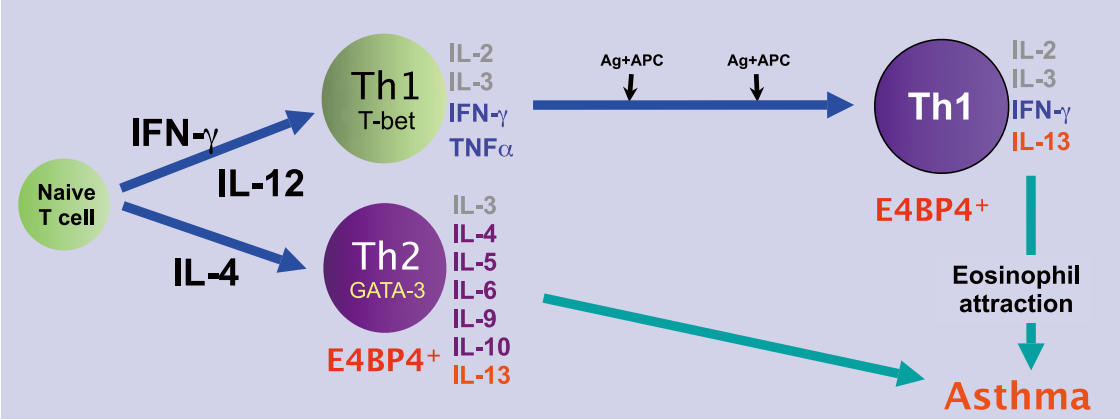

Figure **Chronic antigen stimulation induces E4BP4 mediated IL-13 production**
Significant IL-13 expression was induced in mouse Th1 cells following chronic antigenic stimulation. This chronic stimulation initiated expression of E4BP4, leading to IL-13 expression in conventional Th1 cells. The IL-13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

Il13 gene is controlled independently of the *Il4* gene normally silent in Th1 cells by E4BP4. Our findings provide novel insight into the traditional dogma of allergic incidents in otherwise chronic Th1 biased situations.

Naturally occurring IL-17 producing T cells regulate the initial phase of neutrophil mediated airway responses.

Effector Th17 cells are a major source of IL-17, a critical inflammatory cytokine in autoimmune diseases and for regulating host defense during bacterial infections. We recently found that the immune system contains a unique set of naturally occurring IL-17 producing cells, "natural" Th17 (nTh17), which have a memory-like T cell phenotype. The nTh17 cells can develop in the absence of the IL-6-STAT3 signaling axis needed for the inducible type of Th17 (iTh17) cells. The nTh17 cells are a distinct population from conventional iTh17, because nTh17 expressed substantial amounts of IL-17 (IL-17A) but not IL-17F under the influence of the master transcriptional regulator, RORγt, whereas nTh17 cells simultaneously produced IL-17A and IFN-γ. DO11.10 transgenic (Tg) mice on a *Rag* -/- background (DO11.10 *Rag* -/-) lack nTh17 cells and, following intranasal administration of OVA, IL-17 dependent-neutrophil infiltration occurred in DO11.10 Tg mice but not in the DO11.10 *Rag* -/- mice. The impaired neutrophil dependent airway response was restored by adaptive transfer of nTh17 cells. These results demonstrate that a novel T cell subset, nTh17, facilitates the early phase of antigen-induced airway responses and host defenses against pathogen invasion prior to the establishment of acquired immunity.

Regulation of Th1 vs. Th17 differentiation: Selective contribution of IRF1 to the IFN-γ-IL-12 axis of signaling networks

Interleukin-12 (IL-12) and interferon-γ (IFN-γ) are two major cytokines that drive Th1 differentiation, but the mechanisms underlying the gene network are complex and even somewhat controversial. Here we report that the IFN-γ-induced IRF1 transcription factor is central to Th1 differentiation by the induction of the gene encoding IL-12 receptor β1 subunit (IL-12Rβ1) in $CD4^+$ T cells. We show that $CD4^+$ T cells from mice deficient in the IRF1 gene ($Irf1^{-/-}$ $CD4^+$ T cells) are defective in Th1 differentiation *in vitro* and *in vivo*, and that IRF1 drives IL12 responsiveness through direct binding to and activation of the ISRE element within the IL-12Rβ1 promoter. We also demonstrate that the retroviral expression of IL-12Rβ1 results in the procurement of $Irf1^{-/-}$ $CD4^+$ T cells for Th1 differentiation via restoration of the IL-12/STAT4-IFN-γ/IRF1 axis of signaling networks, indicating that IL-12Rβ1 is the sole target of IRF1. Interestingly, although IL-12 and IL-23 share IL-12Rβ1 as a common receptor subunit, the IRF1-dependent induction of *IL-12Rβ1* is dispensable for IL-23 signaling in IL-17-producing Th17 cells, indicating a critical aspect of IL-12Rβ1 expression level in Th1 vs. Th17 commitment. Our results *in toto* place IRF1 as a new member of the transcription factors that selectively governs the Th1 limb of $CD4^+$ T cell differentiation, offering a new insight into the complex mechanisms of gene networks operating during IFN-γ and IL-12 signalings.

3. Kohfu, K., **Kubo, M.**, Ichikawa, H., Ohno, S., Habu, S., Sato, T., and Satake, M.; Pleiotropic Roles of Runx Transcription Factors in the Differentiation and Function of T Lymphocytes. ***Current Immunology Reviews*** 4, 101-115, 2008

4. Nakayama, J., Yamamoto, M., Hayashi, K., Satoh, H., Bundo, K., **Kubo, M.**, Goitsuka, R., Farrar, M.A., Kitamura, D., BLNK suppresses pre-B cell leukemogenesis though inhibition of Jak3. ***Blood***, 2008

5. Lu, L-F., Thai, T-H, Dinis Pedro Calado, D.P., Chaudhry, A., **Kubo, M.**, Kentaro Tanaka, K. Gabriel, B. G., Lee, H., Yoshimura, A., Klaus Rajewsky, R., Alexander Y. Rudensky Foxp3-dependent microRNA155 confers competitive fitness to regulatory T cells through targeting SOCS1. ***Immunity***, Jan;30(1):80-91, 2009

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Laboratory for Immunogenetics

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

ENU mutant panel study

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

During the first 5 years of this project we have screened 8,000 recessive inheritances from 88 mouse pedigrees. For basic phenotypic screening, we examined the levels of immunoglobulins, cytokines, and autoantibodies, as well as the 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 characterized the mutant phenotypes by histopathological, cytological and biochemical examinations of affected tissues, lymphoid organs and blood cells.

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

Recent publications

1. 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)

2. Piao JH, Yoshida H, Yeh WC, Doi T, Xue X, Yagita H, Okumura K, Nakano H. TNF receptor-associated factor 2-dependent canonical pathway is crucial for the development of Peyer's patches. ***J Immunol.*** 178(4),2272-7(2007)

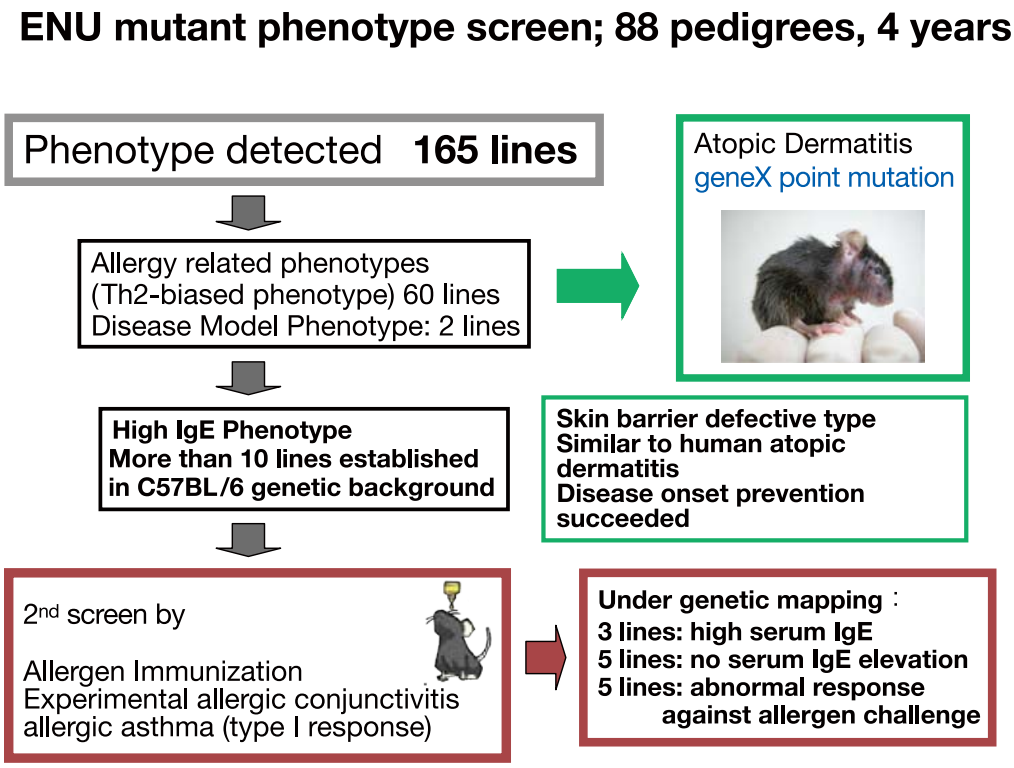


Figure **Summary of four years ENU mutant screening.** In total, approximately 8,000 mutant mice from 88 genomes have been screened and 165 phenotypes have been identified as mutant lines. The analysis of a mutant mouse that developed atopic dermatitis (AD) has clarified a new mechanism for AD development. This mouse model should be a valuable tool for studying human atopic dermatitis prevention and treatment.

Allergic disease model mutant mouse model

By ENU recessive mutant screening, we identified and established a mutant mouse line with phenotypic features reminiscent of a typical human allergic disease. The ear skin became thicker and red and, as the mice aged, they began to scratch the ear skin and/or face. The serum IgG1 or IgE levels of these mice gradually increased after the onset of dermatitis. Histopathologically, the epidermal layer was hypertrophic and many lymphocytes were found in the dermis of the lesion. These symptoms and findings are compatible with the diagnostic criteria for human atopic dermatitis (AD). A bone marrow chimerism analysis indicated the disease is caused by problems with the skin and not bone marrow-derived cells, i.e. immune cells. We have mapped the phenotype responsible region to within a few cM and identified a candidate gene mutation. We have confirmed that the mutation is responsible for the phenotype using genetic manipulation.

Perspectives

By ENU recessive mutant screening, we have identified and established more than 165 mutant lines with immunologic or hematologic defects. In keeping with our expectation, more than 60 lines showed phenotypes related to allergic diseases. We are now backcrossing some of the mice to the C3H/HeJ strain in order to map the responsible loci. As of December 2008, we have mapped ten independent mutant loci to distinct regions, and five of them have been further defined as point mutations of known genes.

3. Sato T., Endoh M., Yoshida H., Yasuo S., Katsuno T., Saito Y., Isono K., Koseki H. Mammalian Polycomb complexes are required for Peyer's patch development by regulating lymphoid cell proliferation. ***Gene.*** 379, 166-74(2006)

4. Tosaki H.,Kunisada T.,Motohashi T.,Aoki H.,Yoshida H.,Kitajima Y. Mice Transgenic for KitV620A:Recapitulation of Piebaldism but not Progressive Depigmentation Seen in Humans with this Mutation. ***J Invest Dermato.*** 126, 1111-1118(2006)

5. Yuhki M., Yamada M., Kawano M., Iwasato T., Itohara S., Yoshida H., Ogawa M., Mishina Y. BMP receptor IA signaling is necessary for hair follicle cycling and hair shaft differentiation in mice. ***Development*** 131, 1825-1833(2004)

Laboratory for Vaccine Design

Team Leader
Yasuyuki Ishii, PhD.

Visiting Scientists : **Omar Duramad**
Kenichi Masuda
Nobuomi Yasuda

Technical Staff : **Risa Nozawa**
Annabelle Teng
Haruka Katagiri

Allergen-specific immunotherapy (AIT) is a globally recommended treatment for hay fever and various forms of allergic rhinitis. However, in Japan, the only current AIT is subcutaneous immunotherapy (SCIT) for Japanese cedar pollinosis and it is not widely accepted due to the requirement for long term treatment and the obscure mechanism of its action.

We are now researching new vaccine technologies aimed at the practical application of AIT for cedar pollinosis. The first candidate vaccine is a recombinant Cryj 1 & 2 fusion protein designed so as to have no risk of anaphylaxis. It will be utilized as a safe antigen for both SCIT and sub-lingual immunotherapy (SLIT). The second candidate vaccine is liposomal α-GalCer, a ligand for invariant natural killer T (iNKT) cells, encapsulating the Cry j1&2 fusion protein. The liposomes system not only reduced on-going IgE antibody formation but also suppressed tertiary antibody responses. Moreover, oral administration of the vaccine also showed efficacy for IgE antibody suppression. To elucidate the detailed mechanisms of both vaccines, several experiments are being carried out.

PEGylated recombinant Cry j1/2 fusion protein

Our recombinant Cry j1/2 fusion protein is composed of full length mature Cry j1 and Cry j2 proteins containing all potential human and mouse T-cell epitopes. This strategy was meant to overcome the problems with the recombinant polypeptide vaccines so far developed, most notably the lack of antigenic peptides for particular MHC haplotypes. For the purposes of drug development, we must prepare homogenous samples of the vaccine. However, there are 23 target cysteine residues for PEGylation in the native recombinant fusion protein; therefore the PEGylated proteins will by definition be heterogeneous. In order to generate a uniform product, we generated a mutant fusion protein in which all but one of the cystein residues was replaced by serines. Importantly, the fusion protein does not contain any structural IgE-binding epitopes because the native Cry j1 and Cry j2 tertiary structures are destroyed by the fusion of these two proteins. In fact, binding of IgE antibodies derived from 100-cedar pollinosis patients to the fusion protein was completely lost, although almost all patients were strongly reactive to native Cry j1 protein (Fig. 1). These results suggests that the PEGy-

Recent publications

1. Tashiro T, Hongo N, Nakagawa R, Seino K, Watarai H, Ishii Y, Taniguchi M, and Mori K. RCAI-17, 22, 24-26, 29, 31, 34-36, 38-40, and 88, the analogs of KRN7000 with a sulfonamide linkage: their synthesis and bioactivity for mouse natural killer T cells to produce Th2-biased cytokines. ***Bioorg. Med. Chem.*** 16 (19):8896-8906(2008)

2. Ito T, Hasegawa A, Hosokawa H, Yamashita M, Motohashi S, Naka T, Okamoto Y, Fujita Y, Ishii Y, **Taniguchi M**, Yano I, Nakayama T. Human Th1 differentiation induced by lipoarabinomannan/li*pomannan from Mycobacterium bovis BCG Tokyo-172* . ***Int. Immunol.*** 20(7):849-860 (2008)

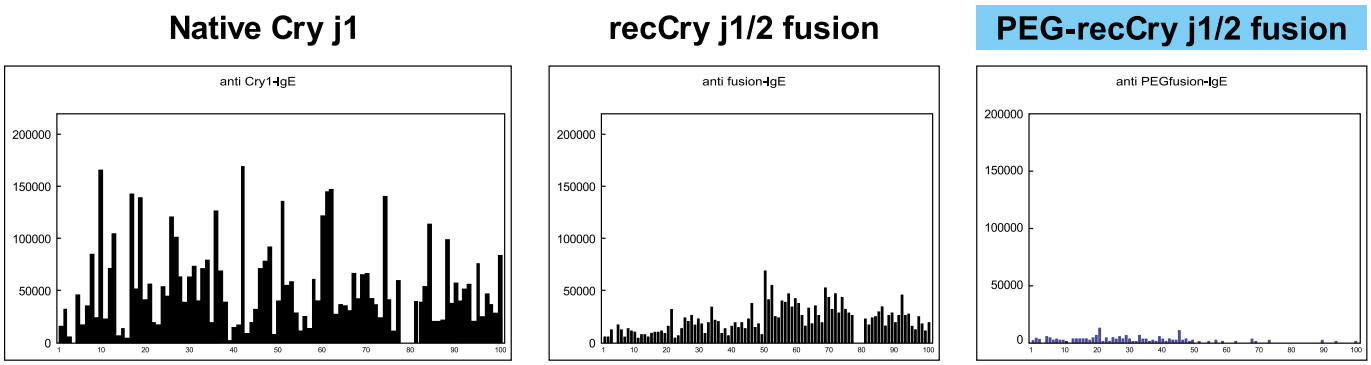


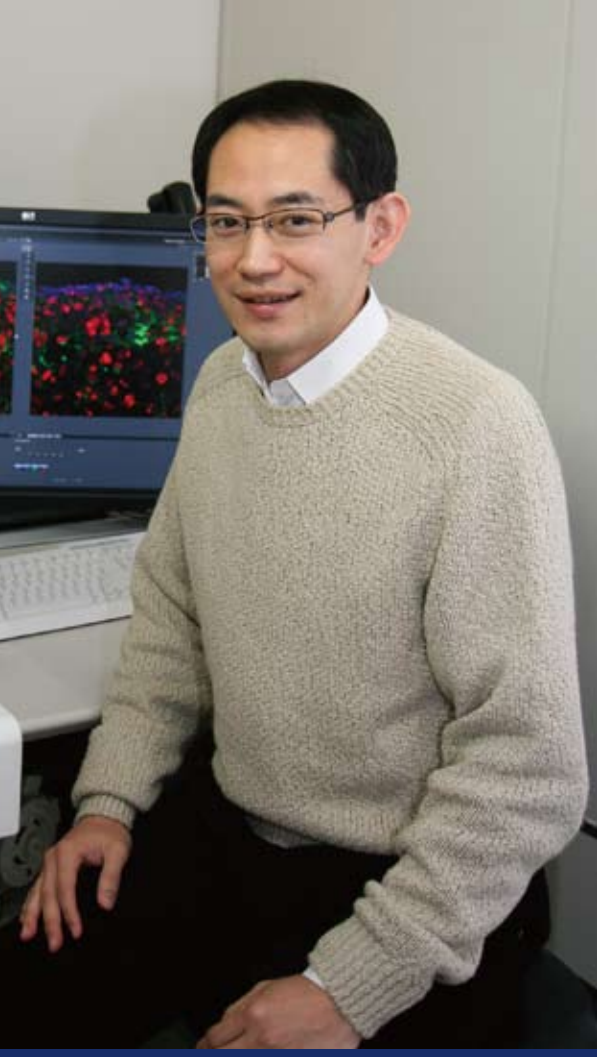
Figure **Binding of recCry j1/2 fusion protein to patient IgE antibodies – IgE-capturing ELISA –**
Binding capability of PEGylated recCry j1/2 fusion protein to IgE antibodies in sera of 100 patients of Japanese cedar pollinosis was remarkably lost, suggesting that the protein would not induce anaphylaxis.

lated recombinant fusion protein will not bind to IgE/FcεRI on mast cells and basophiles, indicating that this fusion protein will not have the risk of inducing anaphylactic shock. Next, to test whether PEGylated recombinant Cry j1/2 might enhance antibody responses against native cedar pollen antigens, mice were immunized with alum-adsorbed PEGylated recombinant protein and boosted with native Cry j1 protein. Anti-native Cry j1 IgE antibody formation was not generated by priming with the PEGylated recombinant protein. On the other hand, anti-native Cry j1-IgG1 antibody formation was observed after priming with the recombinant Cry j1/2, although the titer was remarkably low compared to native Cry j1-priming. Although anti-recombinant IgG1 antibody formation was observed, IgE antibody was not. These results collectively indicate that PEGylated recombinant Cry j1/2 is safe because there is no binding to the patients IgE antibodies and no priming for cedar antigen-specific IgE antibody formation.

Liposomal α-GalCer -Cry j1/2 fusion protein

The original idea behind the liposome vaccine was that α-GalCer activates iNKT cells to induce iNKT cell-mediated adjuvant activity together with activation of Cry j-specific Th1 cells to suppress Th2 cells, resulting in the suppression of IgE responses. To evaluate the antigen-specific suppressive activity of the liposomal α–GalCer-recombinant Cry j1/2 fusion protein, the liposomes were injected intravenously into native Cry j1&2-primed mice. Anti-Cry j1 antibody responses of all the immunoglobulin isotypes examined were markedly suppressed after native Cryj antigen challenge. To understand the detailed mechanism of liposome vaccine-mediated antigen specific suppression of antibody responses, target cells of the liposome vaccine were first analyzed by using liposomal GFP. The data suggest that the major target cells are $B220^{+}CD1d^{high}$ cells and also that the liposome rather than the aqueous form is effectively take up by the $B220^{+}$ cells. Since the liposomes contain α-GalCer, which is a ligand for iNKT cells, we anticipated that there would be an interaction of iNKT cells with $B220^{+}CD1d^{high}$ cells. It has been reported that CXCR6, which is a major chemokine receptor for iNKT cells, is highly expressed on iNKT cells. Thus, we investigated CXCR6 expression on various lymphocytes and found that CXCR6 is selectively expressed on iNKT cells but not on other cells such as NK, $CD4^{+}$T and $CD8^{+}$T cells. Moreover, antibodies against the CXCR6 ligand CXCL16 significantly inhibited NKT cell migration. Analysis showed that GFP-positive $B220^{+}CD1d^{high}$ cells expressed CXCL16 but GFP-negative cells did not, suggesting that iNKT cells migrate to the areas of splenic $B220^{+}$ cells and uptake liposome. To evaluate bioactivity after interaction of liposome vaccine-target cells with iNKT cells, mice were intravenously injected with liposomal or aqueous α-GalCer. In contrast to $CD11c^{+}$ DCs, iNKT cells produced mainly IL-10 when co-cultured with $B220^{+}$ $CD21^{high}$ cells. Importantly, the cells without iNKT cells failed to produce IL-10. This IL-10 production was remarkably inhibited in the presence of anti-CD40L neutralizing antibody, indicating that interaction of iNKT cells with $B220^{+}CD21^{high}$ cells through CD40/CD40L is essential for IL-10 production.

3. Ishii Y, Nozawa R, Takamoto-Matsui Y, Teng A, Katagiri-Matsumura H, Nishikawa H, Fujita H, Tamura Y, Taniguchi M. Alpha-galactosylceramide-driven immunotherapy for allergy. ***Front. Biosci.***, 13:6214-6228.(2008)
4. Son A, Nakamura H, Okuyama H, Oka SI, Yoshihara E, Liu W, Matsuo Y, Kondo N, Masutani H, Ishii Y, Iyoda T, Inaba K, Yodoi J.Dendritic cells derived from TBP-2-deficient mice are defective in inducing T cell responses. ***Eur. J. Immunol.*** 38(5):1358-1367(2008)
5. Tamura Y, Teng A, Nozawa R, Takamoto-Matsui Y, Ishii Y. Characterization of the immature dendritic cells and cytotoxic cells both expanded after activation of invariant NKT cells with alpha-galactosylceramide *in vivo*. ***Biochem. Biophys.Res. Commun.*** 369(2):485-492(2008)



Research Unit for Human Disease Model

Unit Leader

Fumihiko Ishikawa, M.D., PhD.

Research scientist : **Yoriko Saito**
Takehiko Doi

Technical scientist : **Hidetoshi Ozawa**

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

Administrative staff : **Toshiko Nakamrura**

Studying normal human hematopoietic & immune systems

To understand how homeostasis is maintained in the human hematopoietic and immune systems, we have been trying to these systems in mice. We successfully obtained high levels of human hematopoietic chimerism and multi-lineage differentiation of immune subsets from injected hematopoietic stem cells (HSCs).

Within immune subsets, we carefully analyzed differentiation and function of human B cells engrafted in the recipient NOD/SCID/IL2rgKO mice. Multi-color FACS enabled us to identify developmental stages of human B cells such as pro-B, pre-B, immature B, transitional B, and mature B. Our xenotransplantation model was found to support the maturation of transitional to mature B cells in the recipient spleen and lymph nodes. Consistent with the full maturation of human B cells in mice, we detected human IgM, IgG and IgA in the recipient sera. Additionally, splenic B cells undergo cell division and class switching following stimulation using CD40Ab, IL4, and IL21. These data indicate that the problem of human B cell maturation arrest encountered when using NOD/SCID recipients has been overcome by using the current xenotransplantation model. The NOD/SCID/IL2rgKO system should therefore be useful in investigating human B cell maturation and function *in vivo*.

We are beginning the analysis of human immunity reconstituted in the next generation humanized mouse which, in addition to being severely immunocompromised, expresses human MHC class I antigens.

Stemness in human acute leukemia

As a human disease model, we created a mouse model for acute myeloid leukemia (AML). While advances in chemotherapeutic agents and stem cell transplantation have improved survival of patients with AML over the last 30 years, two serious problems, chemoresistance and relapse, continue to adversely affect clinical outcomes. We confirmed that $CD34^{+}CD38^{-}$ AML cells possess exclusive potential to initiate leukemia *in vivo*, self-renew, and give rise to $CD38^{+}$ and $CD34^{-}$ leukemic cells. Therefore, $CD34^{+}CD38^{-}$ AML cells satisfy the criteria for “leukemia/cancer stem cells” proposed by the AACR. Moreover, we demonstrated that AML stem cells preferentially reside within the bone marrow endosteal

Recent publications

1. Saito Y and Ishikawa F. The origins of Blood: Induction of hematopoietic stem cells from different sources. ***Cell Stem Cells*** 3:8-10, 2008.

2. Kong Y, Yoshida S, Saito Y, Doi T, Nagatoshi Y, Fukata M, Saito N, Yang SM, Iwamoto C, Okamura J, Liu KY, Huang XJ, Lu DP, Shultz LD, Harada M, Ishikawa F. CD34+CD38+CD19+ as well as CD34+CD38-CD19+ cells are leukemia-initiating cells with self-renewal capacity in human B-precursor ALL. ***Leukemia*** 22:1207-13, 2008.

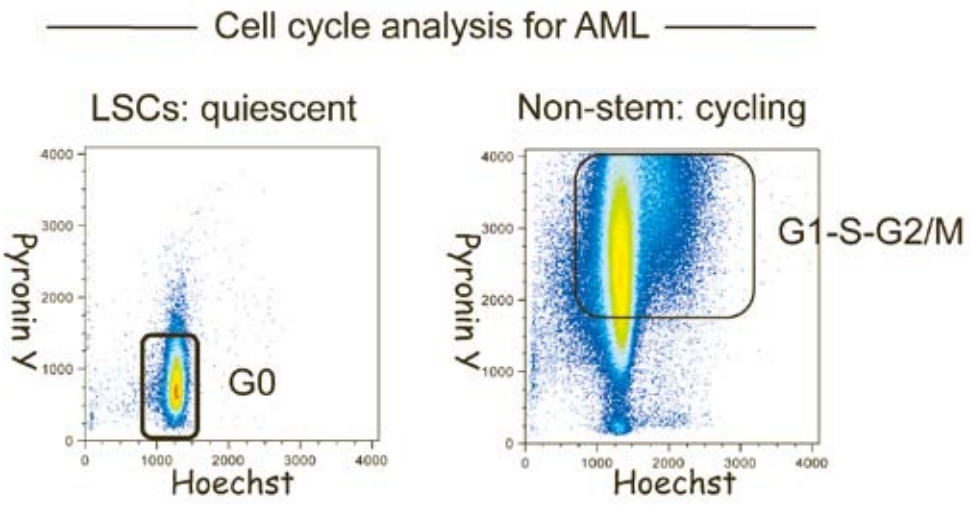


Figure **The majority of LSCs are quiescent and chemo-resistant.**
(*left*) Scheme for cell cycle progression (*right*) The majority of leukemic stem cells (LSCs) are quiescent, while non-stem leukemic cells are mainly cycling.

niche and show increased chemoresistance compared with non-stem AML cells residing in the center of the bone marrow. Cell cycle analysis revealed that quiescence of AML stem cells could be one of the major mechanisms underlying chemoresistance and relapse. We are currently working on novel strategies to eliminate AML stem cells to overcome relapse in this disease entity.

Modeling primary immunodeficiency diseases

Primary immunodeficiency (PID) is usually caused by single gene mutations and results in opportunistic infections and impaired quality-of-life in affected children. XLA is one of the most common PIDs, and the lack of Btk protein leads to impaired B cell production, B cell maturation arrest, and hypoglobulinemia. As the severity of B cell development and maturation is very different between Btk gene mutated mice and XLA patients, we set out to create a humanized mouse model for this PID. Using five different patient BM HSCs, we confirmed that transplantation of XLA patient HSCs into newborn NOD/SCID/IL2rgKO mice results in impaired B cell production, B cell maturation arrest, and hypoglobulinemia. This humanized mouse model may serve as a useful tool to examine the immune system in spleen, lymph node, and thymus, organs difficult to obtain directly from patients for research purposes. In the future, we plan to use this *in vivo* PID model to examine immune cell function in the setting of various infections and to study the safety/efficacy of gene or cellular therapy for PIDs.

3. 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. ***Nature Biotechnology***, 25(11):1315-21, 2007.

4. Ishikawa F, Niiro H, Iino T, Yoshida S, Saito N, Onohara S, Miyamoto T, Minagawa H, Fujii SI, Shultz LD, Harada M, Akashi K. The developmental program of human dendritic cells is operated independently of conventional myeloid and lymphoid pathways. ***Blood***, 110: 3591-3660, 2007 in press.

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

Research Unit for Cellular Immunotherapy

Unit Leader

Shin-ichiro Fujii, M.D., Ph.D.

Technical staff : **Yohei Shozaki**

Student Trainee : **Asako Kikuchi**

Administrative Staff : **Mariko Iwaki**

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

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

Phase I/IIa immunotherapy for advanced non-small cell lung cancer (NSCL) patients refractory to standard treatment as a post second line 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 (stage IIIB, IV or recurrence). α-GalCer-pulsed APCs ($1x10^9/m^2$ PBMC-DCs) were intravenously administered four times. Twenty-three patients were enrolled and 17 patients (73.9%) completed the study. We found no remarkable adverse effects as a primary endpoint, and evaluated immunological and clinical responses. Based on the immunological findings tested, we identified two patient groups, responder and poor-responder. The

Recent publications

1. Motohashi S, Ishikawa A, Ishikawa E, Otsuji M, Lizasa T, Shimizu N, Horiguchi S, Okamoto Y, Fujii S, Taniguchi M, Fujisawa T, Nakayama T. Phase I study of α-galactosylceramide-pulsed antigen presenting cells administration to the nasal submucosa in unresectable or recurrent head and neck cancer. ***Clin Cancer Res.*** 12, 6079-86 (2006)

2. Fujii S. Exploiting dendritic cells and naturatl killer T cells in immunotherapy against malignancies. ***Trends Immunol.*** 29, 242-9 (2008)

responder group (11/17 patients) had an increased number of IFN-γ producing NKT cells (>1.5 fold after the administration of α-GalCer-pulsed APCs), whereas there was a lesser increase in NKT cells in the poor-responder group (6/17 patients). The MST (median survival time) was longer in the responder group (31.9 months) than in the poor responder group (9.7 months). In addition, the mean time to progression (TTP) was longer in the responder group (5.3 months) than in the poor responder group (2.6 months). Thus, the increased IFN- γ production by NKT cells upon α-GalCer stimulation was significantly associated with clinical outcome. The results of these phase I/IIa trials are encouraging and warrant further evaluation of the survival benefit of this immunotherapy.

Induction of Adjuvant Effects of NKT Cells Leading to Adaptive Immunity in Cancer Therapy

(A collaboration with Dr. Shimizu, Therapeutic model research unit)

Initially, we studied the different types of APCs for loading α-GalCer to induce stronger innate tumor immunity. We have pursed the capacity of CD11c- leukocytes to present α-GalCer by *in vitro* loading, and found that as long as the different types of CD1d expressing leukocytes were able to capture glycolipid, they could stimulate NKT cells. When we used α-GalCer-loaded tumor cells instead of DC/Gal, we found more augmentation of NK cell activity as a "adjunct effect". In addition, we recently found that a low dose of B16 tumor cells loaded with α-GalCer and injected intravenously could induce a protective T cell immune response to subsequent subcutaneous challenge with the B16 melanoma. To analyze the events taking place in this system *in vivo*, we tracked labeled α-GalCer-loaded tumor cells by confocal microscopy and found that α-GalCer did indeed activate NK and NKT cells to kill tumor cells. The tumor debris were captured by DCs in the spleen, which matured to present the tumor antigen peptide to $CD4^+$ and $CD8^+$ T-cells, promoting an adaptive immune response (cross-presentation 1). Also the tumor debris-capturing DCs were able to present the α-GalCer ligand to NKT cells again (cross-presentation 2). This immune response persisted for at least six months.

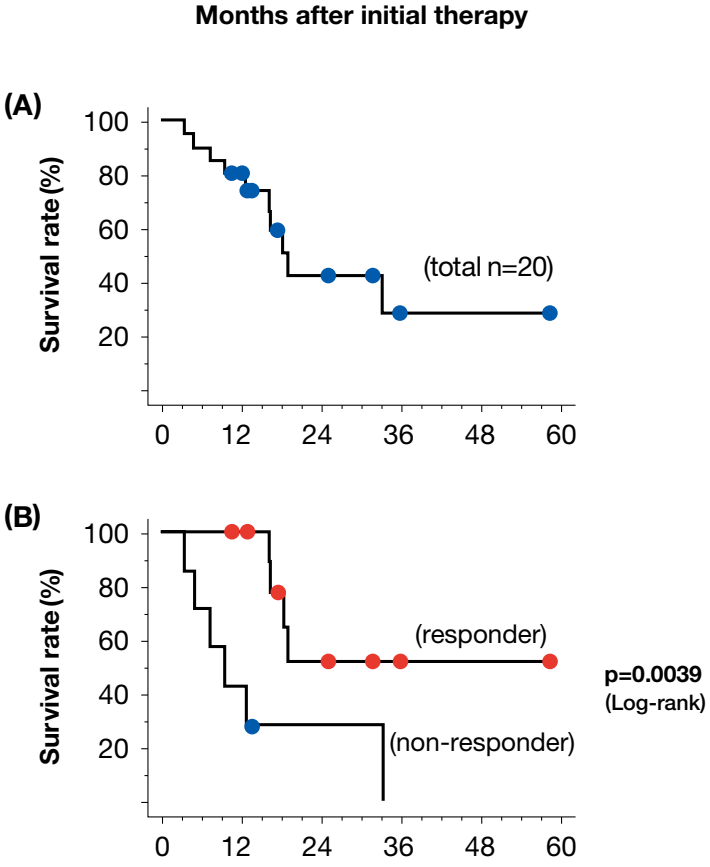


Figure **Correlation of survival of NSLC patients treated with DC/Gal with the increase of IFN-γ producing NKT cells.**

In the immunological findings examined, we indentified two patient groups, responder and poor-responder. The responder group (11/17 patients) had an increased number of IFN-γ producing NKT cells (>1.5 fold increase after the administration of α-GalCer-pulsed APCs), whereas there was a lesser increase in NKT cells in the poor-responder group (6/17 patients). We found a prolonged MST in treated patients (18.3 months) compared to patients treated with chemotherapy alone (4.6 months) (Fig. 1A). When we compared the MST between the responder and poor-responder groups in our phase I/IIa study, the MST was longer in the responder group (31.9 months, range 14.5 to 36.3 months) than in the poor responder group (9.7 months, range 3.8 to 25.0 months) (Fig. 1B). In addition, although the overall 2-year survival rate was 41.2%, that for the responder group was 60% and the poor responder group was 14.3%.

3. Fujii S, Shimizu K, Hemmi H, Steinman RM. Innate Vα14+ naturatl killer T cells mature dendritic cells, leading to strong adaptive immunity. ***Immunol Rev.*** 220, 183-98 (2007)

4. Shimizu K, Kurosawa Y, Taniguchi M, Steinman RM, Fujii S. Cross-presentation of glycolipids from tumor cells loaded with α-galactosylceramide leads to potent and long-lived T cell mediated immunity via dendritic cells. ***J. Exp. Med.*** 204, 2641-53 (2007)

5. Shimizu K, Goto A, Fukui M, Taniguchi M, Fujii S. Tumor cells loaded with α-galactosylceramide induced innate NKT and NK cell-dependent resistance to tumor implantation in mice. ***J Immunol.*** 178, 2641-53 (2007)

Laboratory for Immunogenomics

Group Director
Osamu Ohara, Ph.D.

Senior Research Scientist	: **Hiroshi Kitamura**
Research Scientist	: **Yayoi Kimura**
Research Associate	: **Atsushi Hijikata** **Koichi Oshima** **Mai Yamagishi** (September~)
Technical Staff	: **Masatoshi Ito** **Miho Izawa** **Chisato Kikuguchi** **Tomoko Yuasa** (~September) **Ryo Yokoyama** (~August)
Temporary employee	: **Seiko Watanabe** **Nobutake Suzuki** **Kayoko Nagata** **Aoi Ozawa** **Atsuo Kobayashi** **Tomoko Hasegawa** **Saori Hayashi** **Ryosuke Yashi** **Eri Yamamoto** (August~) **Satomi Takahashi** (~June)
Students Trainees	: **Nanako Shimura** **Noriko Nakagawa**
Administrative Staff	: **Kazuyo Nomura**

An important and basic mission of our research group since it was launched has been to function as a "Gateway" to genomics for immunologists. To achieve this goal, our group has taken a three-pronged approach: (1) central support activities; (2) strategic and collaborative research activities; and (3) exploratory research activities aimed at new technology development. We believe that the interactions among our three-pronged research activities, which are driven by collaborations with immunologists, will play a crucial role in development of Immunogenomics at RCAI and have thus made every effort to keep a good balance among them. While much of our group's efforts have focused on strategic and collaborative research endeavors, we have also carried out some exploratory research with an emphasis on protein structure, translational control of gene expression, and single-cell analysis. In this context, we describe below three of our research activities in 2008 as examples of research topics in categories (2) and (3).

Development of a new bioinformatics tools for mutation analysis in Primary Immunodeficiency Diseases

Based on our experience in diagnosis of Primary Immunodeficiency Diseases (PIDs), we have come to recognize one of the most difficult questions in the area of DNA diagnosis. How does one evaluate genetic variation in a particular gene in a patient with PID in terms of whether it can impact the structure/function of the gene product sufficiently to cause the disease? In this regard, we have developed a new web-based bioinformatics tool, termed Mutation@A Glance (http://rapid.rcai.riken.jp/mutation/: Fig. 1). This tool has a user-friendly graphical interface to allow visualization of the mutation data of known PID genes at levels of the gene structure as well as the primary and tertiary structure of the protein, together with various types of annotation information such as single nucleotide polymorphisms (SNP) and functionally important amino acid residues. Mutation@A Glance also provides a facility to evaluate the genetic variations in the DNA sequences of patients with PID as to whether or not they affect the structure/function of the proteins encoded by the PID genes. Mutation@A Glance is coordinately linked to another PID database termed RAPID (Resource of Asian Primary Immunodeficiency Diseases: http://rapid.rcai.riken.jp:8080/sites/RAPID/index_html) generated by a collabora-

Recent publications

1. Fukada T, Civic N, Furuichi T, Shimoda S, Mishima K, Higashiyama H, Idaira Y, Asada Y, Kitamura H, Yamasaki S, et al. The zinc transporter SLC39A13/ZIP13 is required for connective tissue development; its involvement in BMP/TGF-beta signaling pathways. ***PLoS ONE.*** 3, e3642 (2008)
2. Sasuga Y, Iwasawa T, Terada K, Oe Y, Sorimachi H, Ohara O, Harada Y. Single-Cell Chemical Lysis Method for Analyses of Intracellular Molecules Using an Array of Picoliter-Scale Microwells. ***Anal Chem.*** 80, 9141-9149 (2008)

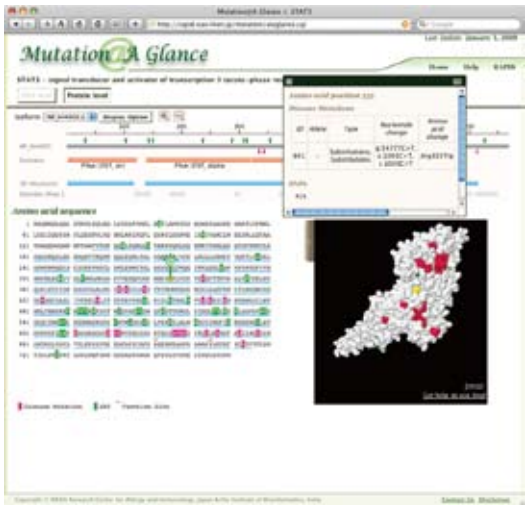


Figure 1 **A screenshot of Mutation@A Glance.** The mutation positions (magenta) of STAT3 are visualized along the primary amino acid sequence and on the three-dimensional structure. The location of SNPs are indicated in green.

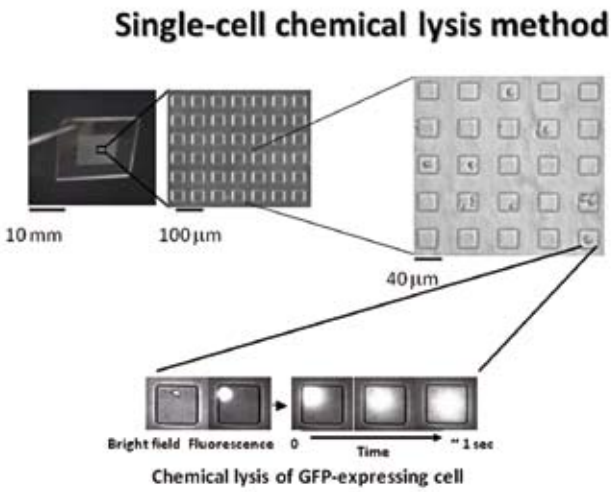


Figure 2. **Chemical lysis of single cells followed by quantification of proteins or enzymatic activity**

tion with the Research Unit for Immunoinformatics at RCAI and The Institute of Bioinformatics (Bangalore, India).

Novel function of c-Jun N-terminal kinase (JNK) in translational control

During the course of our analyses of translational control of gene expression, we observed that a c-Jun N-terminal kinase (JNK) inhibitor SP600125 (SP) suppressed translation of several well known "housekeeping" genes. This translational suppression seems to be global because SP treatment uniformly weakened all visible bands from radiolabeled nascent proteins in SDS-polyacrylamide gels, and there was a concomitant 40% decrease in the total amount of nascent proteins. Reporter assays revealed that SP specifically inhibited cap-dependent translation not but IRES-dependent translation, indicating that JNK functions to maintain translation under non-stressed conditions. We also investigated the relationship between JNK and the phosphatidylinositol 3-kinase (PI3K) / Akt kinase pathway, which is proposed to be a major regulatory pathway for cap-dependent translation. Although SP treatment did not significantly affect phosphorylation of PI3K, it drastically attenuated phosphorylation of AKT and its downstream molecules, namely mammalian target of rapamycin (mTOR) kinase, eukaryotic translation initiation factor 4E binding protein 1, p70S6kinase, and eukaryotic translation initiation factor 4B. The translational repression caused by SP was reproduced by treatment with short hairpin RNA targeting JNK1 but not for JNK2, although the both shRNAs effectively reduced their respective targets. Taken together, we concluded that JNK1 is essential for maintenance of cap-dependent translation through AKT phosphorylation under non-stressed conditions.

A simple single-cell chemical lysis method for analyses of intracellular molecules using an array of picoliter-scale microwells

Analysis of intracellular contents and enzymatic activities of single cells has long been a dream of biologists for studying physiological and pathological activities at the cellular level. To achieve this, we developed a simple single-cell lysis method for quantification of intracellular proteins and enzymatic activities at the single-cell level. We engineered a dense array of microwells of 10-30-pL volume fabricated from polydimethylsiloxane (PDMS) and a commercially available cell lysis reagent (Fig. 2). To demonstrate the performance of this single-cell lysis method, we carried out two different assays at the single-cell level: detection of proteins by antibody conjugated-microbeads and measurement of protease activity by fluorescent substrates using a method we had previously developed. The results indicated that this method readily enabled us to monitor protein levels and enzymatic activities in a single cell. Because this method required only an array of PDMS microwells situated under a fluorescence microscope, the simplicity of this platform opens a means to explore biochemical characteristics of single cells even by those who are not familiar with microfluidics technology.

3. Keerthikumar S, Raju R, Kandasamy K, Hijikata A, Ramabadran S, Balakrishnan L, Ahmed M, Rani S, Selvan LD, Somanathan DS, Ray S, et al. RAPID: Resource of Asian Primary Immunodeficiency Diseases. ***Nucleic Acids Res.*****37**, D863-7 (2008)
4. Ehrhardt GR, Hijikata A, Kitamura H, Ohara O, Wang JY, Cooper MD. Discriminating gene expression profiles of memory B cell subpopulations. ***J Exp Med,*** **205**, 1807-17(2008)
5. Kitamura H, Ito M, Yuasa T, Kikuguchi C, Hijikata A, Takayama M, Kimura Y, Yokoyama R, Kaji T, Ohara O. Genome-wide identification and characterization of transcripts translationally regulated by bacterial lipopolysaccharide in macrophage-like J774.1 cells. ***Physiol Genomics.*** 14, 121-32 (2008)

Research Unit for Immunoinformatics

Unit Leader

S. Sujatha Mohan, Ph.D.

Research Scientist : **Subhashri Ramabadran**

Research Associate : **Lavanya Balakrishnan**

An important mission of our research unit is to develop and maintain an open resource bioinformatics platform and data resources in order to gain immunological insights into Primary immunodeficiency diseases (PID) through genomic, transcriptomic, proteomic data and its analysis. Our ultimate goal is to provide relevant, up-to-date and validated information on PID as per global community standards in an easily decipherable and usable format.

Generation of Primary Immunodeficiency Disease Database from Asia

Primary Immunodeficiency diseases result from mutations in genes important in the development and maintenance of the immune system. Patients with these intrinsic defects may have increased susceptibility to recurrent and persistent infections and, in some cases, elevated susceptibility to autoimmune diseases and cancer. Our main aim is to maintain an informational platform that brings PID clinicians and researchers in Asia together and also to establish an active Asian network among groups interested in PID that will further link to other established PID groups from other parts of the world. No such community involvement has so far been initiated for PID in Asia. Towards this, we have constructed a web-based compendium of molecular alterations in PID, named Resource of Asian Primary Immunodeficiency Diseases (RAPID), which is available as a worldwide web resource at http://rapid.riken.jp/.

RAPID architecture

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

A primary information page is the default main page of every PID gene in the RAPID. It summarizes the external links available in the public domain such as Entrez, HPRD, IDR, RefDIC, NetPath, OMIM, HGNC, PDB, Ensembl and Swiss-Prot. It also includes the disease phenotype linked to the given gene along with the mode of inheritance, alternative names of the gene function and the associated features.

Recent publications

1. Gopa R. Mishra, M. Suresh, K. Kumaran, N. Kannabiran, Shubha Suresh, P. Bala, K. Shivakumar, N. Anuradha, Raghunath Reddy, T. Madhan Raghavan, et al. Human Protein Reference Database – 2006, Update. ***Nucleic Acids Research***. 34: D411-414 (2006).

2. T K B Gandhi, Jun Zhong, Suresh Mathivanan, L Karthick, K N Chandrika, S. Sujatha Mohan, Salil Sharma, Stefan Pinkert, Shilpa Nagaraju, Balamurugan Periaswamy, et al. Analysis of the human protein interactome and comparison with yeast, worm and fly interaction datasets. ***Nature*** Genetics. 38: 285-293 (2006).

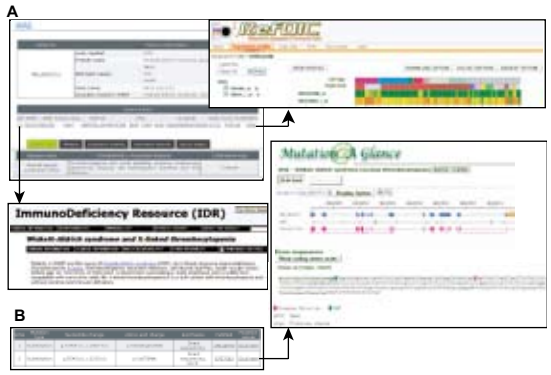


Figure 1

A screenshot of the primary information page and mutation data of the WAS gene in RAPID. (A) Primary information of W gene along with its external resources to IDR (Immunodeficienc resource) and RefDIC (Reference database of Immune cells) is shown. (B) Mutation data curated from the literature is shown along with the mutation viewer tool called 'Mutation@A Glance' to visualize the mutations both at DNA and protein levels.

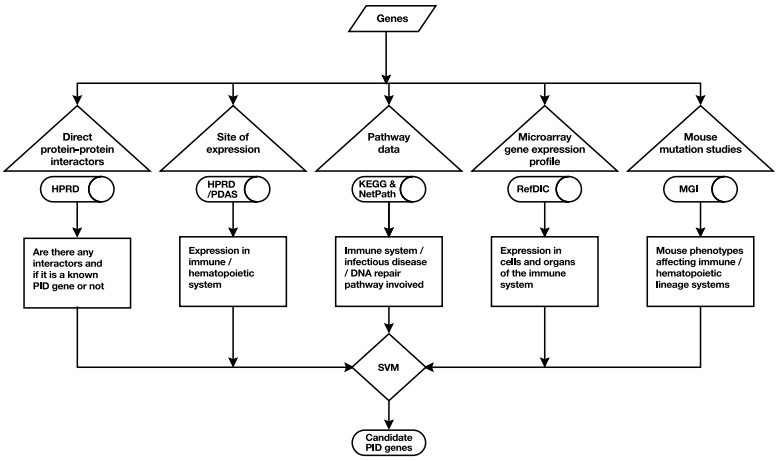


Figure 2

An overview of proposed algorithm for the prediction of candidate PID genes using a Support Vector Machine (SVM) learning approach with various gateways used for analysis.

Annotation of mutation Data

The sequence variations in PID genes reported from patients are manually curated by expert biologists from the published literature and mapped to the NCBI RefSeq genomic, cDNA and protein sequences to aid the scientific community as per the recommendations by Human Genome Variation Society (HGVS). Each entry on the mutation has a link to the mutation viewer, a web-based graphical user interface (GUI) enabled tool, named Mutation@A Glance (http://rapid.rcai.riken.jp/mutation/). It allows the user to visualize the mutation position both at the level of DNA and protein sequences as well as homology based three-dimensional structures with various types of information such as SNP, protein domains and functional sites (Fig. 1).

Highlights of other features

RAPID has also been provided with information about gene expression profiles from Gene expression omnibus (GEO) and Reference Database of Immune cells (RefDIC); protein-protein interaction networks from Human Protein Reference Database (HPRD) and also all the available PID genes are mapped to their corresponding mouse gene counterpart from Mouse Genome Informatics (MGI) to catalogue any lethality and/or immune system/hematopoietic phenotypes resulting from knock in/out of genes or spontaneous mutations.

Prediction of novel PID gene candidates using a Support Vector Machine (SVM) approach

Using the information contained in the RAPID, we have used support vector machines to predict candidate PID genes. This should allow physicians and other biomedical investigators to experimentally determine if any of these genes leads to a PID phenotype. Initially, this analysis was carried out with 148 known PID genes and 3,162 negative genes, where each gene had 69 binary features associated with it. Using this approach, we tested all genes encoded by the human genome to identify candidate PID genes and predicted 1,445 candidate PID genes from the human genome and these genes can be prioritized further and analyzed for their role in PID (manuscript in preparation). An overview of this PID gene prediction algorithm along with various gateways used in this approach is shown in Fig. 2.

Future perspectives

In addition to keeping this resource updated on a regular basis along with further elucidation of the role of PID genes at molecular level, we will initiate global community standard formats for PID data exchange and validation. We include signaling pathways that involve specific PID genes using the e-Path visualization software tool, which should facilitate identification of potential candidate genes that may have a role in the pathogenesis of related immunodeficiency diseases.

Future research will focus on the clinical description, genotype characterization, associated diseases and immunological investigation of novel PIDs that are generated using our in-silico approach. Depending on its priority, the most probable candidate PID genes would further be processed by biomedical investigators using available high throughput experiments. Thus, RAPID will serve as a model for genomics-based immune disease databases in the future.

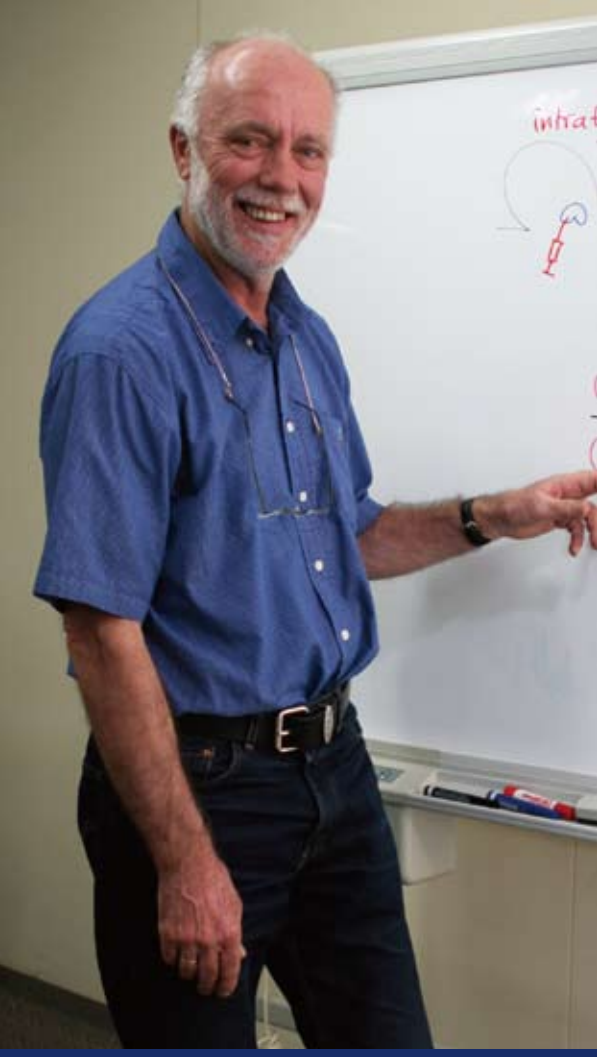
The PID project was initiated in collaboration with the Institute of Bioinformatics (IOB, Bangalore, India), the Immunogenomics research group at RIKEN Research Center for Allergy and Immunology (RCAI, Japan) and the Kazusa DNA Research Institute (KDRI), Japan.

This research unit is supported by Grant-in aid for Scientific Research, The Special Coordination Funds for promoting Science and Technology, from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) Japan.

3. Mathivanan S, Ahmed M, Ahn NG, Alexandre H, Amanchy R, Andrews PC, Bader JS, Balgley BM, Bantscheff M, Bennett KL, et al. Human Proteinpedia enables sharing of human protein data. ***Nature Biotechnology***, 26(2): 164-7 (2008).

4. Shivakumar Keerthikumar, Rajesh Raju, Kumaran Kandaswamy, Atsushi Hijikata, Subhashri Ramabadran, Lavanya Balakrishnan, Mukhtar Ahmed, Sandhya Rani, Lakshmi Dhevi N Selvan, Devi S Somanathan, RAPID: Resource of Asian Primary Immunodeficiency diseases. ***Nucleic Acids Research,*** 37 (Database issue): D863-7 (2009).

5. Prasad, T. S. K., Goel, R., Kandasamy, K., Keerthikumar, S., Kumar, S., Mathivanan, S., Telikicherla, D., Raju, R., Shafreen, B., Venugopal, A., Balakrishnan, L., et al. Human Protein Reference Database - 2009 update. ***Nucleic Acids Research,*** 37 (Database issue): D767-72 (2009).



Unit Leader
Willem van Ewijk, Ph.D.

Research Scientist : **Kathryn Ischi-Schrade**

Research Unit for Thymic Microenvironment

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

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

The immunodeficient thymus.

In 2008, we have studied the development of the thymic environment in absence of developing thymocytes, specifically focusing on the origin and generation of the aberrant epithelial celltypes in the immunodeficient thymus. It can be speculated that these cells are not intrinsic to the thymus and that they have been incorporated in the thymic stroma at early embryonic stages. However, these aberrant cell types derive from the thymic environment itself. In studies using FoxN1 reporter mice, we have shown that the cyst lining epithelial cells have passed through a FoxN1 dependant developmental pathway, univocally demonstrating that they have “trans-differentiated” from normal TECs.

We then analyzed the thymic origin of cyst lining epithelial cells in an *in vitro* experimental system, where cortical and medullary TECs were tested for their potential to induce thymic cysts under influence of de-oxyguanosin, a potent blocking agent for T cell development. We showed that medullary TECs, rather than cortical TECs formed cysts. Moreover, cyst lining TECs have the capacity to proliferate, arguing that epithelial progenitor cells reside within the cyst lining population (Fig 2.).

In separate studies, we have analyzed the molecular nature of factors involved in thymic crosstalk and we have shown that Notch-1 activation on TECs by DLL1 expressing developing thymocytes drives the restoration of the immuno-

References

1. Ewijk W van, Shores EW, Singer A. Crosstalk in the mouse thymus. ***Immunol Today.*** 1994; 15: 214-217.

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

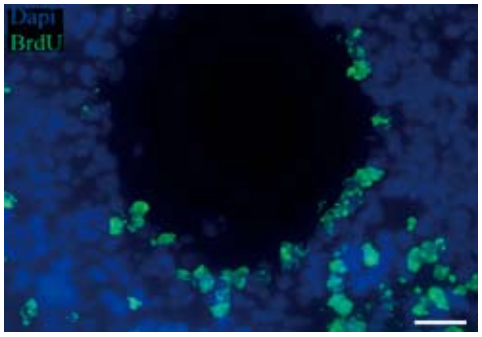


Figure 1
TECs proliferate during thymic cyst formation. BrdU was added during dGuo treatment. Thymi were snap frozen in OCT compound, and stained with anti-BrdU antibody (*green*).

compromised thymus (5). Thus, Notch-1-DLL1 interaction is a prime molecular example of thymic crosstalk, because both types of molecules are reciprocally expressed on TECs and developing T lymphocytes, and this molecular interaction drives both TEC development as well as T cell development. This latter aspect can be employed to generate an artificial thymic environment.

The artificial thymus.

Importantly, Notch signaling in developing hemato-progenitor cells induces restriction of these cells along the T-lineage (5). We have set-up an *in vitro* system to generate T lymphocytes, based on Notch signaling. By infecting the fibroblast cell line Tst-4 with a DLL1 containing retrovirus, hematopoietic progenitor cells added to the monolayer culture are commited into the T cell lineage, generating $CD4^+CD8^+$ thymocytes. However, CD4 and CD8 T cells did not develop. To promote final steps in T cell development we have added MHC expressing cells to the Tst-4/DLL1 culture. Thus, bone marrow dendritic cells (BMDCs) were generated *in vitro* from Rag $1^{-/-}$ mice, using GM-CSF and LPS. $CD11c^+$ MHC II^+ BMDCs were sorted by FACS and added with T cell precursors to TSt-4/DLL1 monolayers. Such isolated BMDC's nicely develop as MHC II expressing cells within the Tst-4/DLL1 monolayer, but sofar these cells could partially induce final steps in T cell maturation.

An alternative approach to generate an artificial thymus includes studies on the trans-differentiation capacity of non-thymic epithelial cells into thymic epithelial cells. We are pursuing this novel approach in two different experimental systems. Moreover, in collaboration with Dr. Wilfred Germeraad (University of Maastricht, the Netherlands) we investigate the role of keratinocytes in T cell development.

In our *in vivo* approach we have shown that intra-thymic transfer of epithelial cells derived from the intestine (Fig. 2a) results in integration of these extra-thymic cells in the thymic environment. Interestingly, these cells develop morphologically into three dimensionally organized TECs (Fig. 2b). The important observation here is that the thymic environment overrules the endogenous developmental program in other epithelial cell types, forcing those cells to express a thymic phenotype.

Using an vitro approach, where we have implanted tissue fragments or cell suspensions of other organs into fetal thymi, we have confirmed the inducing capacity of the thymic environment. We found that epithelial cells derived both from the fetal intestine and lung can trans-differentiate into TECs (Fig. 3).

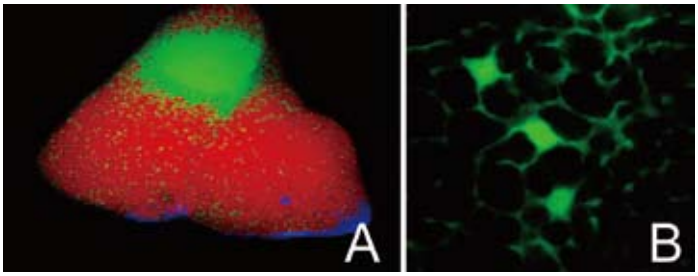


Figure 2a Intra-thymic injection of GFP expressing epithelial intestinal cells in one thymic lobe of a 6wk old C57BL mice.

Figure 2b Intra-thymic trans-differentiation of intestinal epithelial cells into a thymus specific 3-D oriented epithelial network.

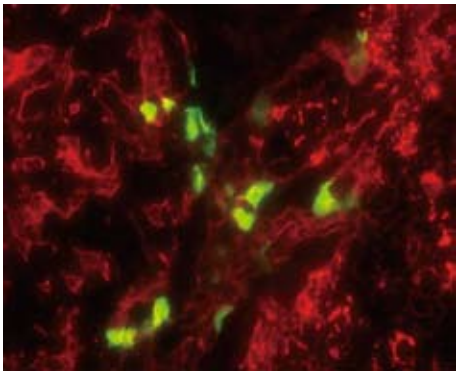


Figure 3
Integration of lung epithelial cells (*green*) in the epithelial thymic network (*red*).

Future aspects.

The construction of an artificial thymic environment generating a balanced T cell repertoire is not a simple task. We have set the first steps on the way to define minimal requirements for the development of T lymphocytes outside the body. In our *in vitro* approach we need to search for molecules and cell types completing final steps in T cell maturation. Our *in vivo* approach will concentrate both on the plasticity of thymus-induced epithelial cell differentiation as well as the complete functional maturation of intra-thymic implanted epithelial cells. Importantly, these experiments will generate insight in molecular aspects of epithelial trans-differentiation. Once such trans-differentiating factors have been identified, other types of epithelia can be converted into a thymic environment.

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

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

5. 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.*** **In press.**

6. 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. **Re-submitted for publication.**

Research Unit for Immunoepigenetics

Unit Leader

Miguel Vidal, Ph.D.

Research Scientist : **Kaori Hisada**

Technical staff : **Asako Shibano**

The products of the Polycomb group (PcG) of genes form multiprotein complexes that act as chromatin modifiers for epigenetic regulation of stem cell pluripotency, cell differentiation, genomic imprinting or X-chromosome inactivation. Two types of PcG-dependent histone modifications are found in separate complexes: trimethylation of histone H3 lysine 27 and the monoubiquitylation of histone H2AK119, both of which are typically associated to transcriptionally repressed states. Histone H2A monoubiquitylation depends on RING finger proteins Ring1A and Ring1B, that act as E3 ubiquitin ligases. Initially identified as members of the so-called Polycomb Repressive Complex 1 (PRC1, Ring1A and Ring1B also associate to various sets of subunits in independent complexes. To understand Ring1A-Ring1B function in the light of this biochemical heterogeneity we set out to investigate the role of these subunits. One of the subunits identified in my lab in Madrid is RYBP (Ring1 and YY1 binding protein) which was isolated by means of its direct binding to Ring1A-Ring1B.

Genetic analysis of RYBP (Ring1 and YY1 binding protein)

After gene inactivation, RYBP turned up to be an essential gene for embryonic development. We then, in collaboration with H. Koseki (Developmental Genetics, RCAI), generated a conditionally mutant mouse line in which RYBP deletion occurs upon Cre-mediated excision of coding sequences flanked by loxP sites. These mice were mated to another mouse line that expresses ubiquitously the Cre recombinase in a tamoxifen-dependent manner and embryonic stem (ES) cells were derived. Currently we are studying the function of RYBP in ES cells by looking into its targets using both chromatin immunoprecipitation assays and transcriptome analysis of mutant cells.

Previously we have investigated the hematopoietic phenotype of Ring1B-deficient mice, mostly characterized by proliferative alterations resulting from upregulation of components of the cell cycle machinery that promote or inhibit cell cycle progression. To investigate the possible contribution of RYBP to these phenotypes we are using an inducible (interferon-responsive) Cre transgenic line to inactivate RYBP in the bone marrow. Preliminary results from my lab in Madrid showed affectation of the B-cell lineage that merits further investigation. This will be done in collaboration with H. Koseki and H. Kawamoto in order to characterize the function of RYBP during hematopoietic development.

Recent publications

1. Calés, C., Román-Trufero, M., Pavón, L., Serrano, I., Melgar, T., Endoh, M., Pérez, C., Koseki, H. and Vidal, M. (2008) Inactivation of the Polycomb group protein Ring1B unveils an antiproliferative role in hematopoietic cell expansion and cooperation with tumorigenesis associated to *Ink4a* deletion. *Mol. Cell. Biol.* 28:1018-28

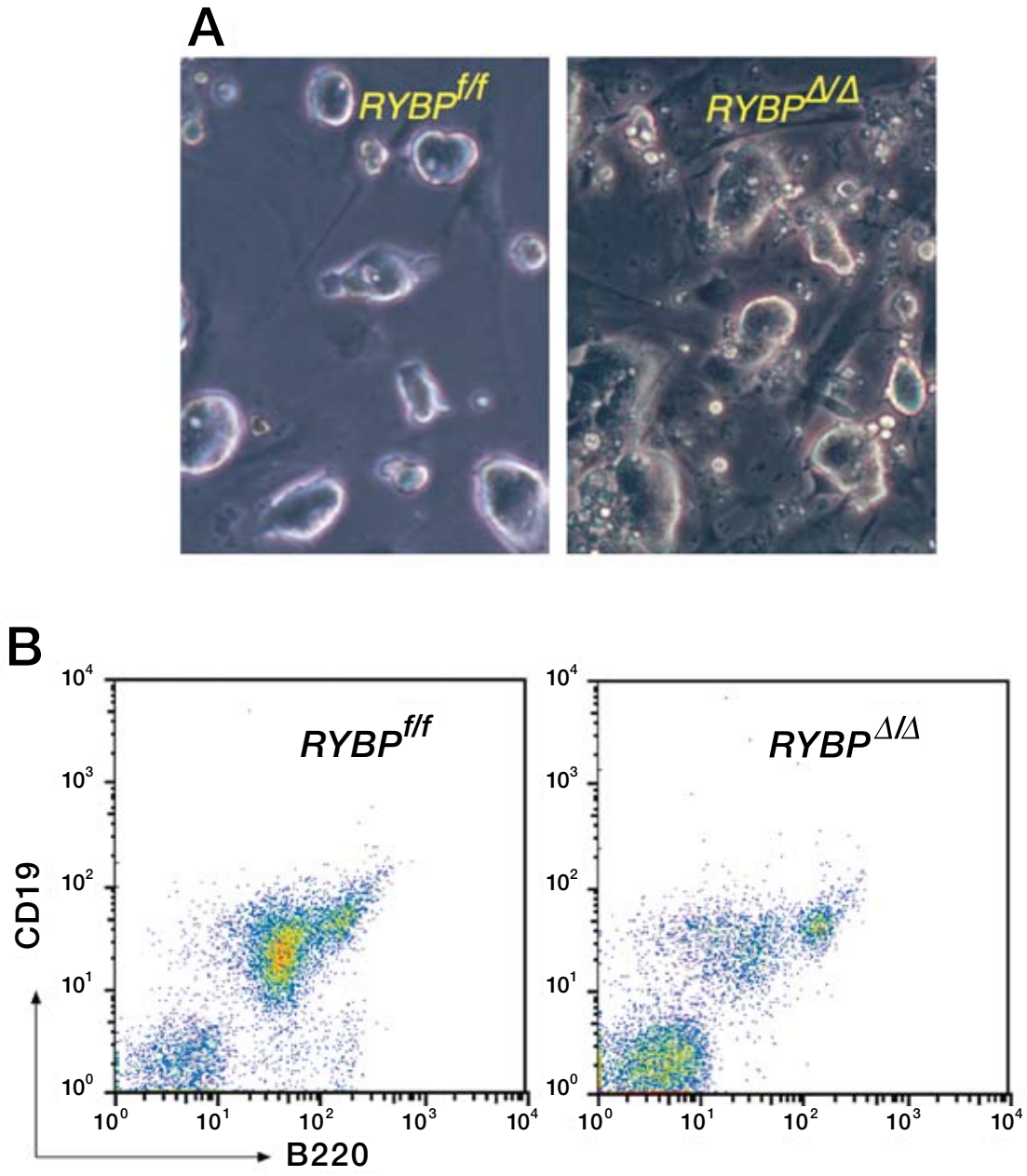


Figure Conditional inactivation of RYBP results in (A) enhanced apoptotic rate of ES cells growing under non-differentiating conditions, and (B) in decreased number of B-cells in the bone marrow of mutant mice.

Central Facilities

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

FACS Lab

Technical Scientist: Hanae Fujimoto
Technical Staff: Yukiko Hachiman

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

1. **Technical support and training**
   In 2008, the facility offered 20 technical courses (14 for cell sorting and 6 for cell analysis). Courses were held at 7 different levels, LSR, Calibur basic, Calibur option, Vantage basic, Vantage option, Aria basic and Aria option. A total of 49 researchers in RCAI took these courses in 2008.
2. **Cell sorting operation service**
   The FACS Lab provides a cell sorting operation service, through which researchers request an experienced operator to conduct the sorting experiment. In 2008, there were 504 such requests (Fig.), a 7.7% increase from the previous year.

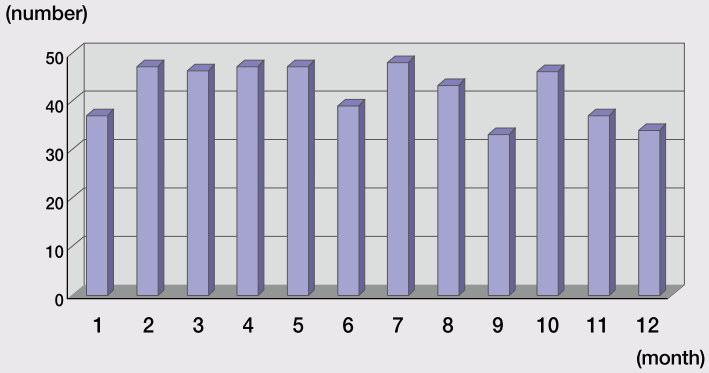


Figure : Cell sorting operation services provided per month in 2008

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

Monoclonal Antibody Lab

Technical Staff: Tomomi Aoyama, Mayuko Matsuda

The Monoclonal Antibody (mAb) Lab restarted its activity in November, 2008 after a 7 months hiatus. The new activity is partially supported by Becton Dickinson. Two technical staff members, Ms. Aoyama and Ms. Matsuda, are engaged to strategically develop mAb, such as those recognizing regulatory cells, stem cells, and allergy related molecules, that would meet the needs of RCAI researchers and society. They especially target the development of mAb for FACS staining in collaboration with RCAI researchers. These products will be considered by Beckton Dickinson for global commercialization.

Confocal Lab

Collaborative Researcher: Akiko Furuno and Yasutaka Wakabayashi (Leica Co. Ltd.)

The Confocal Lab provides imaging equipment and technical support and is managed in collaboration with Leica Microsystems. There are 5 Conforcal microscope systems and a new multiphoton system is now under development:

1. Inverted system with visual and multiphoton MP laser suitable for time-lapse imaging of living cells and organs.
2. Inverted system with a 405 nm laser suitable for a time-lapse imaging of living cells in a controlled environment (CO2, temperature, and humidity).
3. Inverted system with visual and UV lasers, which can be used for calcium detection.
4. Upright system with visual and UV lasers suitable for standard fixed specimen observation.
5. Intravital upright system with visual laser and MP laser, which can be used for *in vivo* imaging of various tissues such as lymph nodes.
6. Intravital upright system with single visual laser and double MP lasers, which can be used for *in vivo* imaging. Its scanning is faster and more sensitive, therefore applicable to more gentle observation.

During 2008, Dr. Furuno and Dr. Wakabayashi from Leica provided training courses for 24 people. The total running time of the microscopes was over 3230 hours.

Genomics Lab

Senior Research Scientist: Hiroshi Kitamura
Research Scientist: Yayoi Kimura
Research Associate: Mai Yamagishi
Technical Staff: Tomoko Yuasa, Masatoshi Ito, Tomoko Hasegawa, Atsuo Kobayashi, Saori Hayashi, Ryosuke Yashi, Satomi Takahashi, Ryo Yokoyama, Nobutake Suzuki, Kayoko Nagata

The Genomics Lab provides various services to the members of the Center: Protein identification by mass spectrometry, two-dimensional gel electrophoretic analyses (including differential image analysis), DNA microarray experiments, DNA sequencing, and cDNA/ Genomic clone distribution

1. **Proteome analysis**
   Mass spectrometry analysis (132 samples) and 2-D gel electrophoresis (50 samples) were carried out in 2008.
2. **DNA microarray analysis**
   Microarray analysis is conducted using the GeneChip system (Figure). In 2008, 91 miRNA assays and 470 expression/ exon assays were conducted.
3. **Multiplex suspension array**
   The Genomics Lab launched a new central support activity for cytokine assays using xMAP technology (Luminex). This array allows us to simultaneously measure multiple cytokines and chemokines in each sample (50- and 32- plexed cytokine/ chemokine assays for human and mouse, respectively). In 2008, 351 assays were carried out.
4. **DNA Sequencing**
   In 2008, the lab conducted 24,857 DNA sequencing runs.
5. **Clone distribution**
   The lab provides cDNA and genomic clones in FANTOM (Functional Annotation of the Mouse) projects and/or in the genomic resource of Kazusa DNA Research Institute. In 2008, 86 clones were provided to RCAI researchers.

Figure : GeneChip system

Animal Facility

Senior Research Scientist: Takanori Hasegawa
Technical Scientist: Yasuyuki Murahashi, Shinobu Mochizuki
Technical Staff: Masashi Matsuda, Tomoyuki Ishikura, Isamu Hisanaga, Momoko Ookoshi, Natsumi Saitou, Yusuke Iizuka, Naomi Ootsuka
Administrative Staff: Hiroko Iwamoto
Animal care takers (out sourced): 21 people

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

1. **Generation of SPF mice and cryopreservation of fertilized embryos**
   In order to maintain its SPF level, the facility performs *in vitro* fertilization to sanitize mice obtained from outside institutes. In 2008, 3,507 mice of 187 lines were obtained by this procedure. Cryopreservation of fertilized embryos reached 50,282 embryos of 392 lines.
2. **Generation of transgenic mice**
   The facility generates transgenic mice on users' requests. 72 transgenic mouse lines were generated using 35 vectors in 2008.
3. **Generation of chimeric mice**
   1) injection method
   818 male chimeras were generated using 45 vectors from 12 laboratories.
   2) aggregation method
   1,069 male chimeras were obtained using 67 vectors from 12 laboratories.

Figure : SPF mice maintained in the Animal Facility

4. **Cryopreservation of sperm**
   Twenty-four tubes of 9 lines were cryopreserved in 2008.
5. **Support for the ENU mutant mice project**
   The facility produced third generation (G3) offspring for phenotype-driven screening of the ENU-induced mutants. During 2008, 6 lines of G3 (363 mice) were generated by *in vitro* fertilization.
6. **Support for the Humanized Mice project**
   In 2007, the facility installed 16 racks of vinyl isolators to support the generation of humanized mice. In 2008, 20 racks were added and a total of 36 racks are maintained in the facility.

Immune iPS Project is Launched

Core members :

Haruhiko Koseki
Ruken Deveci-Yaman
Kyoko Masuda
Midori Iida-Kobayashi
Momoko Okoshi-Sato
Mai Sugiyama
Chieko Tezuka

Adjunct members :

Hiroshi Kawamoto
Fumihiko Ishikawa
Osamu Ohara
Hiroshi Watarai

Induced pluripotent stem (iPS) cells may possess tremendous therapeutic potential not only in the field of regenerative medicine but also for immune therapy. RCAI has launched a new activity to apply iPS technology for mouse and human immunology research and therapeutic development.

The main aim of the project is to clarify genetic/epigenetic profiles of iPS clones which reflect tumorgenicity and thus to establish criteria that can be used to select risk-free iPS cells (Figure A). This can be done by transferring hematopoietic cells derived from profiled iPS cells to humanized mice, and by subsequently monitoring development of leukemia in these mice. Other projects include trials to reprogram mature lymphocytes into iPS cells and then re-induce them back into mature lymphocytes, aiming to expand a lymphocyte bearing a specific antigen receptor, such as an NKT cell (Figure B). These trials have been combined into the immune iPS project. The core facility for iPS research is engaged in providing technical support for any studies using iPS cells on a collaborative basis with individual research activities in RCAI. This activity is partly supported by CREST from JST.

Photo : Staff members

(*from left to right*) Midori Iida-Kobayashi, Haruhiko Koseki, Kyoko Masuda, Mai Sugiyama, Hiroshi Kawamoto and Chieko Tezuka

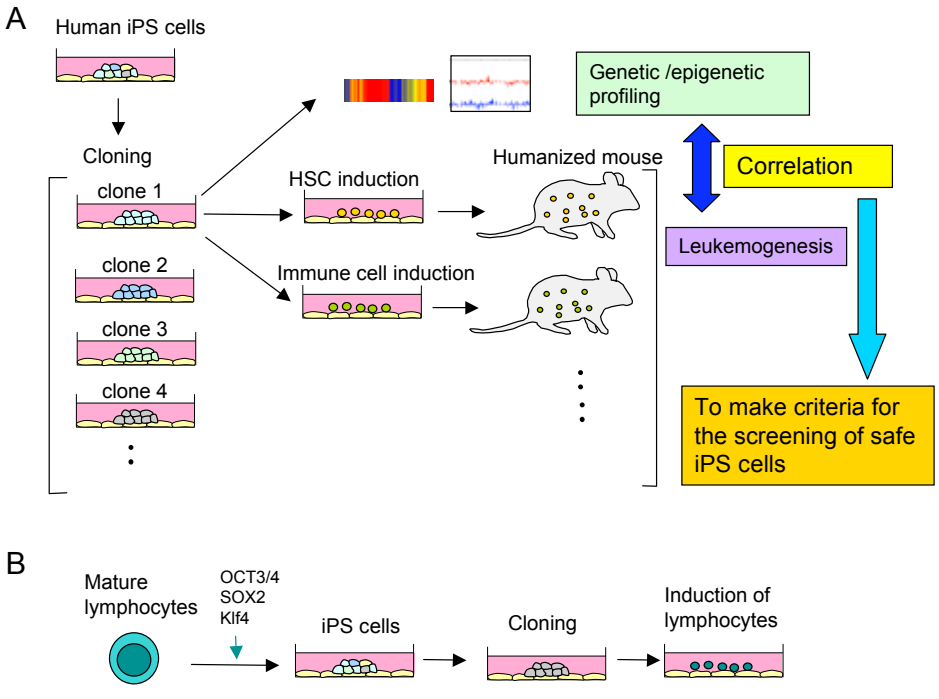
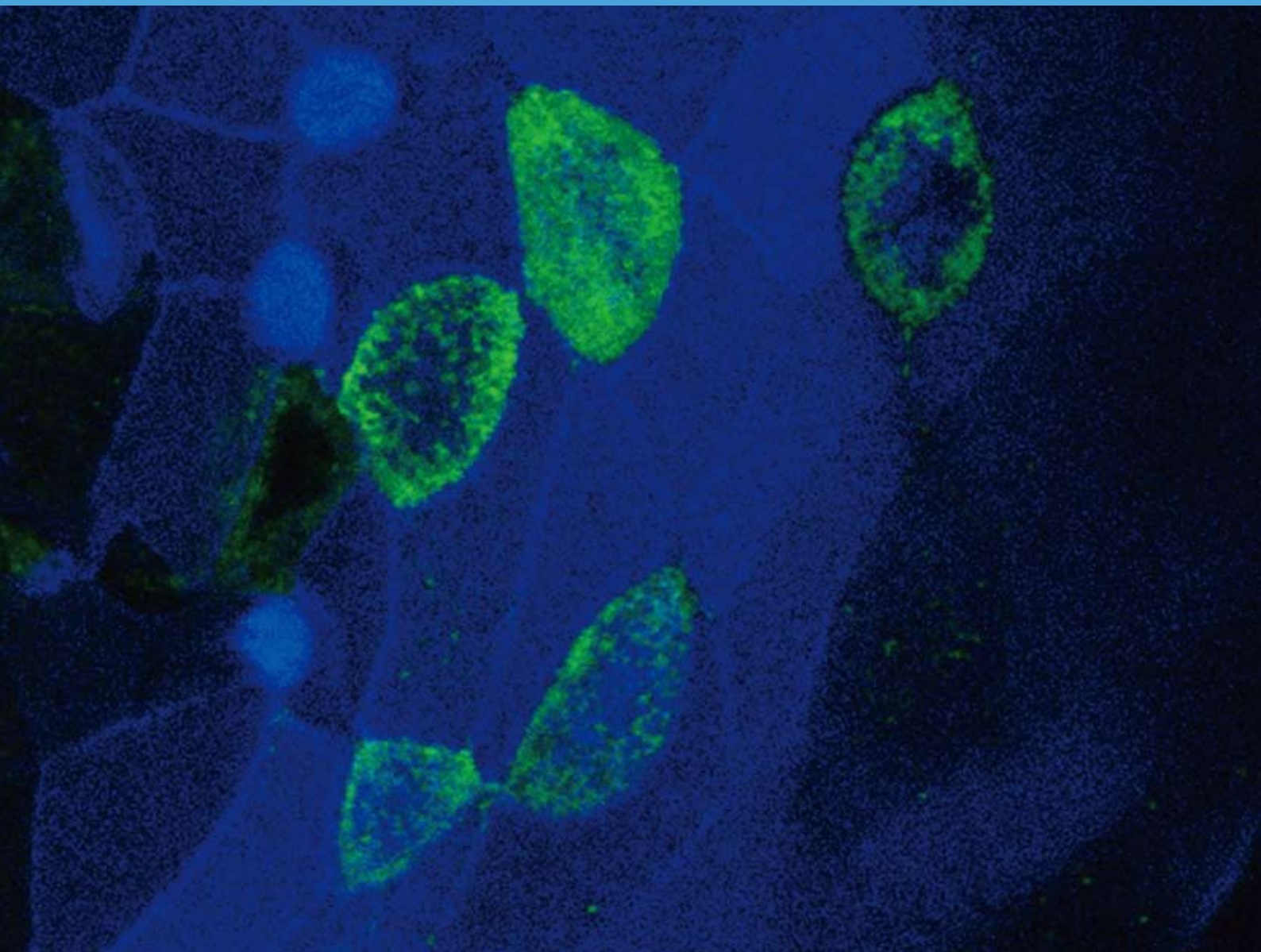


Figure Schemes of the Immune iPS project

2008

Research Activities



The immune cell march

A color-shifting fluorescent protein allows researchers to observe immune cell migration that occurs in living animals

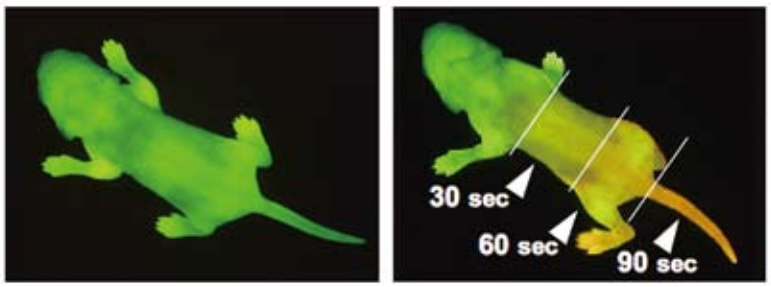


Figure A newborn mouse (*top*) expressing the color-swapping Kaede protein in its cells. When the mouse is exposed to violet light for the indicated times (*bottom*), its green cells turn red, and can be tracked as they move through the body.

Immune cells migrate throughout the body to monitor different organs and rapidly respond to invading pathogens. Now it is possible to monitor immune cell transport in a line of genetically engineered mice that was created by a team of researchers led by Osami Kanagawa at RCAI, and including Yoshihiro Miwa at the University of Tsukuba, and Atsushi Miyawaki at the RIKEN Brain Science Institute in Wako. The mice were modified to express a new color-shifting fluorescent protein called 'Kaede' in all their cells. Normally, the Kaede protein glows green. But when cells expressing the Kaede protein are exposed to violet light, it glows red (Figure), without any effect on cellular function. To track the cells, the researchers made a small incision in the skin near the groin of the mice to expose the inguinal lymph node to violet light, which caused all of the Kaede protein in the cells to become red. This effectively flagged the origin of the red cells. Using this technique, the researchers determined the speed of transport of different types of lymph node immune cells—T cells, B cells, and dendritic cells—to different tissues and organs in the body. They also located where the cells would migrate. The different types of immune cells migrated from place to place at different speeds, and migrated to different locations, including the blood, other lymph nodes, the spleen, bone marrow, liver and lung. Many of the inguinal lymph node cells migrated to the axillary lymph node in the armpit, suggesting that these two lymph nodes may be directly connected to each other through a lymphatic vessel. Kanagawa and colleagues confirmed this by injecting blue dye into the inguinal lymph node, which was rapidly detected in the axillary lymph node. The researchers also were able to observe the migration of immune cells from the skin, an organ that is not part of the immune system. When they exposed the skin to violet light, the immune cells found there migrated into a nearby lymph node. Future experiments could use the mice expressing the Kaede protein to monitor cell movement during autoimmune disease induction, and during immune responses to pathogens. "We recently made another mouse line, in which the Kaede protein can be expressed in a tissue-specific manner, and we would like to use these mice to study when and where the initial immune response starts," says Kanagawa.

Osami Kanagawa

Michio Tomura

ORIGINAL RESEARCH PAPER

Tomura, M., Yoshida, N., Tanaka, J., Karasawa, S., Miwa, Y., Miyawaki, A. & Kanagawa O. Monitoring cellular movement *in vivo* with photoconvertible fluorescence protein "Kaede" transgenic mice. *Proceedings of the National Academy of Sciences USA* **105,** 10870–10875 (2008).

Heterogeneity of Foxp3$^+$ T cells

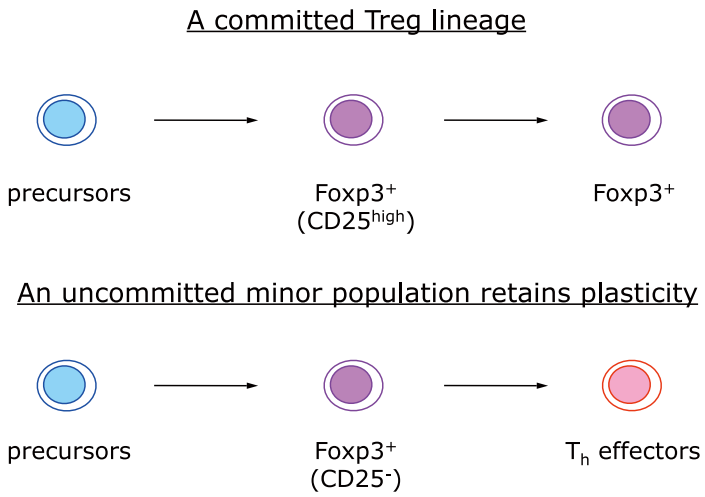
A small subpopulation of T lymphocytes known as regulatory T cells (T_{reg}) plays a central role in preventing pathological immune responses such as autoimmunity, inflammation, allergy and transplant rejection, thus ensuring tolerance to self- and innocuous environmental antigens.

T_{reg} predominantly express the transcription factor Foxp3, and its expression has been thought to specifically identify this lineage. However, recent studies in human T cells have shown that Foxp3 can be transiently expressed without inducing a T_{reg} phenotype and function.

To reevaluate the stability of Foxp3 expression in T_{reg}, Shohei Hori and his team analyzed natural Foxp3$^+$ T cells in normal mice. Surprisingly, they found that, expression of Foxp3 was down-regulated in some of the cells and that these Foxp3-down-regulated T cells lost the immuno-regulatory functions of T_{reg}. The majority of the Foxp3-down-regulated T cells produced inflammatory cytokines and adopted helper T cell functions, whereas some others retained "memory" of their previous Foxp3 expression and reacquired Foxp3 expression upon activation. These Foxp3-down-regulated T cells were generated preferentially in lymphopenic conditions through so called homeostatic proliferation.

To distinguish this unstable population of Foxp3$^+$ T cells, Hori's group focused on the CD25 marker, a molecule that has been shown to be associated with T_{reg} phenotype and to be directly regulated by Foxp3. Sorted CD4$^+$Foxp3$^+$CD25$^-$ and CD4$^+$Foxp3$^+$CD25^{high} T cells were compared in terms of their stability of Foxp3 expression and function. The team found that Foxp3 expression in CD4$^+$Foxp3$^+$CD25$^-$ cells was unstable and that these cells retain plasticity to differentiate into helper T cells in response to cytokine signals such as IL-4 and IL-6. On the other hand, CD4$^+$Foxp3$^+$CD25^{high} cells maintained Foxp3 expression and were resistant to such conversion to helper T cells even after many rounds of cell division. The results indicate that Foxp3$^+$ T cells are a heterogeneous population consisting of a committed T_{reg} lineage and an uncommitted subpopulation with developmental plasticity.

Adoptive cell therapy using T_{reg} has emerged as a potential novel treatment for autoimmune diseases, allergy, and transplant rejection. The isolation of functionally stable T_{reg} is a crucial step for this therapy. Identifying the molecular mechanisms that determine stability versus plasticity of T_{reg} and molecular markers that allow the isolation of stable T_{reg} cells will be crucial for this type of therapy.



Figure

Heterogeneity of Foxp3$^+$ T cells in normal non-manipulated mice
The majority of Foxp3$^+$ T cells isolated from normal unmanipulated mice represent a committed Treg lineage. These cells stably maintain Foxp3 expression and a T_{reg} phenotype irrespective of the cytokine milieu, and are enriched in the CD25^{high} subset. However, a minor subpopulation of Foxp3$^+$ T cells contained within the CD25$^-$ subset remain uncommitted to the T_{reg} fate and retain plasticity to re-differentiate into other effector Th subsets, including Th1, Th2, and Th17, in response to cytokine signals.

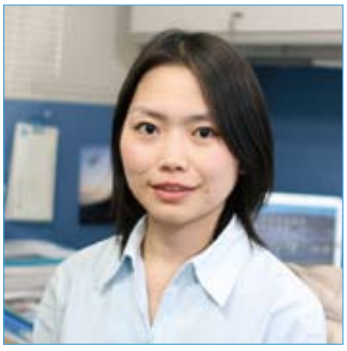
ORIGINAL RESEARCH PAPER

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

RELATED READING

Tsuji, M., * Komatsu, N.,* Kawamoto, S.,* Suzuki K., Kanagawa, O., Honjo, T., Hori, S.,† Fagarasan, S.† Preferential Generation of Follicular B Helper T (T_{FH}) Cells from Foxp3$^+$ T Cells in Gut Peyer's Patches. *Science* 323, 1488-1492 (2009)

Shohei Hori



Noriko Komatsu

Specifying the specialized mucosal ‘M cells’ by Glycoprotein 2

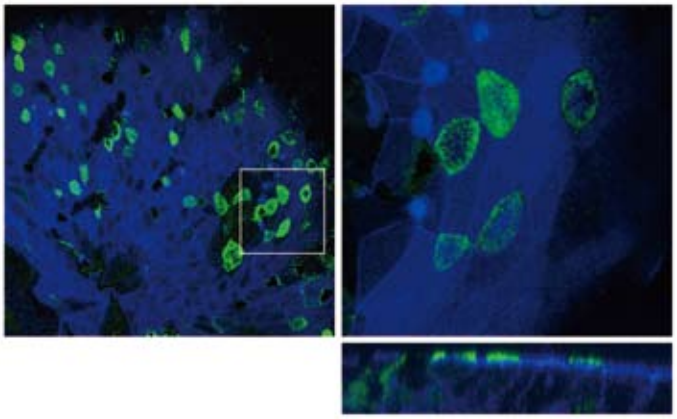
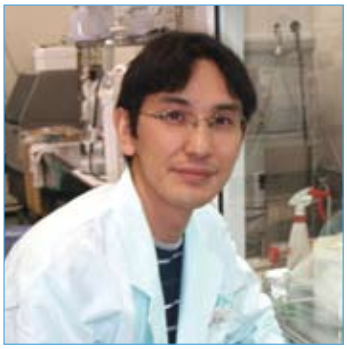


Figure **Whole-mount staining of murine Peyer’s patch shows M-cell-specific expression of GP2.**
The upper left panel shows one FAE region of a murine Peyer’s patch. GP2-positive M cells (*green*) are scattered. The tissues are counterstained with phalloidin to depict the outline of all the cells. The right panels are the magnification of the white rectangle in the left. The X-Z section (*bottom*) indicates that GP2 is localized on the apical plasma membrane of M cells.

The intestinal immune system is the largest component of our immune system, containing approximately three-quarters of all lymphocytes and harboring organized lymphoid structures of the gut-associated lymphoid tissue (GALT) such as Peyer’s Patches (PPs). To evoke the proper mucosal immune response against a vast array of food-borne and microbial antigens, they must be transported across the gut epithelium into the GALT. This ‘antigen transcytosis’ is mediated by specialized epithelial M cells scattered amongst the follicle-associated epithelium (FAE) that overlays the PP. Although M cells are believed to play an important role in mucosal immunity, no M-cell-specific surface marker has been identified, thus we are completely in the dark concerning the molecular mechanisms of M-cell function. In a recently published paper, Koji Hase and Hiroshi Ohno of the laboratory for Epithelial Immunobiology, in collaboration with Dr. Kiyono’s group in the University of Tokyo, reported that Glycoprotein 2 (GP2) is exclusively expressed by Peyer’s patch M cells in the mouse intestine.

GP2 is a glycosylphosphatidylinositol protein originally identified as being highly expressed in pancreatic acinar cells, although its function has long been unknown. However, the cDNA microarray with subsequent qPCR and *in situ* hybridization analyses revealed that GP2 is specifically expressed in M cells among the intestinal epithelial cells. Immunostaining with anti-GP2 monoclonal antibodies raised by these investigators confirmed the M-cell-specific expression of GP2 and provided further insight into its possible function in that GP2 protein is localized on the apical plasma membrane of M cells (see figure). Further studies by Dr. Ohno’s laboratory indicate that GP-2 is expressed by both murine and human M cells, suggesting that it could serve as a novel, universal M-cell marker. His team’s data also suggest that GP-2 may function as an uptake receptor for antigens of the intestinal lumen. The molecular dissection of GP2-mediated antigen transcytosis could lead to development of more efficient oral vaccination strategies against mucosal infectious diseases and/or food allergies.

Hiroshi Ohno



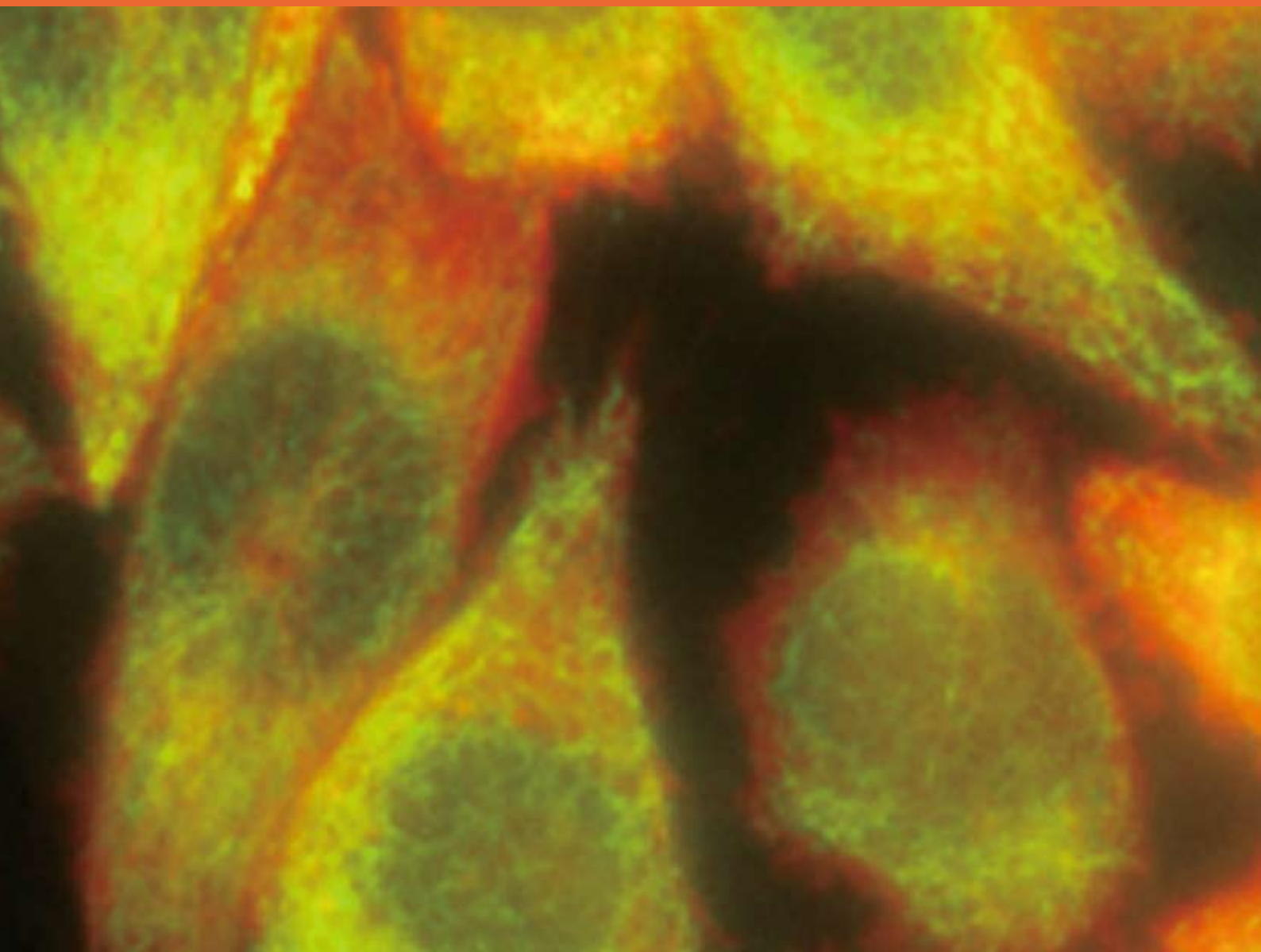
Koji Hase

ORIGINAL RESEARCH PAPER

Terahara K, Yoshida M, Igarashi O, Nochi T, Pontes GS, Hase K, Ohno H, Kurokawa S, Mejima M, Takayama N, Yuki Y, Lowe AW, Kiyono H. Comprehensive gene expression profiling of Peyer's patch M cells, villous M-like cells, and intestinal epithelial cells. *Journal of Immunology* **180,** 7840-7846 (2008)

2008

Collaborative Networks



Allergy Vaccine Project is Designated as the First RIKEN Translational Research Study

In June, 2008, RCAI's Allergy Vaccine Project, led by Dr. Yasuyuki Ishii of the Vaccine Design Research Team, was designated as the first RIKEN Translational Research (TR) Project.

Pollinosis is a serious and growing problem in Japan, where an estimated 22 million people or 16% of the population is suffering from symptoms of the allergy. Until now there has been no treatment except for symptomatic treatments, such as antihistamines, which suppress the symptoms of itchy eyes, runny nose and sneezing. A new allergy vaccine would offer the hope of a cure, or at least a way to regulate the allergic response caused by pollinosis.

In the RIKEN TR project, RCAI will collaborate with phamaceutical companies for clinical application of two types of cedar pollinosis vaccines developed by Dr. Ishii and his colleagues. One is a protective vaccine, which is a recombinant fusion protein of two cedar (*cryptomeria japonica*) pollen antigens (Cry j1/Cry j2). The other is a therapeutic vaccine, which is a liposome vaccine containing the cedar pollen antigens and α-GalCer glycolipids.

Through animal trials, the team found that the vaccines were effective in both treating the condition and reducing the likelihood of anaphylactic shock.

The project design is that, RIKEN, in association with the universities and the hospitals in the Allergy Network (explained below) will cooperate for pre-translational/ translational research and proof of concept studies using GMP samples generated by a pharmaceutical company (Figure). Their endeavors are expected to be an important bridge between immunological and clinical studies, and contribute to the cure for human diseases.

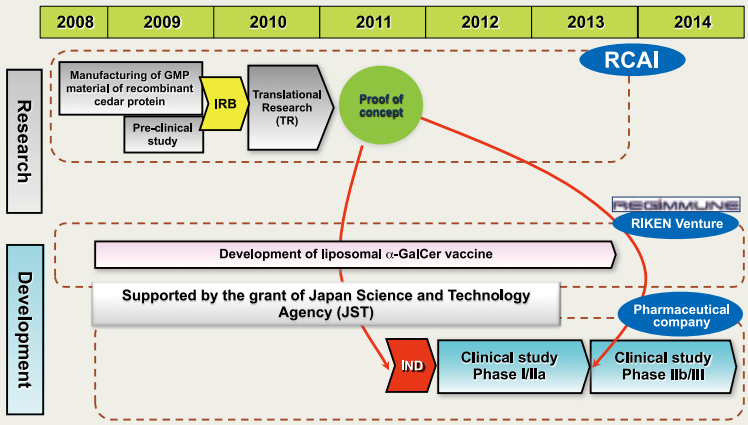


Figure R & D of allergy vaccines for Japanese cedar pollinosis

Establishment of an Allergy Research Network

In order to translate the results of basic science into clinical applications, RCAI established a network on clinical allergy in 2008, with linkage to seven universities (Table), Sagamihara Hospital, Kanagawa Children's Hospital, and a pharmaceucal company, Torii Co. Ltd.

The first meeting was held at RCAI on April 8, 2008 to discuss the issues that needed to be solved for understand ing the pathogenesis of allergies and the development of new therapies for prevention of these diseases. According to a decision at the meeting that one of their goals is to discover allergy biomarkers, which will be useful tools to evaluate the efficacy of allergy therapies, they analyzed immunological and clinical features of allergic patients, and RCAI created a database of the immunological status and clinical characteristics of the patients. In addition, to identify genetic markers linked to therapeutic mechanisms of sublingual desensitization therapy, now being tested in pre-clinical trials, RCAI investigators used microarray of peripheral T cells in clinical samples for gene expression profiling. Several candidate genes, probably as sociated with some aspects associated with clinical improve ment after therapy, have been extracted by this approach.

It has been recently recognized that rapid desensitization therapy is an effective treatment method for food allergy, however, the underlying mechanism remains unknown. In this context, RCAI established a network from basic to clinical sites to analyze the immunological aspects of rapid desensitization and to develop the technique for applications to otherallergies.

In June 2008, RIKEN President Noyori declared the development of a cedar pollen allergy vaccine as the first translational research project in RIKEN (see above). In collaboration with pharmaceutical companies, the network also agreed to cooperate for pre-translational/translational research and proof of concept studies of the novel allergy vaccines.

Table : Members of the Allergy Research Network

Keisuke Masuyama	University of Yamanashi
Kimihiro Okubo	Nippon Medical School
Shigeharu Fujieda	University of Fukui
Norimitsu Tanaka	Kagoshima University
Mitsuhiro Okano	Okayama University
Yuichi Majima	Mie University
Reiko Honma	Research Institute of Torii Pharmaceutical Co.
Yoshitaka Okamoto	Chiba University

Open Laboratory for Systems Immunology

Ever since the days of Jerne's network theory, it has been thought that theoretical and/or computational approaches could be particularly useful and important in immunology, because the immune system is one of the most complex biological systems. Advances in genomics, bioimaging, and computational science have made this idea more realistic now than ever before. How to understand complex immune reactions from a systematic viewpoint is a serious and intriguing issue for the future of immunology. Considering this, RCAI has launched an open laboratory for systems immunology where multidisciplinary researchers can interact with each other at RCAI. In the first phase, RCAI invited top researchers in the fields of mathematical/theoretical biology and systems engineering of biological systems (Table).

A kick-off meeting of the open laboratory for systems immunology was held at RCAI on January 16, 2009. Drs. Junichi Imura (Tokyo Institute of Technology), Yoshiharu Yamamoto (The Univ. of Tokyo), Shinji Nakaoka (The Univ. of Tokyo), Hidenori Kimura (RIKEN Bio-Mimetic Control Research Center), Mariko Hatakeyama (RIKEN Advanced Science Institute) participated in the meeting together with RCAI researchers.

At the meeting, the investigators discussed how to understand the basic principles underlying the normal structure and dynamics of the immune system as well as the mechanisms by which the system fails, causing disease. They will approach these subjects from the computational, mathematical, and statistical aspects of systems biology, integrate the data obtained by various technologies, such as a single molecule microscopy, gene expression profiling at a single cell level, or deep-imaging microscopy, and build up a model for understanding the spatio-temporal dynamics of the immune system. They will especially focus on molecular and cellular behavior, including single molecule and cell movement, and thereby try to understand higher order functions such as immunological memory.

To establish models enabling us to predict Immune system behavior under environmental or genetic alterations, the participants have begun to discuss various immune events with non-immunologists in a face-to-face manner from experimental and theoretical/computational viewpoints.

The predictive nature of such models is expected to provide reliable intervention strategies to control immune system functions.

Table : The members of Open Laboratory for Systems Immunology in RCAI

Kazuyuki Aihara	The University of Tokyo
Shinji Hara	The University of Tokyo
Hidenori Kimura	RIKEN Bio-Mimetic Control Research Center
Yohiharu Yamamoto	The University of Tokyo
Kenko Uchida	Waseda University

Harvard Summer School Program at RIKEN Center for Allergy and Immunology

RCAI launched a summer school program for undergraduate students of Harvard College in collaboration with the Reischauer Institute of Japanese Studies at Harvard University.

The first two students to be accepted into the Harvard Summer Program 2008 were Ms. Tzu-Ying Chuang and Ms. Iddoshe Hirpa. They spent two months (Jun. 9-Aug. 7) in the RCAI Autoimmune Regulation Research Group and Immune Regulation Research Group, respectively, as interns. They also participated in a series of basic immunology lectures, RCAI's International Summer Program (RISP), the RCAI-JSI International Symposium on Immunology, basic Japanese classes, and a few excursions.

The RCAI Harvard Summer School Program 2009 is planned for June 8–August 16.

http://www.summer.harvard.edu/2009/programs/abroad/yokohama/

Photo (*from left*) **Ms. Tzu-Ying Chuang, Dr. Shigeo Koyasu (a guest lecturer), Ms. Iddoshe Hirpa, and Dr. Masaru Taniguchi.**

Symposium for Primary Immunodeficiency Diseases in Asia

Primary Immunodeficiecy Diseases (PID) are heterogeneous genetic defects that cause immune abnormalities of varying severity. The PID are an important health problem because they are rare and complex and thus often misdiagnosed and inadequately treated, despite their sometimes grave prognoses. Given its mission of improving the immune health of the Japanese populace, RIKEN RCAI began to establish a Primary Immunodeficiency Database in Japan (PIDJ) in 2006. The PIDJ network is in the relatively early stages of development and is currently directed toward Japanese patients and their physicians. Plans for the next phase are already in place and involve the expansion of the network into Asia. To get this effort underway, RIKEN RCAI held an Asian immunodeficiency conference at RCAI on December 11-12, 2008.

On the Japanese side, the meeting was organized by Dr. Taniguchi (RCAI) and Dr. Hara (Leader, PID Study Group in Japan, Ministry of Health, Labour and Welfare) and included some of the top immunodeficiency experts in Japan. Immunodeficiency experts also attended from many Asian countries including Korea, China, Taiwan, Thailand, Australia, and Iran. Dr. Hans Ochs, a well-known PID specialist from Seattle Children's Research Institute, USA, also attended the meeting.

The meeting began with an overview of PID networks in the US and Europe and then described the basic setup of the PIDJ and how it might be expanded into Asia. The immunodeficiency experts from the other countries then described the state of PID diagnosis in their homelands, in some cases describing specific immunodeficiency diseases. This session was followed by talks describing basic science approaches to understanding PID and then a special lecture by Dr. Ochs, "Regulatory T Cells and the Clinical Syndrome of Immune Dysregulation, Polyendocrinopathy, Enteropathy, X-linked Inheritance (IPEX)". The second day of the meeting began with a poster session, followed by talks on pathogenesis/pathophysiology of PID, diagnostics, both current and in the future, when diagnosis will hopefully become more rapid and accurate by incorporating advanced software and database platforms. The final session dealt with the very important topic of therapeutic approaches to cure PID. The first successful human gene therapy was for a PID, X-linked severe combined immunodeficiency. This fatal disease is caused by mutations in the common γ-chain gene, which encodes a protein utilized by many cytokine receptors. The retroviral gene therapy was successful and the B and T cell systems in the patients were restored. However, several of the patients developed T cell leukemia due to insertional oncogenesis by the therapeutic vector. This outcome had a chilling effect, but research has continued and was described, to develop safer vectors or stem cell therapies.

The Symposium participants were uniformly in favor of continuing efforts to establish the Asian PID network, ultimately integrating it into worldwide networks. After the close of the Symposium, a satellite meeting to discuss the specifics of forming an Asian PID network was organized by Dr. Ohara. The goal is to introduce a regional web-based system for the registration of patients, share diagnostic tools and methods for disease screening, and set up consultation systems among specialists in the Asia-Pacific Economic Cooperation (APEC) countries. All the participants in the satellite meeting agreed to make an effort to generate such a PID network among APEC countries and have started to take practical steps towards that goal.



Session I : PID Network	
Kosuke Imai (National Defense Medical College)	PID Network in US and Europe
Shigeaki Nonoyama (National Defense Medical College)	PIDJ Network: Clinical and Basic Linkage
Osamu Ohara (RIKEN RCAI/Kazusa DNA Research Institute)	PIDJ Network: DNA Analysis
Session II : PID in Asian Countries	
Toshiro Hara (PID study group in Japan/ Kyushu University)	Primary immunodeficiency in Japan
Joong Kim (Seoul National University, Korea)	Primary immunodeficiency in Korea
Xiaochuan Wang (Fudan University, China)	PID, being unveiled in China
Wen-I Lee (Chang Gung Memory Hospital and University College of Medicine, Taiwan)	Clinical aspects, immunologic assessment and genetic analysis in Taiwanese children with hemophagocytic lymphohistiocytosis
Voravich Luangwedchakarn (Mahidol University, Thailand)	Primary immunodeficiency in Thailand
Tony Roscioli (University of Sydney, Australia)	Molecular and cellular studies in veno-occlusive disease with Immunodeficiency syndrome due to SP110 mutations
Asghar Aghamohammadi (Children's Medical Center, Iran)	Primary immunodeficiency in Asia: today and future
Session III : Basic and Clinical Development in PID	
Fumihiko Ishikawa (Riken RCAI)	Humanized mouse model for primary immunodeficiency diseases
Katsuyuki Minegishi (Tokyo Medical and Dental University)	Molecular origin and pathogenesis of hyper-IgE syndrome
Special Lecture Hans D. Ochs (Immunology Research Seattle Children's Research Institute, USA)	FOXP3, regulatory T cells and the clinical syndrome of Immune Dysregulation, Polyendocrinopathy, Enteropathy, X-linked inheritance (IPEX).
Session IV : Pathogenesis/pathophysiology of PID	
Hideo Kaneko (Gifu University)	Pathogenesis of primary antibody deficiencies
Akihiro Yachie (Kanagawa University)	Omenn disease and related syndrome; variable genetic backgrounds and common pathogenesis
Hirokazu Kanegane (Toyama University)	Flow cytometric diagnosis of primary immunodeficiency diseases
Hidetoshi Takada (Kyushu University)	IRAK4 deficiency in Japan
Sujatha Mohan (RCAI)	Construction of an informational platform for primary immunodeficiency diseases in Asia
Hiroyuki Nunoi (Miyazaki University)	Guideline for hematopoietic stem cell transplantation in chronic granulomatous disease-based on a retrospective survey in Japan, 1992-2006
Satoru Kumaki (Tohoku University)	Safer approach using suicide gene-equipped vector in X-SCID gene therapy
Masafumi Yamada (Hokkaido University)	Stem Cell Gene Therapy without Cytoreductive Conditioning for 2 Patients with ADA Deficiency

LIAI-RCAI Joint Workshop 2009

RCAI and the La Jolla Institute for Allergy and Immunology (LIAI) have had a collaborative arrangement for four years and previously held a joint meeting in La Jolla in Feb. 2006. This year a joint meeting with LIAI researchers, "Regulation of Lymphocyte Function and Immune Responses," was held at RCAI on January 7. This was co-organized with the Chiba University G-COE program, which organized a symposium, "Immune System Regulation and Treatment," with LIAI on Jan.6 in Tokyo.

The January 6th Symposium featured talks by investigators from RCAI, LIAI, Benaroya Research Institute (USA), Chiba University, and the National Institute of Radiological Sciences (JAPAN). The theme of the day's sessions was a unique combination of basic research on immunoregulation and clinical applications. After a keynote lecture by Mitchell Kronenberg (LIAI) on NKT cells, there were sessions on immune system regulation and immunological memory. These were followed by translational research talks on allergy and vascular diseases, and then a session on clinical applications, including the use of NKT cells in tumor immunotherapy.

The LIAI-RCAI workshop on January 7th was organized by Yun-Cai Liu (LIAI) and Takashi Saito (RCAI). The first session focused on lymphocyte development and signaling, with talks on lineage potential during T cell development and molecular aspects of signaling processes in immune cells. The second session on immune regulation and modification featured talks on the roles of ubiquitination in the immune system, structural features of immunodominance in humoral and cellular immunity, and regulation of interferon responses. The final session dealt with mucosal immunity and disease regulation in autoimmunity, allergic diseases, and mouse models of the human immune system.

The RCAI/LIAI collaboration is based on common research interests, from basic immunology to disease, particularly allergy. Future joint symposia are planned to further enhance these collaborations.



I. Lymphocyte development and signaling	
Myeloid potential is retained in T cell progenitors: Revised scheme for hematopoiesis	**Hiroshi KAWAMOTO, RCAI**
Lineage decision in thymus	**Ichiro TANIUCHI, RCAI**
Dynamic regulation of T cell co-stimulation by TCR-CD28 microclusters	**Tadashi YOKOSUKA/ Takashi SAITO, RCAI**
SLAT: A novel GEF coordinating actin cytoskeleton reorganization and Ca^{2+} signaling in T cells	**Amnon ALTMAN, LIAI**
Calcium signal in immune cells	**Tomohiro KUROSAKI, RCAI**
II. Immune regulation and modification	
Protein ubiquitination in immune regulation	**Yun-Cai LIU, LIAI**
E3 ubiquitin ligases for MHC molecules	**Satoshi ISHIDO, RCAI**
How dendritic cell subsets respond to nucleic acid adjuvants?	**Tsuneyasu KAISHO, RCAI**
Structural features of immunodominance in antibody, helper and cytotoxic responses to vaccinia virus	**Alessandro SETTE, LIAI**
PDC-TREM, a novel plasmacytoid dendritic cell receptor, is responsible for augmentation of type I interferon production after Toll-like receptor triggering	**Hiroshi WATARAI/Masaru TANIGUCHI, RCAI**
III. Mucosal immunity and disease regulation	
Controlling tolerance and protective immunity from the outside in	**Hilde CHEROUTRE, LIAI**
Bacterial uptake receptors on intestinal M cells	**Hiroshi OHNO, RCAI**
Dendritic cells activated by viral infection or TLR signaling *in vivo* promote regulatory mechanisms that prevent autoimmune diabetes.	**Matthias von HERRATH, LIAI**
Role of zinc transporter in mast cell mediated-allergic reaction	**Keigo NISHIDA/ Toshio HIRANO, RCAI**
Reconstitution of human immunity and diseases in mice	**Fumihiko ISHIKAWA, RCAI**



International Research Collaboration Award

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

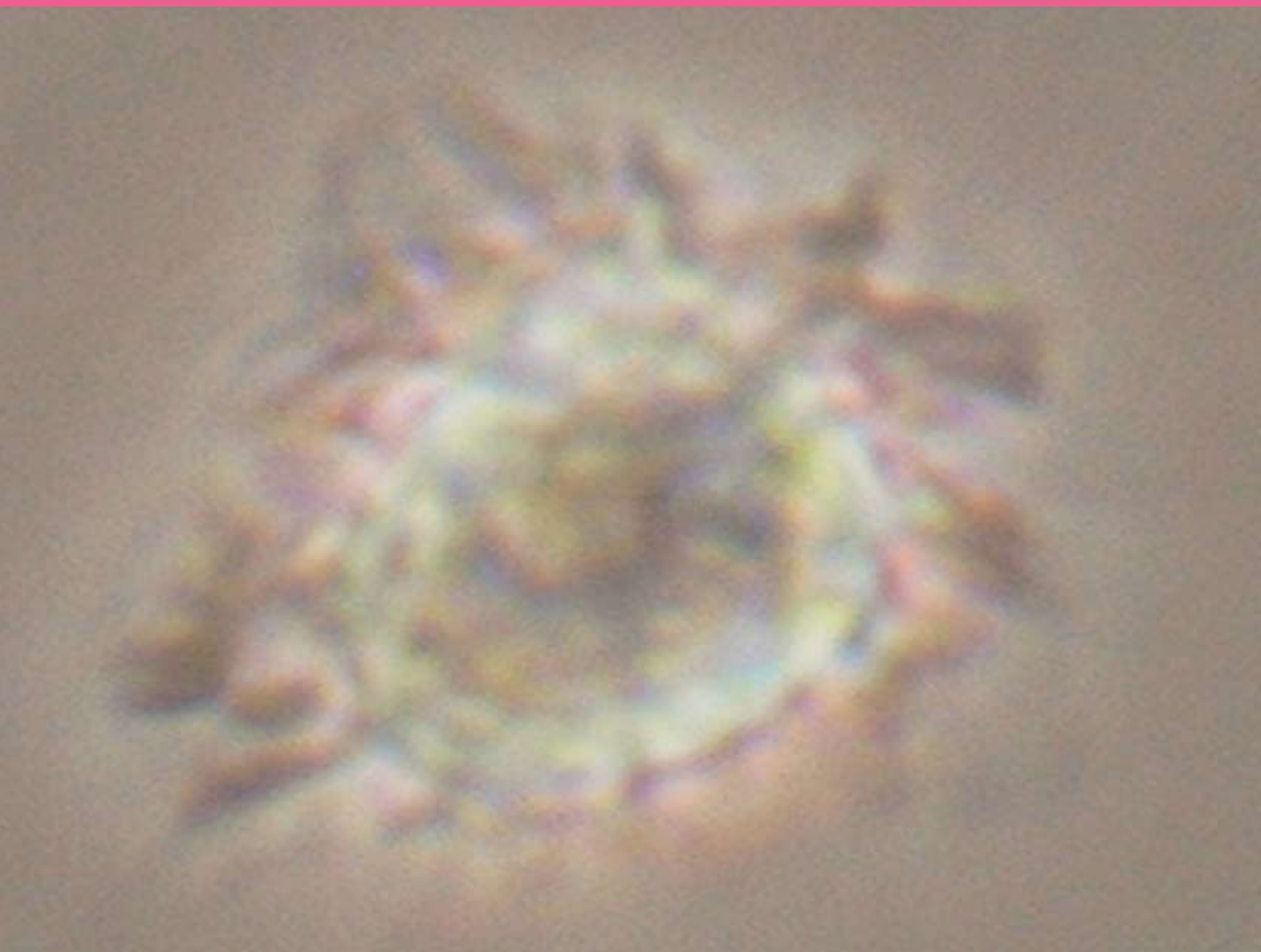
Since the program started in 2004, 14 projects have been funded (Table). In FY2008, Drs. Dustin and Saito published their work on spatiotemporal regulation of T cell microclusters and PKCθ translocation (Yokosuka et al. Immunity, 29, 589-601 (2008)) and Drs. Batista and Kurosaki's work on B cell synapse resulted in successful publication (Weber et al. J. Exp. Med. 205, 853-868 (2008)). Three collaborative projects took place in FY2008, and contributed not only to scientific discoveries but also to international exchange of people and international visibility.

Table : Awardees of RCAI International Collaboration Award Program

Year	Awardee	Title of Research	Host Lab.
2004-2006	**Michael Dustin** New York University School of Medicine	Analysis of dynamism and function of limmunological synapse using planar membrane and knock-in T cells	**Takashi Saito** Lab for Cell Signaling
2004-2006	**Willem van Ewijk** Leiden University Medical Center	Regulatory role of lymphoid progenitors during development of thymic microenvironments	**Hiroshi Kawamoto** Lab for Lymphocyte Development
2004-2006	**Miguel Vidal** Centro de Investigaciones Biologicas, CSIC	Genomic and functional analysis of the role of the Polycomb Ring1 genes in B-cell development	**Haruhiko Koseki** Lab for Developmental Genetics
2004-2005	**Steven F. Ziegler** Benaroya Research Institute at Virginia, Mason Medical Center	Role of NKT cells in TSLP-Mediated Aallergic linflammation	**Masaru Taniguchi** Lab for Immune Regulation
2004-2006	**Peter D. Burrows** University of Alabama at Birmingham	Expression and function of FcRY-a novel Fc receptor-related gene expressed in B cells	**Jiyang O-Wang** Lab for Antigen Receptor Diversity
2005-2007	**Wilfried Ellemeier** Institute of Immunology, Medical University Vienna **Hilde Cheroute** La Jolla Institute for Allergy and Immunology	Study of T cell differentiation mediated by regulated expression of CD8 genes	**Ichiro Taniuchi** Lab for Transcriptional Regulation
2005-2007	**Mark Bix** University of Washington, Seattle, Washington	Understanding genetic regulation of interleukin 4 production by a CD4(+) T cell-intrinsic mechanism.	**Masato Kubo** Lab for Signal Network
2005-2006	**Yun-Cai Liu** La Jolla Institute for Allergy and Immunology	Gene-array analysis and proteomics of Th2 tolerance	**Yasuyuki Ishii** Lab for Vaccine Design
2005-2007	**Kenneth M. Murphy** Howard Hughes Medical Institute, Washington University School of Medicine	Visualization of STAT protein in the cytokine mediated signaling at a single molecular level.	**Osami Kanagawa** Lab for Autoimmune Regulation
2005-2007	**Facundo Damian Batista** Cancer Research UK London	Role of signaling molecules in B cell synapse formation and its maintenance	**Tomohiro Kurosaki** Lab for Lymphocyte Differentiation
2006-	**Sunhwa Kim and Michael Karin** Department of Pharmacology, Univ. of California, San Diego	Identification of Novel Necrotic Molecules from Necrotic Hepatocytes and Examination of Its Effect on the Inflammatory Response	**Masato Tanaka** Lab for Innate Cellular Immunity
2007	**Andrea Brendolan** Cornell University Medical Center, Department of Cell and Developmental Biology	A study on the spleen and lymph nodes mesenchymal cells that participate in the assembly of artificial secondary lymphoid organs	**Takeshi Watanabe** Research Unit for Immune Surveilance
2008-	**Stefano Casola** IFOM-The FIRC Institute of Molecular Oncology Foundation	Nuclear reprogramming of terminally differentiated plasma cells to study the specific role of IgA in mucosal and systemic immunity and B cell development	**Sidonia Fagarasan** Lab for Mucosal Immunity
2008-	**Yoshiaki Ito and Motomi Osato** Institute of Molecular and Cell, National University of Singapore	Understanding of tumor suppressive mechanism of Runx complexes against leukemia and gastrointestinal cancer	**Ichiro Taniuchi** Lab for Transcriptional Regulation

2008

Events



RCAI International Summer Program 2008

The third RCAI international Summer Program (RISP 2008) was held in Yokohama June 20-27. Thirty-nine graduate students and postdoctoral fellows from nineteen countries participated in RISP 2008. The first part of the program was held at RCAI and featured poster and oral presentations by the participants and a series of lectures by invited speakers from RCAI, Japanese universities, and abroad. The second part of the program was the RCAI/Japanese Society for Immunology (JSI) joint meeting. "Regulation of Immune Homeostasis and Diseases" held at the Pacifico Conference Center in Yokohama. Two of the participants stayed on at RCAI for a four week long laboratory internship.

The focus of the lectures at RCAI was on innate immunity but other topics included lymphocyte development/differentiation, immune evasion, mucosal immunity, and immunotherapy. These lectures provided a broad overview of the immune system, with methodologies ranging from single molecule to whole animal analyses. Each of the invited speakers incorporated introductory material pertinent to their topic as well as recent highlights from their own research. The research interests of the participants were also varied, making for a unique opportunity during the oral and poster presentations as well as in more informal settings for cross-fertilization among immunology subdisciplines. The question periods following talks by both invited lecturers and participants were spirited and stimulating.

Several of the invited lecturers also gave talks at the RCAI/JSI meeting, where they described further new developments in their research programs. There were also many new speakers and topics, thus further increasing the breadth of the immunology coverage available to the RISP participants. Awards for best RISP posters were presented at a reception during the RCAI/JSI meeting. All of the participants gathered to express their appreciation to the organizers for inviting them to RISP 2008, an exceptional experience from scientific and cultural perspectives. The success of this unique program is due to the efforts of the Organizing Committee, Dr. Kurosaki (chair), Dr. Ishido, Dr. Tanaka and Ms. Aki (secretariat), who kept the entire operation running smoothly, and in particular to the efforts of the outstanding participants. Planning is already underway for RISP 2009, tentatively scheduled for early July.

Table 1: RISP lectures

Lecturer	Title
Dr. Yoshihiro Kawaoka, The University of Tokyo	Why influenza kills and will kill again
Dr. Satoshi Ishido, RIKEN, RCAI	A novel family of E3 ubiquitin ligase
Dr. Hiroshi Ohno, RIKEN, RCAI	The role of M cells, a specialized subset of intestinal epithelium, in the mucosal immune system.
Dr. Ichiro Taniuchi, RIKEN, RCAI	Lineage choice in thymus
Dr. Hiroshi Kawamoto, RIKEN, RCAI	Lineage restriction pathway in hematopoiesis: towards a unified model
Dr. Ruslan Medzhitov, Yale University School of Medicine	Innate immune system
Dr. Bruce Beutler, The Scripps Research Institute	Innate immune sensing and response: analysis by mutagenesis in mice
Dr. Christian Munz, The Rockefeller University	Human natural killer cell activation by dendritic cells during viral infection
Dr. Takeshi Watanabe, Kyoto University & RIKEN, RCAI	A trial to construct artificially-made secondary lymphoid tissues with a strong immunological function
Dr. Warren S Pear, University of Pennsylvania	The multiple roles of Notch in regulating immune development, function, and transformation
Dr. Andreas Strasser, The Walter and Eliza Hall Institute of Madical Research	FasL mutant knock-in mice delineate the roles of the membrane bound and secreted FasL.
Dr. Shohei Hori, RIKEN, RCAI	Dominant immunological tolerance
Dr. Andre Veillette, Clinical Research Institute of Montreal	NK cell biology and activation
Dr. Jeffrey Ravetch, The Rockefeller University	Role of IgG sialylation in controling inflammation

Table 2: RISP participants

Participants name (country)			
Damiana Alvarez (Argentina/Italy)	Olga Barreiro (Spain)	Stefano Caserta (Italy)	Alejo Chorny (Argentina)
Cristina Costantino (USA)	Jeremy Daniel (USA)	Marei Dose (Germany)	Mark Dowling (Australia)
Thorsten Feyerabend (Germany)	Sandra Gardam (Australia)	Landi Guillermo (Peru)	Yuyuan Han (Singapore)
Colin Havenar-Daughton (USA)	Leo Iwai (Brazil)	Jae-Young Kim (South Korea)	Nicholas Kin (USA)
Hyun-Jeong Ko (South Korea)	Brent Koehn (USA)	Heung Kyu Lee (South Korea)	Koon-Guan Lee (Singapore)
Elena Martinelli (Italy)	Gustavo Martinez (Argentina)	Ponpan Matangkasombut (Thai)	Sonja MeixIsperger (Germany)
Ngozi Monu (Nigeria)	Natalie Nieuwenhuizen (South Africa)	Daniel Patton (UK)	Robert Rigby (UK)
Richard Robinson (USA)	Lynnie Rudner (USA)	Noam Stern (Israel)	Jonathan Tan (Australia)
Jose Luis Vela (USA)	Femke Van Wijk (Netherlands)	Ingela Vikstrom (Sweden)	Di Wang (China)
Johnna Wesley (USA)	Mahesh Yadav (India)	Di Yu (China)	

RCAI-JSI International Symposium on Immunology 2008

Regulation of Immune Homeostasis and Diseases

The fourth RCAI-JSI International Symposium on Immunology 2008 took place on June 26-27 in Yokohama. This annual symposium is jointly organized by RIKEN Research Center for Allergy and Immunology (RCAI) and the Japanese Society for Immunology (JSI). Four hundred people, including 89 from outside of Japan, attended this year's symposium entitled "Regulation of Immune Homeostasis and Diseases". There were 24 prominent invited speakers who gave talks about the hottest topics related to immune regulation.

In the first session "Regulation of lymphocyte activation: molecular events and imaging", Dr. Arup Chakraborty of MIT presented his unique computational approach to analyze the biological experimental data on molecular signaling in T cells. Related to the imaging analysis, Dr. Takashi Saito of RCAI presented his recent data on T cell activation and Dr. Facundo Batista of Cancer Research UK on B cell activation. In the second session "Molecular basis of innate immunity", Dr. Tadatsugu Taniguchi of Univ. of Tokyo nicely summarized his tremendous body of work on the interferon regulatory factor (IRF) family, and Dr. Shizuo Akira of Osaka Univ. introduced the function of Toll-like-receptor 5 for host defense in the intestine. On the second day, a session "Signal transduction pathways regulating cell fates" focused on the cytoskeletal proteins and apoptosis. Dr. Shigekazu Nagata of Kyoto Univ. showed several movies to explain the variety of molecules used by different macrophages for recognition of apoptotic cells. The final session "Molecular mechanisms of immunological diseases and future direction" focused on disease-related approaches. Dr. Michael Lenardo of NIH introduced a human genetic approach to understand abnormalities of immunological tolerance. Dr. Alain Fischer of Hospital Necker introduced genetic disorders resulting in T-cell immunodeficiencies.

The various approaches introduced in this symposium gave a clear impression that research on immune regulation is moving toward the successful integration of multidisciplinary fields.

Session I : Regulation of lymphocyte activation: molecular events and imaging	
André Veillette, Clinical Research Institute of Montreal, Canada	SAP family adaptors in immune regulation
Tomohiro Kurosaki, RCAI and Osaka University, Japan	The PLCγ/calcium system as a regulator of immune cells
Fabienne Mackay, Garvan Institute of Medical Research, Australia	The role of BAFF in autoimmunity is T cell-independent but requires MyD88 signaling
Warren S. Pear, University of Pennsylvania, USA	The myriad functions of Notch1 in T cell development, differentiation, and transformation
Arup K. Chakraborty, MIT, USA	Cooperative effects during selection of the T cell repertoire
Arthur Weiss, UCSF, USA	Structural insights into ZAP-70 regulation and TCR signaling
Takashi Saito, RCAI, Japan	Dynamic regulation of T cell co-stimulation by TCR-CD28 microclusters
Facundo D. Batista, London Research Institute, UK	Initiation of B cell activation and adaptive immune response
Session II : Molecular basis of innate immunity	
Jürg Tschopp, University of Lausanne, Switzerland	NLRs and the inflammasome: danger sensing complexes involved in inflammatory diseases
Tadatsugu Taniguchi, The University of Tokyo, Japan	The role of IRF family transcription factors in innate immune responses
Shizuo Akira, Osaka University, Japan	The role of TLR5-expressing CD11c+ lamina propria cells in induction of adaptive immunity
Jeffrey V. Ravetch, The Rockefeller University, USA	Role of IgG sialylation in controlling inflammation
Session III : Signal transduction pathways regulating cell fates	
Janis K. Burkhardt, University of Pennsylvania, USA	Actin dynamics at the immunological synapse
Toshio Hirano, Osaka University and RCAI, Japan	Roles of zinc and its transporters in immunity and connective tissues
Tatsuo Kinashi, Kansai Medical University, Japan	Rap1-RAPL-Mst1 signaling in lymphocyte cascades and interstitial migration
Christian Münz, The Rockefeller University, USA	Viral regulation of autophagy for antigen presentation on MHC class II molecules
Andreas Strasser, The Walter and Eliza Hall Institute of Medical Research, Australia	FasL mutant knock-in mice delineate the roles of the membrane bound and secreted FasL
Shigekazu Nagata, Kyoto University, Japan	Engulfment of apoptotic cells
Session IV : Molecular mechanisms of immunological diseases and future direction	
Michael J. Lenardo, NIH, USA	Mutation in N-Ras causes ALPS Type IV
Akihiko Yoshimura, Keio University and Kyushu University, Japan	Regulation of Th17 differentiation and TGF-beta signaling by SOCS1
Nagahiro Minato, Kyoto University, Japan	Rap signaling in normal lymphocyte development and leukemia
Virginia Pascual, Baylor Institute for Immunology Research, USA	Old cytokines, novel therapeutic targets: type I interferon and interleukin 1
Hajime Karasuyama, Tokyo Medical and Dental University, Japan	Hyper-IgE syndrome is a compound primary immunodeficiency caused by the signalopathy of multiple cytokines
Alain Fischer, Hospital Necker, France	Human T cell immunodeficiencies

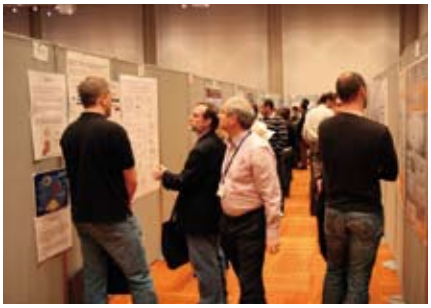




International Symposium on CD1/NKT Cells

The 5th International Symposium on CD1/NKT Cells was held March 23-27, 2009 in Kamakura. This meeting was previously designated a workshop and focused mainly on NKT cells, but this year, because of the large number of participants (~180), it was elevated to Symposium status. The 2009 meeting had several new features. In addition to talks by invited speakers and short talks selected from the submitted abstracts, there were two luncheon seminars, where the speakers were able to give a comprehensive overview of two hot topics, NKT cell development and NKT cells in cancer immunotherapy. The goal of these seminars was to broaden the knowledge base of young investigators who have recently joined the NKT/CD1 research field. In the same vein, due to current economic conditions, scholarships were awarded to all fifty-three graduate students and postdoctoral fellows who had applied for them.

This symposium brought together researchers focusing on an array of topics, and the sessions were notable for their intensity and lively discussion. The Keynote address was delivered by Dr. Shizuo Akira of Osaka University, a highly cited researcher well-known for his seminal work on the innate immune system. The meeting then started with a session on development and homeostasis of NKT cells, where new findings were presented about the origin of NKT precursors, the identification of a master transcription factor that directs NKT cells into that lineage, and the regulation of the pathway by micro RNA. A session on the structure of the NKT cell receptor and its ligand recognition provided evidence for the presence of physiologically important ligands on several bacterial pathogens. A session on whether there are endogenous selecting ligands for NKT cells was particularly lively and thought provoking. On the next day the focus shifted to CD1, the MHC-like restricting molecule for the NKT cells, with new data on how the glycolipid NKT antigens are loaded and stabilized in complex with CD1. The Thursday session was devoted to effector functions, which for NKT cells are many and seemingly ever increasing. There was discussion of the role of these cells in regulating inflammation, B cell help, IL17 production, and suppression. NKT cells were also shown to be involved in autoimmune diseases and tissue damage. The final session dealt with what is the holy grail of NKT cell researchers; given their potent activity in many physiological contexts, these cells have the potential to become powerful clinical weapons for controlling cancer and allergic diseases. Their use in cancer cellular immunotherapy was shown by several speakers to be efficacious, although there is clearly a long way to go before the full potential of NKT cells can be realized in the clinic.



4th JSI-RCAI Workshop

The Front-line of Allergy Research: Cytokines, Innate Immunity and Mast Cells

The 4th JSI-RCAI Workshop “The Front-line of Allergy Research: Cytokines, Innate Immunity and Mast Cells” took place on May 20, 2008 in Yamanashi prefecture.

Allergic diseases including asthma, allergic rhinitis, atopic dermatitis, and food allergy have become one of the epidemics of the 21st century. However, this burgeoning problem has not been met, in general, with any fundamental preventive/therapeutic options. Patients must attempt to avoid known allergens and treat any allergic reactions with symptomatic treatments.

Over the past one decade, the understanding of the immune system has been greatly advanced and several new paradigms have emerged such as Toll-like/NOD-like receptors, new T cell subsets, Treg and Th17 cells, and a novel cytokine IL-33. In this workshop, organized by Atsuhito Nakao (Univ. of Yamanashi), 46 participants gathered to discuss the roles of these cytokines, receptors, and T cell subsets in allergic diseases. Under the aims to significantly advance the studies of allergy for the worldwide health, the workshop provided a chance for immunology physicians, scientists and investigators to discuss the recent radical progress in the field of allergy and fostered their communications to work together.

Speaker	Title
Dr. Tomohiro Yoshimoto (Hyogo College of Medicine)	The roles of IL-33 in mast cells/basophils activation
Dr. Susumu Nakae (The University of Tokyo)	IL-33 in allergic inflammation
Dr. Hiroshi Nakajima (Chiba University)	Development and characterization of IL-21-producing CD4 T cells
Dr. Kenji Izuhara (Saga University)	Roles of IL-4 and IL-13 in allergic diseases
Dr. Kenji Kabashima (Kyoto University)	The roles of lipid mediators in atomic dermatitis
Dr. Toshiro Takai (Juntendo University)	What makes an allergen an allergen?: Importance of protease activity of allergen sources
Dr. Naohiro Inohara (University of Yamanashi)	Nod family of proteins plays an important role in allergic diseases?
Dr. Atsuhito Nakao (University of Yamanashi)	Oral TGF-β: a novel approach to prevent allergy

RCAI Advisory Council 2008

The RCAI Advisory Council conducts "Annual Reviews" each year and "Term Reviews" every 2-3 years. The RCAI Advisory Council 2008 meeting was an important evaluation session for three reasons. First, most of the Team Leaders were appointed 5 years ago and, in accordance with RIKEN policy, had to be critically evaluated for their achievements as well as contributions to RCAI and society. Second, the RCAI is in the process of formulating its plan for the Independent Administrative Institution (IAI) Tertiary Term and is thus required to establish long term research directions. Third, due to significant budget reductions, the Center must develop a reorganization plan for the long and short term in order to continue as a productive entity. The RCAI Advisory Council meeting was held December 4 and 5, 2008 at the RCAI research facility in the RIKEN Yokohama campus.

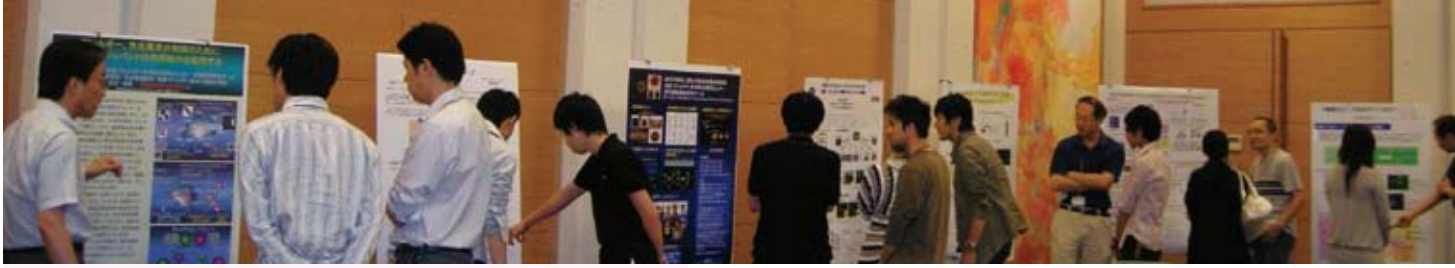
The Advisory Council has been evaluating RCAI on an annual basis since its inception and was very pleased with the way RCAI responded to their previous recommendations. They commented that the RCAI has been using the scientific appraisals and advice by the Advisory Council in a productive manner, continually improving the research quality and operational policies of the Center. The Advisory Council was impressed by the outstanding science that is being done at RCAI, which is reflected by the quality of the publications from both senior and young investigators.

The Advisory Council noted that most of the research groups led by senior investigators are doing very extremely well and the productivity of most is very impressive. More importantly, in terms of the reputation and future of the Center, many of the younger investigators have blossomed in the past five years. The Advisory Council summarized that, with rare exceptions, the quality of research being performed at RCAI is outstanding and internationally recognized and competitive. It is especially impressive given that the Center has only been in existence for five years.

The Advisory Council noted that the RCAI is in an unfortunate and difficult financial situation. This and shifting research priorities will necessitate closing of some labs, immediately in some cases and in the next two to three years in other cases. The Advisory Council was in general agreement with the reorganization plan. Realizing that decisions about individual researchers must be taken by the Director, the Advisory Council provided him with their views on ongoing research activities and their potential. Based on his leadership in the development of a world class immunology center, the Advisory Council expressed full confidence in Dr. Taniguchi's ability to guide the RCAI through this difficult period and to make necessary personnel changes.

The Advisory Council felt that the ongoing and future budget cuts threaten the survival of RCAI. It was not clear to them why drastic budget cuts are being imposed on this fledgling Institute that has rapidly reached a world class level of performance. The science of immunnoregulation is fundamental to understanding allergy, autoimmune and immunodeficiency diseases, and vaccine development. Due to the current economic crisis, research budgets are being strained worldwide. However, the Advisory Council advised that every effort should be made to stabilize the RCAI budget. The Advisory Council considers that the current budget is quite low for such a well performing institute and that the planned cut in financing for an institution that is doing remarkably well after only 5 years will have a negative effect on morale and perception inside Japan and internationally.

The Advisory council concluded that during its short existence, RCAI has developed into an internationally renowned Center for top level immunology research. The Director is to be congratulated for managing this successful effort. This goal has been achieved by recruiting outstanding researchers to RCAI, as well as by establishing novel international programs such as the summer educational program and international collaborative research grant award system. The RIKEN and the Japanese government are to be congratulated for their support of this outstanding Center. Having the unanimous opinion that RCAI will provide exceptionally valuable return on the investment in terms of improved human health and well being, they strongly recommended continuation of that support at the highest level possible.



Briefing Session for the RCAI Adjunct Graduate School Programs

May 24, 2008

RCAI accepts graduate students from various universities through the adjunct professorship mechanism. In 2008, Dr. Ishido (Team Leader, Laboratory for Infectious Immunity) became a new visiting associate professor at Yokohama City University. The total number of adjunct professors/associate professors in RCAI is now 18, and the Center collaborates with five university graduate schools (Table).

On May 24, RCAI held a briefing session on adjunct graduate school programs. The aim of this session was to introduce the adjunct graduate school system at RCAI to students who are interested in studying immunology. Twenty-seven participants gathered from Kyoto, Mie, Shizuoka, Saitama, Kanagawa and Tokyo. Dr. Saito, Deputy Director, first briefly summarized the adjunct graduate school programs, and then representatives from seven laboratories introduced their research topics. After the oral session, 15 laboratories had poster presentations, and then each of the participants had the opportunity to visit the laboratories of his/her interest.

The session provided an opportunity for students to visit and talk directly with lab leaders, and to consider their future directions. "The session was very fruitful for me. More people would be interested in this session if the universities and RCAI were able to publicize it better," a participant said.

Graduate School of Frontier Bioscience, Osaka University	Tomohiro Kurosaki (visiting professor)
	Ichiro Taniuchi (visiting professor)
	Keigo Nishida (visiting associate professor)
Graduate School of Medicine, Osaka University	Osami Kanagawa (visiting professor)
	Tsuneyasu Kaisho (visiting professor)
	Toshiyuki Fukada (visiting associate professor)
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)
School of Biomedical Science, Tokyo Medical and Dental University	Takashi Saito (visiting professor)
	Tomohiro Kurosaki (visiting professor)
	Masato Kubo (visiting professor)
	Sidonia Fagarasan (visiting associate professor)
International Graduate School of Arts and Sciences, Yokohama City University	Hiroshi Ohno (visiting professor)
	Satoshi Ishido (visiting associate professor)

RIKEN Yokohama Open Campus

June 5, 2008

The RIKEN Yokohama Institute Open Campus was held on July 5, 2008. The fine weather brought a record high number of visitors (2,064) including 523 children and 1,541 adults. Compared to last year, this represented an increase of greater than 240 participants. Twelve RCAI laboratories exhibited posters, and researchers explained their research topics to the visitors. The visitors could also study the basics of the immune system through digital content produced by RCAI. (The content "Karada wo Mamoru Shikumi" is available to the public at RCAI's Japanese webpage http://www.rcai.riken.go.jp/). Two other hands-on learning opportunities were part of the program. The Laboratory for Immunogenetics prepared differentially stained samples of fetal mice. The visitors, ranging in age from kindergarteners to retirees, observed the samples through microscopes and asked many questions. The Laboratory for Lymphocyte Development arranged a sophisticated cell-sorting experiment for those in high school or above. After hearing a lecture on lymphocyte development and the principles of the fluorescent activated cell sorter, participants were divided into groups and isolated $CD4^{+}CD8^{+}$ T lymphocytes from mice.

Super Science High School

July 29, 2008

"Super Science High Schools" are schools designated by the Ministry of Education, Culture, Sports, Science and Technology to focus specifically on science education. There are currently 102 Super Science High Schools in Japan. Because more young people in Japan, as well as in other countries, are moving away from the sciences, the ministry aims to strengthen and improve scientific education especially at these schools. RCAI holds a special workshop every year for students from the Super Science High Schools in Kanagawa prefecture. This year, 22 students and four teachers from Hakuyoh, Seisho, Yokosuka and Kanagawa Sohgoh High Schools were invited. After lectures on "AIDS Virus and Immunity" and "Immunological Organs" by Drs. Tomohiro Kurosaki and Hisahiro Yoshida, the students were divided into groups for a hands-on experience in the dissection and histological staining of immunological organs. After this session, the students had presentations and discussions. "I enjoyed the practical aspects and discussions. It was nice to talk with researchers during lunch, too. I feel closer to the research careers now," one student said. "I would like to apply the discussions and presentations to my classes," a teacher said. The workshop provided good opportunities to experiment, think together, and open the minds of both students and teachers.

RCAI Retreat 2008

September 1-2, 2008

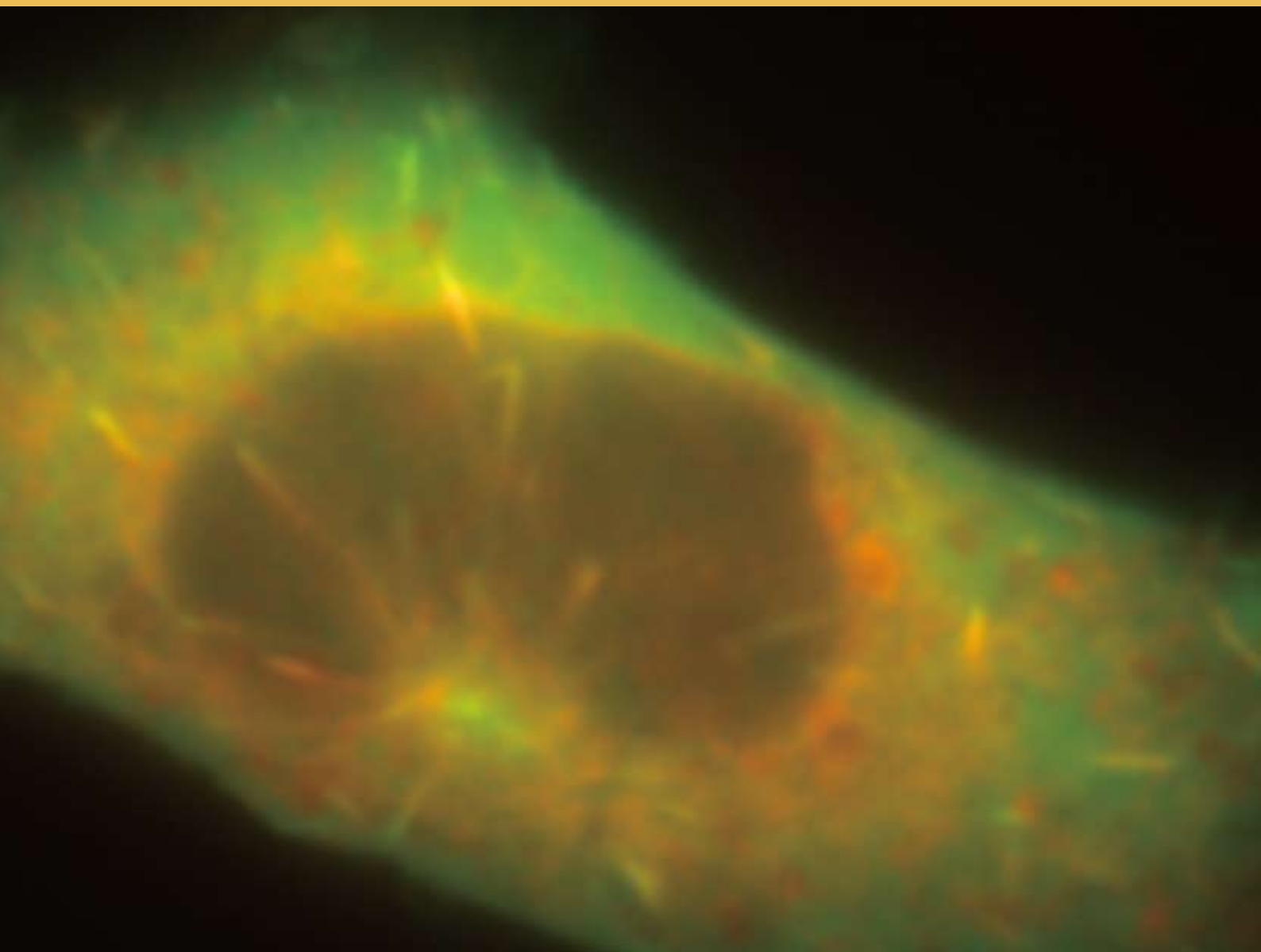
The aim of the RCAI Retreat is to improve the activities of the Center by gathering the members at a quiet place with few distractions. This setting provides the RCAI scientists with the opportunity for in-depth discussion and critiques of ongoing research projects, and this open communication promotes interactions and collaborations. At the 2008 RCAI Retreat, all in attendance including researchers and staff members (technical, and assistant) were encouraged to exchange information and initiate new collaborations. This year, two senior editors from *Science* and *The Journal of Experimental Medicine (JEM)* attended the meeting at the Nihon Aerobics Center in Chiba Prefecture together with 147 RCAI members.

On September 1, two poster presentation sessions were held. There were 88 posters in total, and both new unpublished data and ongoing projects were introduced to the retreat participants. Nine top posters were selected by the vote of the laboratory heads and these received the Excellent Poster Awards from the Director. The first-place winner, Dr. Tomokatsu Ikawa, also received an award from *Science* magazine, a one-year free subscription to the journal. Oral presentations were given on September 2. Dr. Thompson, the editor of *JEM* gave a talk about the manuscript submission process and the recent problems of data manipulation and the efforts of journal editors to deal with the problem. Ten young postdoctoral fellows then introduced their projects, which stimulated active discussions from the floor.

The dedicated efforts of the organizers contributed to the success of the retreat. Drs. Kaisho and Sato were in charge of the general organization of the meeting. Ms. Haraguchi's coordination of travel, food, and accommodation ensured that the program went very smoothly. The organizer, Dr. Kaisho commented that it was very difficult this year to select the best 10 presentations because the quality of each presentation was very high and seemed to have advanced since the 2007 RCAI Retreat.

2008

Data and Statistics



Publications (Apr. 2008-Mar. 2009)

Table : RCAI Publidations (Apr. 2008-Mar. 2009)

Journal Title	IF(2007)	FY2008
Nature	28.8	1
Nature Reviews Immunology	28.3	1
Science	26.4	1
Nature Immunology	26.2	6
Nature Genetics	25.6	2
Immunity	19.3	7
Journal of Clinical Investigation	16.9	1
Journal of Experimental Medicine	15.6	5
Blood	10.9	3
Immunol Rev	10.5	1
PNAS	9.6	6
Trends in Immunology	9.5	3
Current Opinion in Immunology	9.3	3
PLoS Genet	8.7	1
J Allergy Clin Immun	8.1	1
J Neurosci	7.5	1
Development	7.3	1
Nucleic Acids Res	7.0	3
Molecular and Cellular Biology	6.4	1
Advances in Immunology	6.3	1
Journal of Immunology	6.1	6
Journal of Biological Chemistry	5.6	5
J Virol	5.3	1
Anal Chem	5.3	1
International Immunology	3.3	7
Other Journals		43
Total		112

1. Ato, M., Ikebe, T., Kawabata, H., Takemori, T. & Watanabe, H. Incompetence of neutrophils to invasive group A streptococcus is attributed to induction of plural virulence factors by dysfunction of a regulator. ***PLoS ONE*** Oct. 2008, 3(10):e3455.

2. Atsumi, T., Sato, M., Kamimura, D., Moroi, A., Iwakura, Y., Betz, U., Yoshimura, A., Nishihara, M., Hirano, T. & Murakami, M. IFN-gamma expression in CD8+ T cells regulated by IL-6 signal is involved in superantigen-mediated CD4+ T cell death. ***Int Immunol*** Jan. 2009, 21(21):73-80.

3. Collins, A., Littman, D. & Taniuchi, I. RUNX proteins in transcription factor networks that regulate T-cell lineage choice. ***Nat Rev Immunol*** Feb. 2009, 9(2):106-115.

4. Conley, M., Farmer, D., Dobbs, A., Howard, V., Aiba, Y., Shurtleff, S. & Kurosaki, T. A minimally hypomorphic mutation in Btk resulting in reduced B cell numbers but no clinical disease. ***Clin Exp Immunol*** Apr. 2008, 152(151):139-144.

5. Deng, L., Adachi, T., Kitayama, K., Bungyoku, Y., Kitazawa, S., Ishido, S., Shoji, I. & Hotta, H. Hepatitis C virus infection induces apoptosis through a Bax-triggered, mitochondrion-mediated, caspase 3-dependent pathway. ***J Virol*** Nov. 2008, 82(21):10375-10385.

6. Ehrhardt, G., Hijikata, A., Kitamura, H., Ohara, O., Wang, J. & Cooper, M. Discriminating gene expression profiles of memory B cell subpopulations. ***J Exp Med*** Aug. 2008, 205(208):1807-1817.

7. Ellison, C., Makar, B., Wiseman, J., Gheorghiu, I., Taniguchi, M. & Gartner, J. Palifermin mediates immunoregulatory effects in addition to its cytoprotective effects in mice with acute graft-versus-host disease. ***J Clin Immunol*** Sep. 2008, 28(25):600-615.

8. Endoh, M., Endo, T., Endoh, T., Fujimura, Y., Ohara, O., Toyoda, T., Otte, A., 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*** Apr. 2008, 135(138):1513-1524.

9. Fagarasan, S. Evolution, development, mechanism and function of IgA in the gut. ***Curr Opin Immunol*** Apr. 2008, 20(22):170-177.

10. Fujii, S. Exploiting dendritic cells and natural killer T cells in immunotherapy against malignancies. ***Trends Immunol*** May. 2008, 29(25):242-249.

11. Fukada, T., Civic, N., Furuichi, T., Shimoda, S., Mishima, K., Higashiyama, H., Idaira, Y., Asada, Y., Kitamura, H., Yamasaki, S., Hojyo, S., Nakayama, M., Ohara, O., Koseki, H., Dos, Santos, H., Bonafe, L., Ha-Vinh, R., Zankl, A., Unger, S., Kraenzlin, M., Beckmann, J., Saito, I., Rivolta, C., Ikegawa, S., Superti-Furga, A. & Hirano, T. The zinc transporter SLC39A13/ZIP13 is required for connective tissue development; its involvement in BMP/TGF-beta signaling pathways. ***PLoS ONE*** Nov. 2008, 3(11):e3642.

12. Fukagawa, A., Hiroshima, M., Sakane, I. & Tokunaga, M. Direct observation of multiple and stochastic transition states by a feedback-controlled single-molecule force measurement. ***Anal Sci*** Jan. 2009, 25(21):25-27.

13. Grigorova, I., Schwab, S., Phan, T., Pham, T., Okada, T. & Cyster, J. Cortical sinus probing, S1P1-dependent entry and flow-based capture of egressing T cells. ***Nat Immunol*** Jan. 2009, 10(11):58-65.

14. Hara, H., Ishihara, C., Takeuchi, A., Xue, L., Morris, S., Penninger, J., Yoshida, H. & Saito, T. Cell type-specific regulation of ITAM-mediated NF-kappaB activation by the adaptors, CARMA1 and CARD9. ***J Immunol*** Jul. 2008, 181(182):918-130.

15. 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, D. & Koseki, H. FGF9 monomer-dimer equilibrium regulates extracellular matrix affinity and tissue diffusion. ***Nat Genet*** Mar. 2009, 41(43):289-298.

16. Hase, K., Takahashi, D., Ebisawa, M., Kawano, S., Itoh, K. & Ohno, H. Activation-induced cytidine deaminase deficiency causes organ-specific autoimmune disease. ***PLoS ONE*** Aug. 2008, 3(8):e3033.

17. Hida, S., Yamasaki, S., Sakamoto, Y., Takamoto, M., Obata, K., Takai, T., Karasuyama, H., Sugane, K., Saito, T. & Taki, S. Fc receptor gamma-chain, a constitutive component of the IL-3 receptor, is required for IL-3-induced IL-4 production in basophils. ***Nat Immunol*** Feb. 2009, 10(12):214-222.

18. Hijioka, K., Sonoda, K., Tsutsumi-Miyahara, C., Fujimoto, T., Oshima, Y., Taniguchi, M. & Ishibashi, T. Investigation of the role of CD1d-restricted invariant NKT cells in experimental choroidal neovascularization. ***Biochem Biophys Res Commun*** Sep. 2008, 374(371):338-343.

19. 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*** Mar. 2009, 206(203):681-209.

20. Hirahara, K., Yamashita, M., Iwamura, C., Shinoda, K., Hasegawa, A., Yoshizawa, H., Koseki, H., Gejyo, F. & Nakayama, T. Repressor of GATA regulates TH2-driven allergic airway inflammation and airway hyperresponsiveness. ***J Allergy Clin Immunol*** Sep. 2008, 122(123):512-120.e111.

21. Hirano, T., Murakami, M., Fukada, T., Nishida, K., Yamasaki, S. & Suzuki, T. Roles of zinc and zinc signaling in immunity: zinc as an intracellular signaling molecule. ***Adv Immunol*** May. 2008, 97:149-176.

22. Hong, Z., Jiang, J., Lan, L., Nakajima, S., Kanno, S., Koseki, H. & Yasui, A. A polycomb group protein, PHF1, is involved in the response to DNA double-strand breaks in human cell. ***Nucleic Acids Res*** May. 2008, 36(39):2939-2947.

23. Hori, S. Rethinking the molecular definition of regulatory T cells. ***Eur J Immunol*** Apr. 2008, 38(34):928-930.

24. Hoshino, K. & Kaisho, T. Nucleic acid sensing Toll-like receptors in dendritic cells. ***Curr Opin Immunol*** Aug. 2008, 20(24):408-413.

25. Hossain, M., Hosokawa, H., Hasegawa, A., Watarai, H., Taniguchi, M., Yamashita, M. & Nakayama, T. Lymphoid enhancer factor interacts with GATA-3 and controls its function in T helper type 2 cells. ***Immunology*** Nov. 2008, 125(123):377-186.

26. Ichiyama, K., Yoshida, H., Wakabayashi, Y., Chinen, T., Saeki, K., Nakaya, M., Takaesu, G., Hori, S., Yoshimura, A. & Kobayashi, T. Foxp3 inhibits RORgammat-mediated IL-17A mRNA transcription through direct interaction with RORgammat. ***J Biol Chem*** Jun. 2008, 283(225):17003-17008.

27. Inoue, T., Kon, T., Ohkura, R., Yamakawa, H., Ohara, O., Yokota, J. & Sutoh, K. BREK/LMTK2 is a myosin VI-binding protein involved in endosomal membrane trafficking. ***Genes Cells*** May. 2008, 13(15):483-495.

28. Ito, H., Ando, K., Ishikawa, T., Nakayama, T., Taniguchi, M., Saito, K., Imawari, M., Moriwaki, H., Yokochi, T., Kakumu, S. & Seishima, M. Role of Valpha14+ NKT cells in the development of Hepatitis B virus-specific CTL: activation of Valpha14+ NKT cells promotes the breakage of CTL tolerance. ***Int Immunol*** Jul. 2008, 20(27):869-879.

29. Ito, T., Hasegawa, A., Hosokawa, H., Yamashita, M., Motohashi, S., Naka, T., Okamoto, Y., Fujita, Y., Ishii, Y., Taniguchi, M., Yano, I. & Nakayama, T. Human Th1 differentiation induced by lipoarabinomannan/lipomannan from Mycobacterium bovis BCG Tokyo-172. ***Int Immunol*** Jul. 2008, 20(27):849-860.

30. Jensen, K., Su, X., Shin, S., Li, L., Youssef, S., Yamasaki, S., Steinman, L., Saito, T., Locksley, R., Davis, M., Baumgarth, N. & Chien, Y. Thymic selection determines gammadelta T cell effector fate: antigen-naive cells make interleukin-17 and antigen-experienced cells make interferon gamma. ***Immunity*** Jul. 2008, 29(21):90-100.

31. Jin, G., Hirano, T. & Murakami, M. Combination treatment with IL-2 and anti-IL-2 mAbs reduces tumor metastasis via NK cell activation. ***Int Immunol*** Jun. 2008, 20(26):783-789.

32. Kaisho, T. Type I interferon production by nucleic acid-stimulated dendritic cells. ***Front Biosci*** May. 2008, 13:6034-6042.

33. Kaisho, T., Sugiyama, T. & Hoshino, K. Nucleic acids in innate immunity, in Nucleic Acids in Innate Immunity, in ***Nucleic Acids in Innate Immunity***. (eds. Ishii, K. & Akira, S.) p43-58 CRC Press USA.

34. Kaisho, T. & Tanaka, T. Turning NF-kappaB and IRFs on and off in DC. ***Trends Immunol*** Jul. 2008, 29(27):329-336.

35. Kaisho, T. & Wagner, H. Host-pathogen interactions. ***Curr Opin Immunol*** Aug. 2008, 20(24):369-370.

36. Kamijuku, H., Nagata, Y., Jiang, X., Ichinohe, T., Tashiro, T., Mori, K., Taniguchi, M., Hase, K., Ohno, H., Shimaoka, T., Yonehara, S., Odagiri, T., Tashiro, M., Sata, T., Hasegawa, H. & Seino, K. Mechanism of NKT cell activation by intranasal coadministration of alpha-galactosylceramide, which can induce cross-protection against influenza viruses. ***Mucosal Immunol*** May. 2008, 1(3):208-218.

37. Keerthikumar, S., Raju, R., Kandasamy, K., Hijikata, A., Ramabadran, S., Balakrishnan, L., Ahmed, M., Rani, S., Selvan, L., Somanathan, D., Ray, S., Bhattacharjee, M., Gollapudi, S., Ramachandra, Y., Bhadra, S., Bhattacharyya, C., Imai, K., Nonoyama, S., Kanegane, H., Miyawaki, T., Pandey, A., Ohara, O. & Mohan, S. RAPID: Resource of Asian Primary Immunodeficiency Diseases. ***Nucleic Acids Res*** Jan. 2009, 37(Database issue):D863-867.

38. Keshava, Prasad, T., Goel, R., Kandasamy, K., Keerthikumar, S., Kumar, S., Mathivanan, S., Telikicherla, D., Raju, R., Shafreen, B., Venugopal, A., Balakrishnan, L., Marimuthu, A., Banerjee, S., Somanathan, D., Sebastian, A., Rani, S., Ray, S., Harrys, Kishore, C., Kanth, S., Ahmed, M., Kashyap, M., Mohmood, R., Ramachandra, Y., Krishna, V., Rahiman, B., Mohan, S., Ranganathan, P., Ramabadran, S., Chaerkady, R. & Pandey, A. Human Protein Reference Database--2009 update. ***Nucleic Acids Res*** Jan. 2009, 37(Database issue):D767-772.

39. Kikushige, Y., Yoshimoto, G., Miyamoto, T., Iino, T., Mori, Y., Iwasaki, H., Niiro, H., Takenaka, K., Nagafuji, K., Harada, M., Ishikawa, F. & Akashi, K. Human Flt3 is expressed at the hematopoietic stem cell and the granulocyte/macrophage progenitor stages to maintain cell survival. ***J Immunol*** Jun. 2008, 180(111):7358-7367.

40. Ko, J., Patel, N., Ikawa, T., Kawamoto, H., Frank, O., Rivera, R., Van, Etten, R. & Murre, C. Suppression of E-protein activity interferes with the development of BCR-ABL-mediated myeloproliferative disease. ***Proc Natl Acad Sci U S A*** Sep. 2008, 105(135):12967-12972.

41. Kohfu, K., Kubo, M., Ichikawa, H., Ohno, S., Habu, S., Sato, T. & Satake, M. Pleiotropic Roles of Runx Transcription Factors in the Differentiation and Function of T Lymphocytes. ***Current Immunology Reviews*** May. 2008; 4, 101-115.

42. Komaniwa, S., Hayashi, H., Kawamoto, H., Sato, S., Ikawa, T., Katsura, Y. & Udaka, K. Lipid-mediated presentation of MHC class II molecules guides thymocytes to the CD4 lineage. ***Eur J Immunol*** Jan. 2009, 39(31):96-112.

43. Komatsu, N., Mariotti-Ferrandiz, M., 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*** Feb. 2009, 106(106):1903-1908.

44. Kometani, K., Moriyama, M., Nakashima, Y., Katayama, Y., Wang, S., Yamasaki, S., Saito, T., Hattori, M. & Minato, N. Essential role of Rap signal in pre-TCR-mediated {beta}-selection checkpoint in {alpha}{beta} T-cell development. ***Blood*** Dec. 2008, 112(112):4565-4573.

45. Koyama, T., Nakaoka, Y., Fujio, Y., Hirota, H., Nishida, K., Sugiyama, S., Okamoto, K., Yamauchi-Takihara, K., Yoshimura, M., Mochizuki, S., Hori, M., Hirano, T. & Mochizuki, N. Interaction of scaffolding adaptor protein Gab1 with tyrosine phosphatase SHP2 negatively regulates IGF-I-dependent myogenic differentiation via the ERK1/2 signaling pathway. ***J Biol Chem*** Aug. 2008, 283(235):24234-24244.

46. Ku, M., Koche, R., Rheinbay, E., Mendenhall, E., Endoh, M., Mikkelsen, T., Presser, A., Nusbaum, C., Xie, X., Chi, A., Adli, M., Kasif, S., Ptaszek, L., Cowan, C., Lander, E., Koseki, H. & Bernstein, B. Genomewide analysis of PRC1 and PRC2 occupancy identifies two classes of bivalent domains. ***PLoS Genet*** Oct. 2008, 4(10):e1000242.

47. Kurosaki, T. Paradox of B cell-targeted therapies. ***J Clin Invest*** Oct. 2008, 118(110):3260-3263.

48. Kurosaki, T. & Hikida, M. Tyrosine kinases and their substrates in B lymphocytes. ***Immunol Rev*** Mar. 2009, 228(221):132-248.

49. Lu, L., Thai, T., Calado, D., Chaudhry, A., Kubo, M., Tanaka, K., Loeb, G., Lee, H., Yoshimura, A., Rajewsky, K. & Rudensky, A. Foxp3-dependent microRNA155 confers competitive fitness to regulatory T cells by targeting SOCS1 protein. ***Immunity*** Jan. 2009, 30(31):80-91.

50. MacFarlane, A., Yamazaki, T., Fang, M., Sigal, L., Kurosaki, T. & Campbell, K. Enhanced NK-cell development and function in BCAP-deficient mice. ***Blood*** Jul. 2008, 112(111):131-140.

51. Maekawa, Y., Minato, Y., Ishifune, C., Kurihara, T., Kitamura, A., Kojima, H., Yagita, H., Sakata-Yanagimoto, M., Saito, T., Taniuchi, I., Chiba, S., Sone, S. & Yasutomo, K. Notch2 integrates signaling by the transcription factors RBP-J and CREB1 to promote T cell cytotoxicity. ***Nat Immunol*** Oct. 2008, 9(10):1140-1147.

52. Masuda, K., Ouchida, R., Yokoi, M., Hanaoka, F., Azuma, T. & Wang, J. DNA polymerase eta is a limiting factor for A:T mutations in Ig genes and contributes to antibody affinity maturation. ***Eur J Immunol*** Oct. 2008, 38(10):2796-2805.

53. Misawa, H., Fujigaya, H., Nishimura, T., Moriwaki, Y., Okuda, T., Kawashima, K., Nakata, K., Ruggiero, A., Blakely, R., Nakatsu, F. & Ohno, H. Aberrant trafficking of the high-affinity choline transporter in AP-3-deficient mice. ***Eur J Neurosci*** Jun. 2008, 27(12):3109-3117.

54. Mizukami, S., Kajiwara, C., Ishikawa, H., Katayama, I., Yui, K. & Udono, H. Both CD4+ and CD8+ T cell epitopes fused to heat shock cognate protein 70 (hsc70) can function to eradicate tumors. ***Cancer Sci*** May. 2008, 99(95):1008-1015.

55. Mori, Y., Kato, T., Kodaka, T., Kanagawa, E., Hori, S. & Kanagawa, O. Protection of IFN-gamma signaling-deficient NOD mice from diabetes by cyclophosphamide. ***Int Immunol*** Sep. 2008, 20(29):1231-1237.

56. Murakami, M. & Hirano, T. Intracellular zinc homeostasis and zinc signaling. ***Cancer Sci*** Aug. 2008, 99(98):1515-1522.

57. 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*** Oct. 2008, 9(10):1113-1121.

58. Nagase, T., Yamakawa, H., Tadokoro, S., Nakajima, D., Inoue, S., Yamaguchi, K., Itokawa, Y., Kikuno, R., Koga, H. & Ohara, O. Exploration of human ORFeome: high-throughput preparation of ORF clones and efficient characterization of their protein products. ***DNA Res*** Jun. 2008, 15(13):137-149.

59. Nakayama, J, Yamamoto, M, Hayashi, K, Satoh, H, Bundo, K, Kubo, M, Goitsuka, R, Farrar, MA & Kitamura, D. BLNK suppresses pre-B cell leukemogenesis through inhibition of JAK3. ***Blood*** Feb. 2009, 113(117):1483-1492.

60. Negishi, H., Osawa, T., Ogami, K., Ouyang, X., Sakaguchi, S., Koshiba, R., Yanai, H., Seko, Y., Shitara, H., Bishop, K., Yonekawa, H., Tamura, T., Kaisho, T., Taya, C., Taniguchi, T. & Honda, K. A critical link between Toll-like receptor 3 and type II interferon signaling pathways in antiviral innate immunity. ***Proc Natl Acad Sci U S A*** Dec. 2008, 105(151):20446-20451.

61. Ogura, H., Murakami, M., Okuyama, Y., Tsuruoka, M., Kitabayashi, C., Kanamoto, M., Nishihara, M., Iwakura, Y. & Hirano, T. Interleukin-17 promotes autoimmunity by triggering a positive-feedback loop via interleukin-6 induction. ***Immunity*** Oct. 2008, 29(24):628-636.

62. Okada, S., Harada, H., Ito, T., Saito, T. & Suzu, S. Early development of human hematopoietic and acquired immune systems in new born NOD/Scid/Jak3(null) mice intrahepatic engrafted with cord blood-derived CD34 (+) cells. ***Int J Hematol*** Dec. 2008, 88(85):476-482.

63. Okazaki, N., Ikeda, S., Ohara, R., Shimada, K., Yanagawa, T., Nagase, T., Ohara, O. & Koga, H. The novel protein complex with SMARCAD1/KIAA1122 binds to the vicinity of TSS. ***J Mol Biol*** Oct. 2008, 382(382):257-365.

64. Osada, K., Tashiro, T., Mori, K. & Izumi, H. The identification of attractive volatiles in aged male mouse urine. ***Chem Senses*** Nov. 2008, 33(39):815-823.

65. Ouchida, R., Ukai, A., Mori, H., Kawamura, K., Dolle, ME., Tagawa, M., Sakamoto, A., Tokuhisa, T., Yokosuka, T., Saito, T., Yokoi, M., Hanaoka, F., Vijg, J. & Wang, J. Genetic analysis reveals an intrinsic property of the germinal center B cells to generate A:T mutations. ***DNA Repair*** Aug. 2008, 7(8):1392-1398.

66. Ouchida, R., Yamasaki, S., Hikida, M., Masuda, K., Kawamura, K., Wada, A., Mochizuki, S., Tagawa, M., Sakamoto, A., Hatano, M., Tokuhisa, T., Koseki, H., Saito, T., Kurosaki, T. & Wang, J. A lysosomal protein negatively regulates surface T cell antigen receptor expression by promoting CD3zeta-chain degradation. ***Immunity*** Jul. 2008, 29(21):33-43.

67. Puschendorf, M., Terranova, R., Boutsma, E., Mao, X., Isono, K., Brykczynska, U., Kolb, C., Otte, A., Koseki, H., Orkin, S., van, Lohuizen, M. & Peters, A. PRC1 and Suv39h specify parental asymmetry at constitutive heterochromatin in early mouse embryos. ***Nat Genet*** Apr. 2008, 40(44):411-420.

68. Saito, Y. & Ishikawa, F. The origins of blood: induction of hematopoietic stem cells from different sources. ***Cell Stem Cell*** Jul. 2008, 3(1):8-10.

69. Sasuga, Y, Iwasawa, T, Terada, K, Oe, Y, Sorimachi, H, Ohara, O & Harada, Y. Single-Cell Chemical Lysis Method for Analyses of Intracellular Molecules Using an Array of Picoliter-Scale Microwells. ***Anal Chem*** Nov. 2008, 80 (23): 9141_9149.

70. Schaefer, M., Reiling, N., Fessler, C., Stephani, J., Taniuchi, I., Hatam, F., Yildirim, A., Fehrenbach, H., Walter, K., Ruland, J., Wagner, H., Ehlers, S. & Sparwasser, T. Decreased pathology and prolonged survival of human DC-SIGN transgenic mice during mycobacterial infection. ***J Immunol*** May. 2008, 180(110):6836-6845.

71. Shimizu, K. & Fujii, S. An adjuvant role of in situ dendritic cells (DCs) in linking innate and adaptive immunity. ***Front Biosci*** May. 2008, 13:6193-6201.

72. Shimizu, K. & Fujii, S. DC therapy induces long-term NK reactivity to tumors via host DC. ***Eur J Immunol*** Feb, 2009; 39, 457-468.

73. Sireci, G., La, Manna, M., Di, Liberto, D., Lo, Dico, M., Taniguchi, M., Dieli, F. & Salerno, A. Prophylaxis of lipopolysaccharide-induced shock by alpha-galactosylceramide. ***J Leukoc Biol*** Aug. 2008, 84(82):550-560.

74. Son, A, Nakamura, H, Okuyama, H, Oka, S, Yoshihara, E, Liu, W, Matsuo, Y, Kondo, N, Masutani, H, Ishii, Y, Iyoda, T, Inaba, K & Yodoi, J. Dendritic cells derived from TBP-2-deficient mice are defective in inducing T cell responses. ***Eur J Immunol*** Apr. 2008, 38(35):1358-1367.

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

76. Tachibana, M., Tezuka, C., Muroi, S., Nishimoto, S., Katsumoto, T., Nakajima, A., Kitabayashi, I. & Taniuchi, I. Phosphorylation of Runx1 at Ser249, Ser266, and Ser276 is dispensable for bone marrow hematopoiesis and thymocyte differentiation. ***Biochem Biophys Res Commun*** Apr. 2008, 368(363):536-342.

77. Tamura, Y., Teng, A., Nozawa, R., Takamoto-Matsui, Y. & Ishii, Y. Characterization of the immature dendritic cells and cytotoxic cells both expanded after activation of invariant NKT cells with alpha-galactosylceramide in vivo. ***Biochem Biophys Res Commun*** May. 2008, 369(362):485-392.

78. Tanaka, M., Miyake, Y. & Asano, K. Maintenance of self-tolerance by apoptotic cell clearance. ***Front Biosci*** May. 2008, 13:6043-6049.

79. Tanaka, Y., Tanaka, N., Saeki, Y., Tanaka, K., Murakami, M., Hirano, T., Ishii, N. & Sugamura, K. c-Cbl-dependent monoubiquitination and lysosomal degradation of gp130. ***Mol Cell Biol*** Aug. 2008, 28(15):4805-4818.

80. Tanuma, N, Kim, SE, Beullens, M, Tsubaki, Y, Mitsuhashi, S, Nomura, M, Kawamura, T, Isono, K, Koseki, H, Sato, M, Bollen, M, Kikuchi, K & Shima, H. Nuclear Inhibitor of Protein Phosphatase-1 (NIPP1) Directs Protein Phosphatase-1 (PP1) to Dephosphorylate the U2 Small Nuclear Ribonucleoprotein Particle (snRNP) Component, Spliceosome-associated Protein 155 (Sap155). ***J Biol Chem*** Dec. 2008, 283(251):35805-35814.

81. Tashiro, T., Hongo, N., Nakagawa, R., Seino, K., Watarai, H., Ishii, Y., Taniguchi, M. & Mori, K. RCAI-17, 22, 24-26, 29, 31, 34-36, 38-40, and 88, the analogs of KRN7000 with a sulfonamide linkage: their synthesis and bioactivity for mouse natural killer T cells to produce Th2-biased cytokines. ***Bioorg Med Chem*** Oct. 2008, 16(19):8896-8906.

82. Tashiro, T. & Mori, K. Synthesis and absolute configuration of the male aggregation pheromone of the stink bug Erysarcoris lewisi (Distant), (2Z,6R,1 ' S,5 ' S)-2-methyl-6-(4 '-methylenebicyclo[3.1.0]hexyl)hept-2-en-1-ol. ***Tetrahedron-Asymmetry*** May. 2008, 19:1215-1223.

83. Tashiro, T., Nakagawa, R., Inoue, S., Shiozaki, M., Watarai, H., Taniguchi, M. & Mori, K. RCAI-61, the 6'-O-methylated analog of KRN7000: its synthesis and potent bioactivity for mouse lymphocytes to produce interferon-g in vivo. ***Tetrahedron Lett*** Aug. 2008, 49:6827-6830.

84. Tashiro, T., Osada, K. & Mori, K. Syntheses of 2-Isopropyl-4,5-dihydrothiazole and 6-Hydroxy-6-methyl-3-heptanone, pheromone components of the male mouse, Mus musculus. ***Biosci Biotechnol Biochem*** Sep. 2008, 72(79):2398-2402.

85. Terahara, K., Yoshida, M., Igarashi, O., Nochi, T., Pontes, G., Hase, K., Ohno, H., Kurokawa, S., Mejima, M., Takayama, N., Yuki, Y., Lowe, A. & Kiyono, H. Comprehensive gene expression profiling of Peyer's patch M cells, villous M-like cells, and intestinal epithelial cells. ***J Immunol*** Jun. 2008, 180(112):7840-7846.

86. Terashima, A., Watarai, H., Inoue, S., Sekine, E., Nakagawa, R., Hase, K., Iwamura, C., Nakajima, H., Nakayama, T. & Taniguchi, M. A novel subset of mouse NKT cells bearing the IL-17 receptor B responds to IL-25 and contributes to airway hyperreactivity. ***J Exp Med*** Nov. 2008, 205(212):2727-2733.

87. Tomura, M., Yoshida, N., Tanaka, J., Karasawa, S., Miwa, Y., Miyawaki, A. & Kanagawa, O. Monitoring cellular movement in vivo with photoconvertible fluorescence protein. ***Proc Natl Acad Sci U S A*** Aug. 2008, 105(131):10871-10876.

88. Tsuji, M., Komatsu, N., Kawamoto, S., Suzuki, K., Kanagawa, O., Honjo, T., Hori, S. & Fagarasan, S. Preferential generation of follicular B helper T cells from Foxp3+ T cells in gut Peyer's patches. ***Science*** Mar. 2009; 323, 1488-1492.

89. Tsuji, M., Suzuki, K., Kitamura, H., Maruya, M., Kinoshita, K., Ivanov, I., Itoh, K., Littman, D. & Fagarasan, S. Requirement for lymphoid tissue-inducer cells in isolated follicle formation and T cell-independent immunoglobulin A generation in the gut. ***Immunity*** Aug. 2008, 29(22):261-271.

90. Tsunoda, I, Tanaka, T, Taniguchi, M & Fujinami, RS. Contrasting roles for Valpha14(+) natural killer T cells in a viral model for multiple sclerosis. J Neurovirol Dec. 2008, 15(11):90-18.

91. Ukai, A., Ishimaru, K., Ouchida, R., Mori, H., Kano, C., Moritan, T. & Wang, J. Induction of a:T mutations is dependent on cellular environment but independent of mutation frequency and target gene location. ***J Immunol*** Dec. 2008, 181(111):7835-7842.

92. Wada, H., Masuda, K., Satoh, R., Kakugawa, K., Ikawa, T., Katsura, Y. & Kawamoto, H. Adult T-cell progenitors retain myeloid potential. ***Nature*** Apr. 2008, 452(7188):7768-7172.

93. Wakayama, S., Ohta, H., Hikichi, T., Mizutani, E., Iwaki, T., Kanagawa, O. & Wakayama, T. Production of healthy cloned mice from bodies frozen at -20 degrees C for 16 years. ***Proc Natl Acad Sci U S A*** Nov. 2008, 105(145):17318-17322.

94. Wang, J. Mutation frequency vs. mutation patterns: a comparison of the results in spleen and Peyer's patches. ***DNA Repair*** Sep. 2008, 7(9):1408-1401.

95. Wang, Y., Shibuya, K., Yamashita, Y., Shirakawa, J., Shibata, K., Kai, H., Yokosuka, T., Saito, T., Honda, S., Tahara-Hanaoka, S. & Shibuya, A. LFA-1 decreases the antigen dose for T cell activation in vivo. ***Int Immunol*** Sep. 2008, 20(29):1119-1127.

96. Weber, M., Treanor, B., Depoil, D., Shinohara, H., Harwood, N., Hikida, M., Kurosaki, T. & Batista, F. Phospholipase C-gamma2 and Vav cooperate within signaling microclusters to propagate B cell spreading in response to membrane-bound antigen. ***J Exp Med*** Apr. 2008, 205(204):853-268.

97. Xing, Y., Wang, X., Igarashi, H., Kawamoto, H. & Sakaguchi, N. Protein phosphatase subunit G5PR that regulates the JNK-mediated apoptosis signal is essential for the survival of CD4 and CD8 double-positive thymocytes. ***Mol Immunol*** Apr. 2008, 45(47):2028-2037.

98. Xu, S., Huo, J., Lee, K., Kurosaki, T. & Lam, K. Phospholipase C{gamma}2 Is Critical for Dectin-1-mediated Ca2+ Flux and Cytokine Production in Dendritic Cells. ***J Biol Chem*** Mar. 2009, 284(211):7038-7046.

99. Yamada, T., Kurosaki, T. & Hikida, M. Essential roles of mgcRacGAP in multilineage differentiation and survival of murine hematopoietic cells. ***Biochem Biophys Res Commun*** Aug. 2008, 372(374):941-376.

100. Yamaguchi, K, Ohara, O, Ando, A & Nagase, T. Smurf1 directly targets hPEM-2, a GEF for Cdc42, via a novel combination of protein interaction modules in the ubiquitin-proteasome pathway. ***Biol Chem*** Apr. 2008, 389(384):405-313.

101. Yamano, T., Mizukami, S., Murata, S., Chiba, T., Tanaka, K. & Udono, H. Hsp90-mediated assembly of the 26 S proteasome is involved in major histocompatibility complex class I antigen processing. ***J Biol Chem*** Oct. 2008, 283(242):28060-28065.

102. Yamano, T., Sugahara, H., Mizukami, S., Murata, S., Chiba, T., Tanaka, K., Yui, K. & Udono, H. Allele-selective effect of PA28 in MHC class I antigen processing. ***J Immunol*** Aug. 2008, 181(183):1655-1664.

103. Yamasaki, S., Ishikawa, E., Sakuma, M., Hara, H., Ogata, K. & Saito, T. Mincle is an ITAM-coupled activating receptor that senses damaged cells. ***Nat Immunol*** Oct. 2008, 9(10):1179-1188.

104. Yamasaki, S., Matsumoto, M., Takeuchi, O., Matsuzawa, T., Ishikawa, E., Sakuma, M., Tateno, H., Uno, J., Hirabayashi, J., Mikami, Y., Takeda, K., Akira, S. & Saito, T. C-type lectin Mincle is an activating receptor for pathogenic fungus, Malassezia. ***Proc Natl Acad Sci U S A*** Feb. 2009, 106(106):1897-1902.

105. Yamasaki, S., Takase-Utsugi, M., Ishikawa, E., Sakuma, M., Nishida, K., Saito, T. & Kanagawa, O. Selective impairment of FcepsilonRI-mediated allergic reaction in Gads-deficient mice. ***Int Immunol*** Oct. 2008, 20(10):1289-1297.

106. Yano, M., Kuroda, N., Han, H., Meguro-Horike, M., Nishikawa, Y., Kiyonari, H., Maemura, K., Yanagawa, Y., Obata, K., Takahashi, S., Ikawa, T., Satoh, R., Kawamoto, H., Mouri, Y. & Matsumoto, M. Aire controls the differentiation program of thymic epithelial cells in the medulla for the establishment of self-tolerance. ***J Exp Med*** Nov. 2008, 205(212):2827-2838.

107. Yasuda, T. & Kurosaki, T. Regulation of lymphocyte fate by Ras/ERK signals. ***Cell Cycle*** Dec. 2008, 7(23):3634-3640.

108. Yasuda, T., Sanjo, H., G., Pages, Kawano, Y., Karasuyama, H., Pouyss_gur, J., Ogata, M. & Kurosaki, T. Erk kinases link pre-B cell receptor signaling to transcriptional events required for early B cell expansion. ***Immunity*** Apr. 2008, 28(24):499-508.

109. Yokosuka, T., Kobayashi, W., Sakata-Sogawa, K., Takamatsu, M., Hashimoto-Tane, A., Dustin, M., Tokunaga, M. & Saito, T. Spatiotemporal regulation of T cell costimulation by TCR-CD28 microclusters and protein kinase C theta translocation. ***Immunity*** Oct. 2008, 29(24):589-601.

110. Yoshiga, Y., Goto, D., Segawa, S., Ohnishi, Y., Matsumoto, I., Ito, S., Tsutsumi, A., Taniguchi, M. & Sumida, T. Invariant NKT cells produce IL-17 through IL-23-dependent and -independent pathways with potential modulation of Th17 response in collagen-induced arthritis. ***Int J Mol Med*** Sep. 2008, 22(23):369-374.

111. Young, L., Wilson, N., Schnorrer, P., Proietto, A., ten, Broeke, T., Matsuki, Y., Mount, A., Belz, G., O'Keeffe, M., Ohmura-Hoshino, M., Ishido, S., Stoorvogel, W., Heath, W., Shortman, K. & Villadangos, J. Differential MHC class II synthesis and ubiquitination confers distinct antigen-presenting properties on conventional and plasmacytoid dendritic cells. ***Nat Immunol*** Nov. 2008, 9(11):1244-1252.

112. Zhu, G., Okada, M., Yoshida, S., Ueno, S., Mori, F., Takahara, T., Saito, R., Miura, Y., Kishi, A., Tomiyama, M., Sato, A., Kojima, T., Fukuma, G., Wakabayashi, K., Hase, K., Ohno, H., Kijima, H., Takano, Y., Mitsudome, A., Kaneko, S. & Hirose, S. Rats harboring S284L Chrna4 mutation show attenuation of synaptic and extrasynaptic GABAergic transmission and exhibit the nocturnal frontal lobe epilepsy phenotype. ***J Neurosci*** Nov. 2008, 28(47):12465-12476.

Invited Presentations

Meeting	Title	Month-Year	Speaker
National Institute for Basic Biology Seminar.Okazaki,Japan	A phospho-protein selectively required for positive-but not negative-selection.	Apr-08	Yamasaki, S.
NIH Seminar. Bethesda, USA	Transcription factors network in CD4/CD8 Lineage Choice	Apr-08	Taniuchi, I.
RIKEN RCAI Special Seminar. Yokohama, Japan	Chemotherapy-resistant human AML stem cells home to and engraft within the bone-marrow endosteal region	Apr-08	Ishikawa, F.
Seminar in National Institute of Biomedical Innovation. Osaka, Japan	Regulation of innate immunity by PDLIM2, a nuclear ubiquitin ligase	Apr-08	Tanaka, T.
The seminar of Tokyo Women's Medical University. Tokyo, Japan	Cancer Immunotherapy by activated NKT cell-mediated DC maturation in situ	Apr-08	Fujii, S.
Seminar in University of Connecticut. Farmington, USA	Is there any link between innate immune system and HSP-mediated anti-tumor immunity	Apr-08	Udono, H.
Symposium on Korean Dendritic Cell Academy Association. Gwangju, Korea	Exploiting dendritic cells and NKT cells in immunotherapy.	Apr-08	Fujii, S.
5th The International Investigative Dermatology. Kyoto, Japan	Roles of zinc and its transporters in immunity and connective tissues	May-08	Hirano, T.
Discovery Strategies Conference: Modeling Human Autoimmune Diseases in the Laboratory Rodent. San Francisco,USA	Modeling Human Diseases Using NOD/SCID/IL2rgKO Mice	May-08	Ishikawa, F.
International Symposium "Frontiers in Allergy and Autoimmunity". Mainz, Germany	Foxp3 and its essential function for T cell regulation	May-08	Hori, S.
National Institute of Health. Bethesda, USA	PLC-g/calcium signaling in immune cells	May-08	Kurosaki, T.
Collegium Internationale Allergologicum 2008. Curaco, Netherlands	Identification of IL-17RB+ NKT cells preferentially producing IL-13 as a novel subset responsible for development of AHR	May-08	Taniguchi, M.
"Lysosomes and Endocytosis" Gordon Research Conference,Andover NH, USA	GPI-anchored protein glycoprotein 2 mediates transcytosis of type 1 fimbriated bacteria in intestinal M cells	Jun-08	Ohno, H.
Annual meeting of 18th Flow Cytometory Society. Tokyo,Japan	Detection of phosphorylation of STAT by Phosflow Technology.	Jun-08	Kubo, M.
Annual Meeting of Protein Science Society of Japan. Tokyo, Japan	From mammalian genes to proteins: How to use Omics information.	Jun-08	Ohara, O.
Chiba biotechnology forum meeting. Chiba, Japan	Affinity proteomics and its applications for diagnosis.	Jun-08	Ohara, O.
Human reproductive medicine and reproductive health of Urals and Siberia. Yekaterinburg, Russia	iPS as alternative or addition to use of ES cells in Regenerative Medicine	Jun-08	Rybouchkin, A.
RCAI-JSI International Symposium on Immunology. Yokohama, Japan	The PLCg/calcium system as a regulator of immune cells	Jun-08	Kurosaki, T.
RCAI-JSI International Symposium on Immunology. Yokohama, Japan	Dynamic Regulation of T Cell Co-stimulation by TCR-CD28 Microclusters.	Jun-08	Saito, T.
RCAI-JSI International Symposium on Immunology. Yokohama,Japan	Roles of zinc and its transporters in immunity and connective tissues	Jun-08	Hirano, T.
Seminar for Developing Cancer Therapy. Tokyo, Japan	Understanding Human Immunity and its Diseases Using Humanized Mouse	Jun-08	Ishikawa, F.
Symposium on 6th Catholic International Stem cell Symposium. Seoul, Korea	NKT cell-based cancer immunotherapy.	Jun-08	Fujii, S.
XVII Wilsede Meeting, Modern Trends in Human Leukemia. Hamburg, German	Mechanism of the Th-POK gene regulation during differentiation of helper-lineage T cells	Jun-08	Taniuchi, I.
6th Frontier Hematology Seminar. Tokyo,Japan Stem cells in AML Jun-08 Ishikawa, F. Open Symposium in Tokyo Medical and Dental University. Tokyo, Japan	Approach of Bioscience to Allergy.	Jun-08	Kubo, M.
US-Japan Cooperative Medical Sciences Program Immunosenescence Workshop. San Francisco, USA	Reconstitution of Human Immunity and Modeling Human Diseases in Mice	Jun-08	Ishikawa, F.
Symposium on 6th Catholic International Stem cell Symposium. Seoul, Korea	NKT cell-based cancer immunotherapy.	Jun-08	Fujii, S.
"Future developed by Omics-based medicine 2008" Japan Association for Omics-based Medicine.Tokyo, Japan	A strategy of construction of an informational basis for analyses of immunological diseases.	Jul-08	Ohara, O.
32th Aso Symposium. Aso,Kyushu	Regulatory T cell control of immune responses	Jul-08	Hori, S.
Memorial Symposium for Meiji Milk Products Endowed Laboratory of Food-Induced Bio-Signaling on "Probiotics and Intestinal Immunity". Tokyo, Japan	Intestinal epithelial M cells and intestinal immunity	Jul-08	Ohno, H.
Department seminar. Dept. Hematology, Erasmus Medical Center Rotterdam, the Netherlands	Crosstalk in the thymus	Jul-08	van Ewijk, W.
The 29th annual meeting of The Japanese Society of Inflammation and Regeneration. Tokyo, Japan	Mechanisms of tolerance induction by apoptotic cell phagocytosis	Jul-08	Tanaka, M.
The 29th Japanese Society of Inflammation and Regeneration. Tokyo, Japan	Zinc and Immunity/ development /inflammation	Jul-08	Hirano, T.
The 2nd Shanghai Symposium of Epigenetics in Development and Diseases / the 3rd Annual Meeting of Asian Epigenome Alliance. Shanghai ,China	Polycomb group proteins Ring1A/B are functionally linked to the core transcriptional regulatory circuitry to maintain ES cell identity	Jul-08	Koseki, H.
The 4th Annual Meeting of Hematology Young Researchers Conference.Tokyo, Japan	Regulatory dendritic cells protect against cutaneous chronic graft-versus-host disease mediated throughCD4+CD25+Foxp3+ regulatory T cells	Jul-08	Fujita, S.
Irving Weissman departmental congres. Stanford, USA.	Thymic microenvironments over the years	Jul-08	van Ewijk, W.
32th Aso Symposium. Aso,Kyushu	Immuneregulation by Suppressor of Cytokine Signaling,	Aug-08	Kubo, M.
Department of Anatomy & Structural Biology Seminar, Albert Einstein College of Medicine . New York, USA	Single molecule imaging by HILO microscopy in living cells and stochastic emergence of multiple intermediates detected by single-molecule protein unfolding	Aug-08	Tokunaga, M., Fukagawa, A., Sakata-Sogawa K.
Gruss-Lipper Biophotonics Center Seminar, Albert Einstein College of Medicine. New York, USA	Single molecule imaging by HILO microscopy in living cells and stochastic emergence of multiple intermediates detected by single-molecule protein unfolding	Aug-08	Tokunaga, M., Fukagawa, A., Sakata-Sogawa K.
40th Molecular Biology Seminar in Saga	Immuneregulation by Suppressor of Cytokine Signaling,	Aug-08	Kubo, M.
JSI Summer School 2008. Minami-Awaji, Japan	Molecular mechanisms in initiating and maintaining immune responses.	Aug-08	Saito, T.
120 Symposium "Outside or inside? –Relationship between microbial flora and the intestinal environment" in Annual meeting 2008 for the Society for Biotechnology Japan. Sendai, Japan	Elucidation of host-bacterial crosstalk via multi comprehensive analysis	Aug-08	Fukuda, S.

The 4th Hakone Conference . Chiba, Japan	Immunotherapy with regulatory dendritic cells for immunopathogenic disease and the clinical application	Aug-08	Sato, K.
15th international RUNX Workshop. Provincetown, USA	Roles of Runx complexes during thymocyte differentiation	Sep-08	Taniuchi, I.
40th Molecular Biology Seminar in Saga	Immuneregulation by Suppressor of Cytokine Signaling	Sep-08	Kubo, M.
EMBO Practical Course on Anatomy and Embryology of the Mouse. Zagreb,Croatia	Mouse development: embryogenesis	Sep-08	Koseki, H.
Post graduate course in Immunology. Erasmus Medical Center Rotterdam, the Netherlands	The thymus, classrooms, teachers and students	Sep-08	van Ewijk, W.
In a symposium of The 25th Annual Meeting of Japanese Society for Thermal Medicine. Nagoya, Japan	Fever and host defense from the view point of the proteasome	Sep-08	Udono, H.
Seminar at Columbia University. New York, USA	Transcription regulations in CD4/CD8 Lineage Choice	Sep-08	Taniuchi, I.
Special seminar series at Institute of Medical Science, University of Tokyo. Tokyo, Japan	Stem cell hierarchy in human leukemia	Sep-08	Ishikawa, F.
The 8th Awaji International Forum of Infection and Immunity. Awaji, Japan	Nucleic acid recognition by dendritic cells: sensors for viral strangers and host dangers	Sep-08	Kaisho, T.
The 8th Awaji International Forum of Infection and Immunity. Awaji, Japan	C-type lectin Mincle is an ITAM-coupled activating receptor sensing damaged cells.	Sep-08	Saito, T.
The 12th Molecular Cardiovascular Conference. Hokkaido, Japan	Regenerative properties of human stem cells	Sep-08	Ishikawa, F.
7th Oncology Forum in Mie University. Mie, Japan	Leukemic stem cells and relapse	Oct-08	Ishikawa, F.
9th International Congress on Cell Biology, Korea	Dendritic cell maintenance by MARCH-I through MHC II ubiquitination.	Oct.08	Ishido, S., Ohmura-Hoshino, M., Matsuki, Y., Mito-Yoshida, M., Goto, E., Aoki, M.
Cantoblanco Workshops on Biology, Initiation of Antigen Receptor Signaling. Madrid, Spain	Dynamic regulation of TCR- and costimulatory signals via microclusters.	Oct-08	Saito, T.
Cantoblanco Workshops on Biology. Madrid, Spain	Calcium signaling in immune cells	Oct-08	Kurosaki, T.
Cold Spring Harbor Laboratory. NewYork, USA	Polycom repression mediates pluripotency of ES cells	Oct-08	Koseki, H.
Symposium on regulation of DNA replication and repair by ubiquitination and SUMO. National Institute of Genetics	Mechanisms of A:T mutations in the germinal center B cells.	Oct.08	Wang, JY.
The 10th International Symposium on Dendritic Cells, DC2008. Kobe, Japan	Critical roles of IKKa in dendritic cell subsets	Oct-08	Kaisho, T.
The 10th International Symposium on Dendritic Cells. Kobe, Japan	Recognition of Necrosis by a C-type lectin Mincle.	Oct-08	Saito, T.
The 10th International Symposium on Dendritic Cells. Kobe, Japan	PDC-TREM, a new plasmacytoid dendritic cell receptor, is responsible for augmentation of type I interferon production	Oct-08	Watarai, H
The 6th Europeean Mucosal Immunology Group Meeting. Milano, Italy	LP Responses	Oct-08	Fagarasan, S.
Thymus workshop. Dept. Hematology, Erasmus Medical Center Rotterdam, the Netherlands.	The thymus, follow me	Oct-08	van Ewijk, W.
Joint seminar by Kyoto University Graduate School of Medical Sciences and AK project. Kyoto, Japan	Humanized mouse research	Oct-08	Ishikawa, F.
Symposium on 36th Annual Meeting of the Japanese Society for Clinical Immunology. Tokyo, Japan	Immune responses in chronic myelogenous leukemia (CML) and its application to cancer immunotherapy.	Oct-08	Fujii, S.
Symposium on 67th Annual Meeting of the Japanese Cancer Accociation (JCA). Nagoya, Japan	Enhanced antitumor immunity by targeting dendritic cells in situ	Oct-08	Fujii, S.
Symposium on 36th Annual Meeting of the Japanese Society for Clinical Immunology. Tokyo, Japan	Immune responses in chronic myelogenous leukemia (CML) and its application to cancer immunotherapy	Oct-08	Fujii, S.
13th Kurume Hematology Conference. Kurume, Japan	Stemness in AML	Nov-08	Ishikawa, F.
14th Ketsueki-Kagaku seminar. Tokyo, Japan	Revised scheme of hematopoiesis,	Nov-08	Kawamoto, H.
17th AnnulMeating of Bioimaging Society. Chiba, Japan	Dynamic regulation of immunological synapse and T cell activation.	Nov-08	Saito, T.
39th NIPS International Symposium & 7th OIB Symposium “Frontiers of Biological Imaging: Synergy of the Advanced Techniques”. Okazaki, Aichi, Japan	Highly inclined thin illumination enables clear single-molecule imaging in living cells	Nov-08	Tokunaga, M., Sakata-Sogawa K.
Japan-German Immunology Seminar 2008: Immune Regulation in Health and Disease. Fukuoka, Japan	Role of NKT cell in innate and acquired immunity.	Nov-08	Taniguchi, M.
The 13th Annual Meeting of the Japanese Association for Dietary Fiber Research. Tokyo, Japan	Molecular basis of antigen transcytosis in intestinal M cells	Nov-08	Hase, K.
The 57th Fall Conference of the Korean Association of Immunobiologists. Seoul, Korea	Calcium signaling in immune cells	Nov-08	Kurosaki, T.
The 58th Annual Meeting of Japanese Society of Allergology. Tokyo, Japan	Immunotherapy with regulatory dendritic cells against allergic asthma	Nov-08	Sato, K.
The 58th Annual Meeting of Japanese Society of Allergology. Tokyo, Japan	Immunotherapy with regulatory dendritic cells against allergic asthma	Nov-08	Sato, K.
The 8th Annual Meeting of the Korean Atopic Dermatitis Association (KADA) . Seoul, Korea	Control of immune response by regulatory dendritic cells	Nov-08	Sato, K.
Gunma Prefectural Cancer Center Cell Therapy Seminar. Gunma, Japan	Leukemic stem cells	Nov-08	Ishikawa, F.
The 2nd Global COE International Symposium joint with the 18th Hot Spring Harbor Symposium of Medical Institute of Bioregulation, Kyushu University . Fukuoka, Japan	Reconstitution of human immunity and modeling human diseases in humanized mouse	Nov-08	Ishikawa, F.
The 8th Hematology Conference in Hokkaido University. Hokkaido, Japan	Role of LSCs in AML relapse	Nov-08	Ishikawa, F.
31st Annual Meeting of the Molecular Biology Society of Japan, 81st Annual Meeting of the Japanese Biochemical Society. Kobe, Japan. “	Histone H2A ubiquitinylation subdivides bivalent genes into PRC1-dependent and -independent domains in ES cells	Dec-08	Koseki, H.
Asia-Pacific Symposium on Primary Immunodeficiency Diseases. Yokohama, Japan	Construction of an Informational Platform for Primary Immunodeficiency Diseases in Asia	Dec-08	Mohan, S.
BMB2008,Kobe, Japan	Mechanisms of ThPOK gene regulation during helper T cell development	Dec-08	Taniuchi, I.

BMB2008. Kobe, Japan	Dissecting Foxp3-dependent molecular program of regulatory T cell differentiation and function	Dec-08	Hori, S.
BMB2008. Kobe, Japan	Single molecule imaging and quantitative analysis of T cell signaling on the cell membrane	Dec-08	Sakata-Sogawa, K. and Tokunaga, M
Departmental seminar Dept. Dermatology Kochi University, Kochi, Japan	The thymus, classrooms, teachers and students	Dec-08	van Ewijk, W.
Hindgut Club. Tokyo, Japan	Analysis of host-bacterial crosstalk at the follicle-associated epithelium	Dec-08	Fukuda, S.
Seminar of Medical Institute of Bioregulation, Kyushu University. Fukuoka, Japan	Regulation of development, maintenance and activation of immunity through ITAM receptors	Dec-08	Yamasaki, S.
Symposium “Intracellular logistics: approach to pathophysiology of membrane traffic, Biochemistry and Molecular Biology 2008. Kobe, Japan	Cell polarity regulates proliferation of intestinal epithelial cells	Dec-08	Ohno, H.
Symposium for PID in Asia. Yokohama,Japan	PIDJ network: DNA Analysis	Dec-08	Ohara, O.
Systems Biology for the Young Scientists, the 1st International Symposium of Nagoya University G-COE program. Nagoya, Japan	Multi-omics based identification of microbial metabolites can prevent enterohemorrhagic Escherichia coli O157:H7 lethal infection through the gut epithelial barrier function	Dec-08	Fukuda, S.
The 15th Takeda Science Foundation Symposium on Bioscience. Tokyo,Japan	Apoptotic cell clearance and self-tolerance.	Dec-08	Tanaka, M.
The 38th Annual Meeting of the Japanese Society for Immunology. Kyoto, Japan	Exploiting dendritic cells and NKT cells in immunotherapy	Dec-08	Fujii, S.
The 38th Annual Meeting of the Japanese Society for Immunology. Kyoto, Japan	PLCg2 is essential for formation and maintenance of memory B cells	Dec-08	Hikida, M. et al.
The 38th Annual Meeting of the Japanese Society for Immunology. Kyoto, Japan	Transcriptional Regulation of Helper Lineage T cell Development In Thymus	Dec-08	Taniuchi, I.
The 38th Annual Meeting of the Japanese Society for Immunology. Kyoto, Japan	B cell signaling and fate decision	Dec-08	Kurosaki, T.
The 38th Annual Meeting of the Japanese Society for Immunology. Kyoto, Japan	Invasive migration of melanoma cells in the metastatic lymph node	Dec-08	Okada, T.
The 38 Annual Meeting of the Japanese Society of Immunology. Kyoto, Japan	Mincle is an ITAM-coupled activating receptor that senses damaged cells.	Dec-08	Yamasaki, S.
The Annual Meeting of the Biophysical Society of Japan. Fukuoka, Japan	Combination of simulation researches with single-molecule detection and imaging	Dec-08	Tokunaga, M., Fukagawa, A., Sakata-Sogawa, K.
Towards a unified model of hematopoiesis symposium in JSI annual meeting. Kyoto, Japan	Myeloid potential retained by early T cell progenitors	Dec-08	Kawamoto, H.
EMBO Workshop “Visualizing Immune System Complexity. Marseille, France	Single molecule imaging and quantitative analysis in living cells	Jan-09	Sakata-Sogawa K. and Tokunaga, M.,
Immune System Regulation and Treatment: LIAIRCAI Workshop. Yokohama, Japan	Reconstitution of human immunity and diseases in mice	Jan-09	Ishikawa, F.
In the second symposium on Primary Immunodeficiency. Tokyo,Japan	Population dynamics underlying memory B cell development	Jan-09	Takemori, T.
IUPS International Conference of Physiological Sciences. Pusan, Korea	Calcium signaling in immune cells	Jan-09	Kurosaki, T.
Keystone symposia: Allergy and asthma. Keystone Resort, USA	A novel subset of mouse NKT cells bearing the IL-17 receptor B responds to IL-25 and contributes to airway hyperreactivity	Jan-09	Watarai, H
LIAI-RCAI Joint Workshop “Regulation of Lymphocyte Function and Immune Responses”. Yokohama, Japan	Role of zinc transporter in mast cell mediated-allergic reaction	Jan-09	Nishida, K.
MHC I molecules. Tsukuba, Japan	Seminar in University of Tsukuba, “Mystery of crosspresentation: how are extracellular antigens	Jan-09	Udono, H.
Seminar in Institute Curie. Paris, France	Single molecule imaging and Quantitative analysis of living cells	Jan-09	Sakata-Sogawa, K.
The Third iCeMS International Symposium, The MEXT Priority Area of Transportsome Open Symposium, The Twelfth Membrane Research Forum. Kyoto, Japan	Dynamic assembly of signaling unit for antigen recognition and activation of immune cells	Jan-09	Saito, T.
LIAI-RCAI Joint Workshop “Regulation of Lymphocyte Function and Immune Responses”. Yokohama, Japan	Dynamic regulation of T cell co-stimulation by TCR-CD28 microclusters	Jan-09	Yokosuka, T.
The 3th Japanese Veterinary allergy and immunology Scociety. Tokyo, Japan	Cancer immunotherapy by NKT cell-mediated mature dendritic cells (DCs).	Jan-09	Fujii, S.
HITS2009 Symposium “Frontiers of Biological Imaging: Synergy of the Advanced Techniques”. Yokohama, Japan	Single molecule imaging in living cells and force detection of molecular interactions	Feb-09	Tokunaga, M., Fukagawa, A., Sakata-Sogawa, K.
The 24th Nagoya International Cancer Treatment Symposium. Nagoya, Japan	Targeting human AML stem cells	Feb-09	Ishikawa, F.
The 2nd International Symposium of WPI-iFReC—Dymanics of Immune Responses. Osaka, Japan	Self and non-self recognition by ITAM-coupled C-type lectin receptor, Mincle.	Feb-09	Yamasaki, S.
Miyazaki Science Camp. Miyazaki, Japan	Control of autoimmunity by regulatory T cells	Feb-09	Hori, S.
Japan-USA Co-operative Program Symposium. Bethesda, MD, USA	Lineage stability and plasticity in regulatory T cell differentiation	Feb-09	Hori, S.
The 31th Annual Meeting of the Japan Society for Hematopoietic Cell Transplantation. Sapporo, Japan	Immune regulation by NKT cells in hematological disorders.	Feb-09	Fujii, S.
Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry 2009. Fukuoka, Japan	Zinc is signaling molecule	Mar-09	Hirano, T.
Biomedical Asia 2009-Translatioonal Research Asia Summit	Case study: Establishing a translational platform for development of novel therapeutics to allergy in Japan	Mar-09	Taniguchi, M.
The 5th International Symposium on CD1/NKT cells. Kamakura, Japan	NKT cell-dendritic cell cross-talk and their potential in immunotherapy.	Mar-09	Fujii, S.
The 33th Dermatology and Immunity. Tokyo, Japan	Zinc and Immunity/Inflammatory: Zinc is an intracellular signaling molecule	Mar-09	Hirano, T.
The 7th Anual Meeting of Japanese Society of Medical Oncology ASCO/JSMO Joint Symposium	Stem cells in human hematopoietic malignancies	Mar-09	Ishikawa, F.
The 8th Congress of the Japanese Society for Regenerative Medicine. Tokyo, Japan	Biology of human hematopoietic and leukemic stem cells	Mar-09	Ishikawa, F.

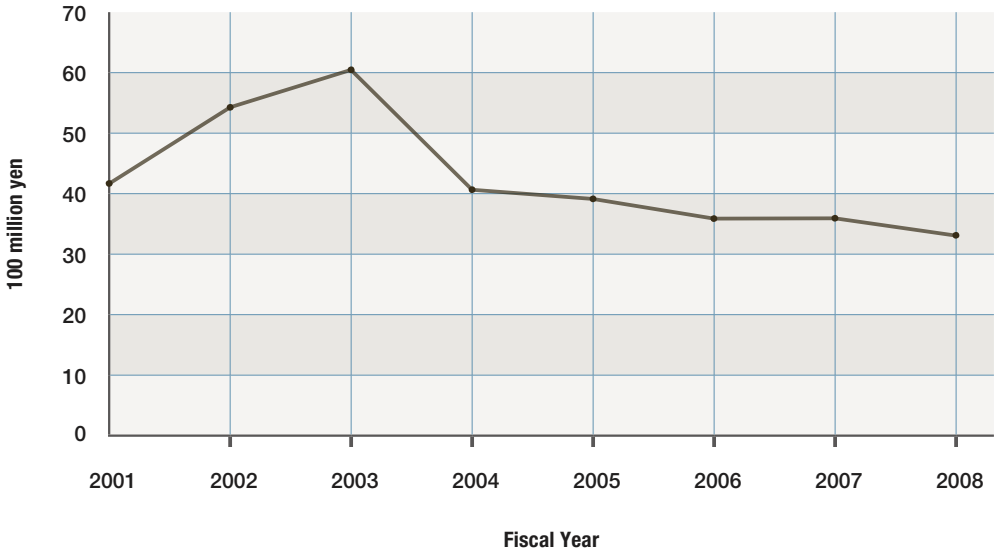
RCAI Seminars

Date	Tittle	Lecturer	Affiliation
4-Apr-08	Research activities at Advanced Science Institute	Dr. Kohei Tamao Dr. Yoshihito Osada Dr. Katsumi Midorikawa Dr. Minoru Yoshida Dr. Hiroyuki Osada	Director, ASI Corporate and International Relations Division, ASI Extreme Photonics Department, ASI Chemical Genomics Research Group, ASI Antibiotics Laboratory, ASI
21-Apr-08	ATM and NFkB activation by RAG-mediated DNA breaks promotes a broadly functional genetic program in developing lymphocytes	Dr. Barry P.Sleckman	Associate Professor,Department of Pathology and Immunology, Washington University School of Medicine,U.S.A
23-Apr-08	The Immunological Genome, stories and projects	Dr. Christophe Benoist	Professor of Medicine Head,Section on Immunology and Immunogenetics, Joslin Diabetes Center,Harvard Medical School
7-May-08	Super Computer Project at ASI	Dr. Makoto Taiji Dr. Mariko Hatakeyama Dr. Shuichi Onami Dr. Ryutaro Himeno	Group Director (Computational Systems Biology, ASI) Team Leader (Cellular Systems Modeling, ASI) Team Leader (Developmental Systems Modeling, ASI) Group Director (Living Matter Simulation, ASI)
13-May-08	The Dark Side of Organelle Biogenesis: Lessons from melanosomes on endosomal protein sorting in the formation of lysosome-related organelles	Dr. Michael S. Marks	Dept. of Pathology and Laboratory Medicine University of Pennsylvania School of Medicine
20-May-08	Unraveling the early hematopoietic hierarchy	Dr. Toshimi Yoshida	CBRC, Massachusetts General Hospital, Harvard Medical School
2-Jun-08	The role of the immune system in prion disease pathogenesis	Dr. Neil A. Mabbott	The Roslin Institute and Royal Dick School of Veterinary Studies, University of Edinburgh, UNITED KINGDOM
9-Jun-08	Positive and Negative Role of Ca2+ Signaling in T Cell Function	Dr. Masatsugu Oh-hora	Instructor at The Immune Disease Institute, Harvard Medical School
30-Jun-08	Dynamic interactions between the nervous and immune systems with the microenvironment regulate hematopoietic stem cells	Dr. Tsvee Lapidot	The Edith Arnoff Stein Professorial Chair in Stem Cell Research, Department of Immunology, Weizmann Institute of Science
30-Jun-08	Visualization of protein interactions and post translational modifications using DuolinkTM	Dr. Erik Nystrom	Application Specialist, Olink Bioscience
28-Sep-08	Dendritic cell Functions revealed by their Conditional and Constitutive In Vivo Ablation	Dr. Steffen Jung	Senior Scientist, Weizmann Institute of Science
28-Oct-08	Multiplex suspension array technology, specifically for cytokines/chemokines	BioRad BioPlex Technology	BioRad BioPlex Technology
6-Nov-08	Balancing act of effector and regulatory T cells	Dr. Makio Iwashima	Associate Professor, Department of Microbiology and Immunology, Loyola University
25-Nov-08	Real time imaging of hematopoietic stem cells	Dr. Motomi Osato	Associate Professor, National University of Singapore, Senior Scientist, Institute of Molecular and Cell Biology
27-Nov-08	Breaking dogmas: CD8+ T helper cells	Dr. Andreas Thiel	Group Leader Clinical Immunology, DRFZ Berlin

27-Nov-08	**The Transcriptional Regulation of Lymphocyte Differentiation**	**Dr. Stephen Nutt**	The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia
28-Nov-08	**Role of the proto-oncogene LRF in the maintenance of hematopoietic stem and cells**	**Dr. Takahiro Maeda**	Department of hematopoietic stem and cell and leukemia research City of Hope National Medical Center, Duarte, CA
8-Dec-08	**Regulatory T cell suppression program co-opts transcription factor IRF4 to control Th2 response**	**Dr. Ye Zheng**	Department of Immunology, University of Washington, Seattle
8-Dec-08	**Competing Ligands of CD22 in B Cell Biology**	**Dr. James C. Paulson**	The Scripps Research Institute
8-Dec-08	**The role of the chemokine receptor CXCR4 in hematopoiesis**	**Dr. Yong-Rui Zou**	Assistant Professor of Microbiology, Columbia University
15-Dec-08	**ESCRT-III Proteins in Hiv budding and cell division**	**Dr. Wesley I.Sundquist**	School of Medicine,University of Utah,
18-Dec-08	**Dynamic assembly and inactivation of centromere chromatin on human artificial chromosomes**	**Dr. Hiroshi Masumoto**	Kazusa DNA Research Institute, Department of Human Genome Research, Laboratory of Cell Engineering
18-Dec-08	**Chromosome architecture and dynamics: a view from condensins**	**Dr. Tatsuya Hirano**	Chromosome Dynamics Laboratory, Advanced Science Institute, RIKEN
22-Dec-08	**Mast Cells: Unique Pathways in Allergy vs T Cell Activation**	**Dr. Taku Kambayashi**	University of Pennsylvania
22-Dec-08	**Subversion of PKCa causes lineage plasticity on pro-B cells**	**Dr. Rinako Nakagawa**	Section of Experimental Haematology, Division of Cancer Science and Molecular Pathology, University of Glasgow
23-Jan-09	**Chemokine-mediated migration control of osteoclast precursors visualized by in vivo bone imaging: A novel point of regulation for osteoimmunology**	**Dr. Masaru Ishii**	Associate Professor at Lab. of Biological Imaging, WPI Immunology Frontier Research Center, Osaka University
9-Feb-09	**Control of homeostasis of dendritic cells and Tregs, and consequences for T cell reactivity**	**Dr. Gunter J. Hammerling**	Director of the Institute for Immunology and Genetics, German Cancer Research Center in Heidelberg, Germany
9-Feb-09	**The linkage between gut and brain: glutamate signaling in the stomach (presented in Japanese)**	**Dr. Kunio Torii**	Executive Fellow, Institute of Life Sciences, Ajinomoto.Co.,Inc.
5-Mar-09	**Lamina propria macrophages and dendritic cells differentially induce regulatory and interleukin 17-producing T cell responses**	**Dr. Timothy L.Denning**	Vaccine Research Center and Yerkes Regional Primate Research Center, Emory University,
30-Mar-09	**Th1 anti-tumor & anti-viral human & mouse NKT cell populations**	**Dr. Mark Exley**	Assistant Professor of Medicine, Harvard Medical School, Boston, USA

Budget, Personnel and Patents

RCAI Budget (JPY 100 Million)

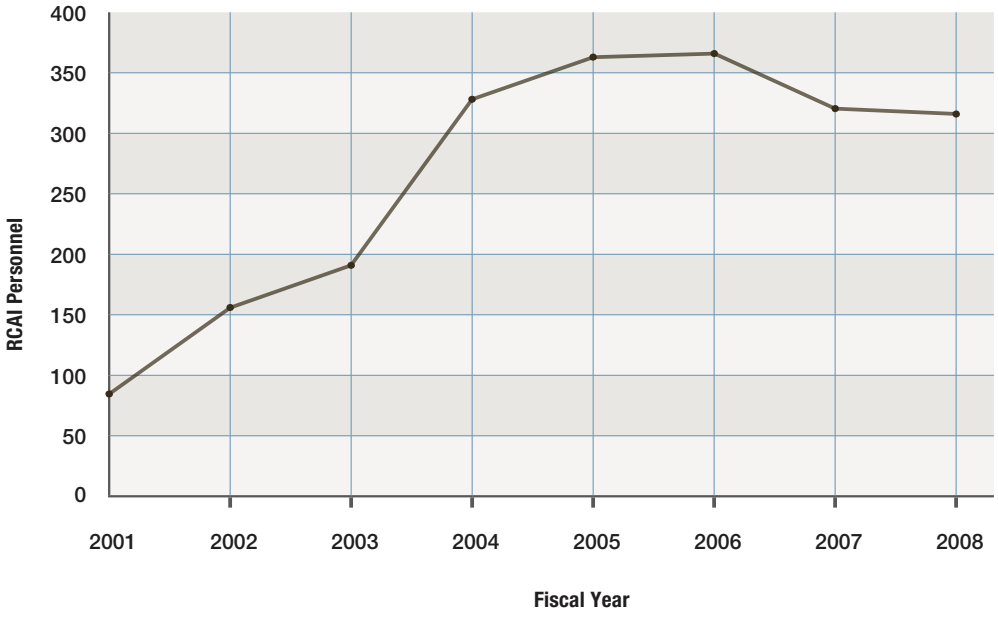


RCAI Budget FY2001-2008 (JPY 100 Million)

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

RCAI Personnel



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

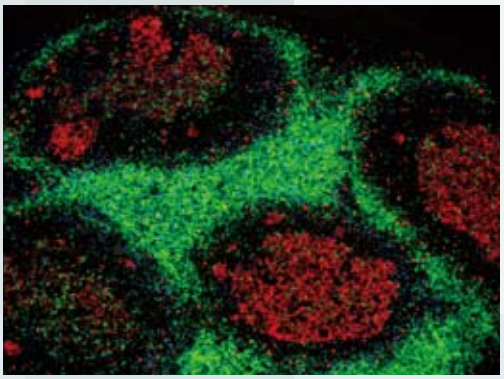
RCAI Staff Composition (as of Nov. 2008)

Category	Number
Director	1
Special Advisor	1
Principal Investigators	33
Science Advisor	1
Coordinator	1
Senior Scientists	8
Scientists	46
Senior Technical Scientist	1
Technical Scientists	4
Technical Staff I	34
Technical Staff II	47
Graduate Students	29
Research Associates	14
Interns	2
Visiting Scientists	31
Research Consultatnts	2
Research Fellows	2
Assistants	21
Part-time Staff	10
Temporary Employment	38
Total	326

RCAI Patents FY 2008 (as of Jan. 2009)

There were 16 patents filed in Apr. 2008-Jan. 2009

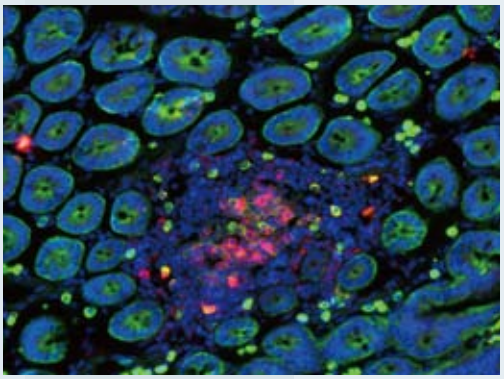
Cover and Section Heading Photo Legends



Front cover

Immunofluorescent microscopy analysis of Peyer's patches from two month old mice, stained to detect AID-expressing germinal center B cells (*red*), $CD3^+$ T cells (*green*), and $Foxp3^+$ T cells (*blue*).
The Laboratory for Mucosal Immunity demonstrated that $Foxp3^+$ T_{reg} cells known for their suppressive function, can trans-differentiate into follicular B helper T cells in Peyer's patches. (See *Research Highlights* section)

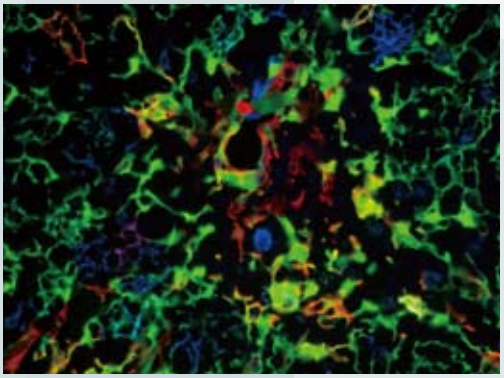
Image courtesy: Laboratory for Mucosal Immunity.



Front page of Research Highlights section

Section from adult small intestine showing an isolated lymphoid follicle (ILF) containing activated B cells expressing AID (*red*) and IgA^+ B cells (*green*) that were generated *in situ* within the ILF. DAPI (*blue*) stains nuclei of all cells.
IgA is generated in the gut by both T cell-dependent and T cell-independent processes, but the sites and the mechanisms for T-independent IgA synthesis remain elusive. The Laboratory for Mucosal Immunity found that isolated lymphoid follicles (ILFs) are sites where induction of activation-induced cytidine deaminase (AID) and IgA class switching of B cells take place even in the absence of T cells. The formation of ILFs is regulated by multiple and complex interactions between lymphoid tissue inducer cells expressing the nuclear receptor RORγt ($ROR\gamma t^+$LTi cells) and stromal cells (SCs). Activation of SCs by $ROR\gamma t^+$LTi cells through the lymphotoxin (LT)-β receptor (LTβR) and simultaneously by bacteria through TLRs induces the recruitment of dendritic cells (DCs) and B cells, and formation of ILFs. These findings provide insight into the crosstalk between bacteria, $ROR\gamma t^+$LTi cells, SCs, DCs and B cells required for ILF formation and establish a critical role of ILFs in T cell-independent IgA synthesis in the gut. (See *Laboratory Activities* section)

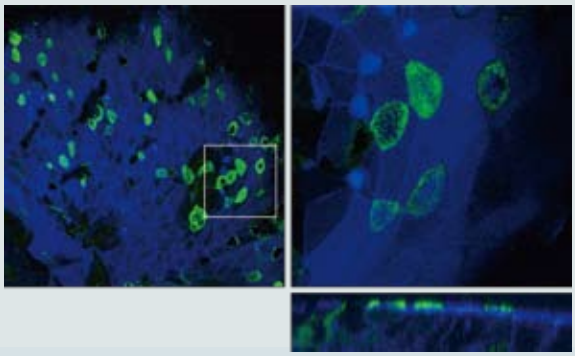
Image courtesy: Laboratory for Mucosal Immunity.



Front page of Laboratory Activities section

FoxN-1 (*green*) expression in subpopulations of thymic epithelial cells in 23 wk old FoxN-1 EGFP transgenic mice, stained by antibodies directed to cytokeratin 5 (*red*; medullary epithelial cells) and cytokeratin 8 (*blue*; cortical epithelial cells)
The Research Unit for Thymic Microenvironment specifically focuses on the origin and generation of aberrant epithelial cell types (ciliated cells, goblet cells) in the immunodeficient thymus. It was speculated that these cells are not intrinsic to the thymus but have been incorporated into the thymic stroma at early embryonic stages. However, in their studies they found that aberrant cell types derive from the thymic environment itself. In studies using FoxN1 reporter mice, they have shown that the cyst lining epithelial cells have passed through a FoxN1 dependant developmental pathway, univocally demonstrating that they have "transdifferentiated" from normal thymic epithelial cells (See *Laboratory Activities* section).

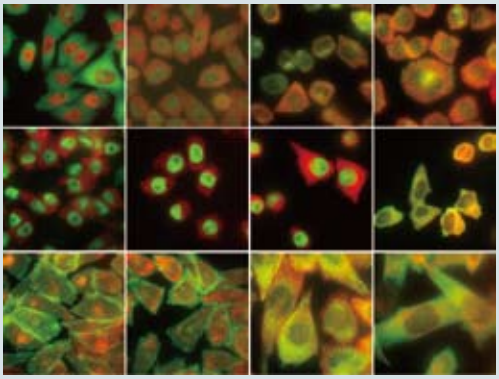
Photo taken by Eric Vroegindewey.
Image courtesy: Research Unit for Thymic Microenvironment.



Front page of Research Activities section

Whole-mount staining of a murine Peyer's patch shows M-cell-specific expression of GP2. (*Upper left panel*) One FAE region of a murine Peyer's patch. GP2-positive M cells (*green*) are scattered throughout the FAE. The tissues are counterstained with phalloidin to depict the outline of all the cells. (*Right panels*) Magnification of the white rectangle in the left. The X-Z section (*bottom*) indicates that GP2 is localized on the apical plasma membrane of M cells.
M cells are believed to play an important role in mucosal immunity. However, no M-cell-specific surface marker had been identified. In a recently published paper, Koji Hase and Hiroshi Ohno of the laboratory for Epithelial Immunobiology, in collaboration with Dr. Kiyono's group in the University of Tokyo, reported that Glycoprotein 2 (GP2) is exclusively expressed by Peyer's patch M cells in the intestine. (See *Research Activities* section)

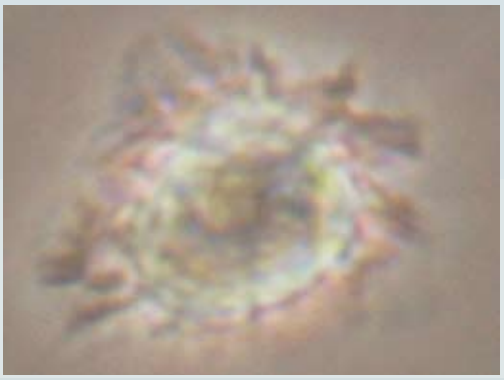
Image courtesy: Laboratory for Epithelial Immunobiology.



Front page of Collaborative Networks section

Dual color imaging of protein interactions in CHO cell lines. EGFP (*green*) -and mCherry (*red*) fusion proteins of two different signaling molecules or transcription factors are stably expressed at low and homogenous levels.
Dual color single molecule imaging is required for quantitative analysis of the interactions of signaling proteins. The Research Units for Single Molecule Imaging and Molecular Systems Immunology have established methods to obtain cell lines expressing two fluorescence tagged proteins at a homogenous and low level. The figure shows examples of dual color expressing CHO cell lines. The expression levels were successfully controlled by selecting suitable promoter sequences.

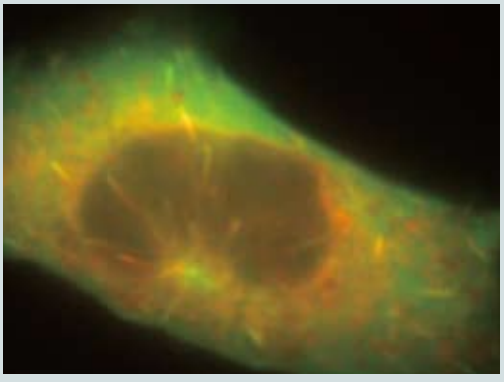
Image courtesy: Research Units for Single Molecule Imaging and Molecular Systems Immunology



Front page of Events section

Morphology of mature dendritic cells (DC)
The Laboratory for Dendritic Cell Immunobiology modified dendritic cells and established tolerogenic antigen presenting cells with potent immunoregulatory properties even under inflammatory conditions. They designated them "regulatory DCs (DC_{reg}) ". Their goal is to identify the molecular mechanisms underlying the function of regulatory DCs and to develop immunotherapy with regulatory DCs for immunopathogenic diseases. (See *Laboratory Activities* section)

Image courtesy: Laboratory for Dendritic Cell Immunobiology



Front page of Data and Statistics section

Imaging interaction of STIM1 and EB1 by HILO microscopy.
The Research Units for Single Molecule Imaging and Molecular Systems Immunology succeeded in imaging the interaction between microtubule plusend-binding protein EB1 and the ER membrane resident Ca^{2+} sensor protein STIM1. Both fusion proteins were stably expressed and observed in CHO cells. Real time imaging showed STIM1 tracking at growing microtubule ends and thus its involvement in remodeling of the ER. (See *Laboratory Activities* section)

Image courtesy: Research Units for Single Molecule Imaging and Molecular Systems Immunology

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