*Center for Experimental Medicine and Systems Biology*

# Laboratory of Molecular Pathogenesis
## システム疾患モデル研究センター分子病態研究分野


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| Professor | Yoichiro Iwakrua, D.Sc. | 教授 | 理学博士 | 岩倉 洋一郎 |
| Assistant Professor | Shigeru Kakuta, Ph.D., D.V.M. | 助教 | 獣医学博士 | 角田 茂 |
| Assistant Professor | Tomonori Kaifu, Ph.D. | 助教 | 医学博士 | 海部 知則 |
| Assistant Professor | Ce Tang, M.D., Ph.D. | 助教 | 医学博士 | 唐 策 |
| Assistant Professor | Guangyu Ma, M.D., Ph.D. | 助教 | 医学博士 | 馬 光宇 |



*Recent development of transgenic techniques has made it possible to directly analyze the functions of a particular gene in a living animal. These techniques have also made it possible to produce various animal disease models as well as tools to analyze them. Immune disorders and infectious diseases are our major concerns, and we are attempting to produce transgenic mouse models for these diseases.*


### 1. Studies on rheumatoid arthritis models


**Shinobu Saijo[1], Masanori Murayama, Kenji Shimizu, Aoi Akitsu, Satoshi Ikeda, Toshimasa Kusaka, and Yoichiro Iwakura: [1]Medical Mycology Research Center, Chiba Univ.**


Rheumatoid arthritis (RA) is one of the most serious medical problems worldwide with approximately 1% of the people in the world affected. The disease is autoimmune in nature and characterized by the chronic inflammation of synovial tissues in multiple joints that leads to joint destruction. High levels of inflammatory cytokine expression in the joints are a characteristic of the disease, although the pathogenesis has not been elucidated completely. We have been studying the pathogenesis of the disease using two arthritis models that we originally developed. One is HTLV-I transgenic (Tg) mice (Iwakura et al., *Science*, 1991) and the other is IL-1 receptor antagonist-deficient (*Il1rn*$^{-/-}$) mice (Horai et al., *J. Exp. Med*., 2000). Both of these models develop autoimmunity and chronic inflammatory arthropathy closely resembling RA in humans.

To identify genes involved in the pathogenesis of arthritis, we analyzed the gene expression profiles of these animal models by using high-density oligonucleotide arrays, and identified genes which are overexpressed in the affected joints in the RA-related gene locus (Fujikado et al., *Arthritis Res. Ther*., 2006). Furthermore, we have also searched for pathogenesis-related genes by analyzing the effect of genetic backgrounds on arthritis development, because BALB/cA strain mice were highly susceptible to develop arthritis in these models, whereas C57 BL/6J strain mice were resistant. We performed linkage-analysis using BALB/c-HTLV-I Tg backcross progenies into C57BL/6J, and identified several RA-related gene loci that affected the strain specificity of arthritis susceptibility. We are now analyzing the roles of these genes in the pathogenesis of arthritis and autoimmunity by generating the gene targeted mice of these genes.

Among these genes identified by microarray analysis and genetic linkage analysis, we are now analyzing the functions of two cytokine re-

ceptors and four novel genes including "*Tora*" and "*Kusa*", which encode cytoplasmic proteins, "*Shimi*", which encodes transmenbrane protein, and "*Mura1*", which encodes secreted protein. We have generated gene-targeted mice for these genes. Both *Tora*$^{-/-}$, *Kusa*$^{-/-}$, *Shimi*$^{-/-}$ and *Mura1*$^{-/-}$ mice were fertile, were born at the expected mendelian ratios, and showed no obvious phenotypic abnormalities. However, we found that both *Mura1*$^{-/-}$ mice and *Kusa*$^{-/-}$ mice were highly susceptible to type 2 collagen-induced arthritis, an experimental model for RA, whereas *Tora*$^{-/-}$ and *Shimi*$^{-/-}$ showed no obvious abnormality to collagen-induced arthritis. We are now analyzing the function of these genes.

## 2. IL-17-producing γδ T cells are important for the development of arthritis in a rheumatoid arthritis mouse model

**Aoi Akitsu, Shinobu Saijo, and Yoichiro Iwakura**

IL-17 is a proinflammatory cytokine that activates T cells and other immune cells to induce a variety of cytokines, chemokines, and cell adhesion molecules. Although it is widely accepted that IL-17 producing-CD4$^+$ T (Th17) cells are involved in the pathogenesis of many autoimmune diseases, the roles of other IL-17-producing T cells, such as γδ T cells, remain to be elusive. We found that IL-17 is mainly produced in γδ T (γδ17) cells rather than Th17 cells in the affected joints of *Il1rn*$^{-/-}$ mice, a model of RA in which IL-17 plays a crucial role. Anti-TCRγδ or anti-CD4 suppressed the development of arthritis in *Il1rn*$^{-/-}$ mice. However, CD4- or TCRδ-gene deficiency did not affect, because γδT cells and CD4$^-$γδ$^-$T cells produced IL-17 in these mutant mice, respectively. Interestingly, a combination of CD4$^+$ T cells and γδ17 cells was required for the development of arthritis in *scid/scid* mice. These observations suggest that γδ17 cells are required for the amplification of inflammation and CD4$^+$ T cells direct the tissue specificity.

## 3. The role of IL-6 in the development of Th17 cells in *Il1rn*$^{-/-}$ mice

**Satoshi Ikeda, Shinobu Saijo, and Yoichiro Iwakura**

*Il1rn*$^{-/-}$ mice develop autoimmune arthritis in which IL-17 plays a crucial role. Although many studies have indicated that Th17 cell differentiation is dependent on TGF-β and IL-6, we found that IL-6 deficiency does not affect the development of arthritis in *Il1rn*$^{-/-}$ mice at all. Interestingly, Th17 cells developed normally in *Il1rn*$^{-/-}$ *Il6*$^{-/-}$ mice. Then, we analyzed the mechanisms of Th17 cell differentiation in *Il1rn*$^{-/-}$*Il6*$^{-/-}$ mice. We found that IL-21 production was increased in the lymph nodes (LNs) of *Il1rn*$^{-/-}$ mice and naïve Il6$^{-/-}$ CD4$^+$ T cells differentiated into Th17 cells when cultured with TGF-β and IL-21. Th17 cell differentiation was much enhanced when IL-1 was added to the culture, although Th17 cell differentiation was not induced by TGF-β or IL-1 alone, or in combination. Furthermore, IL-1 augmented the expression of Th17 cell-specific transcription factors such as *Nfkbiz* and *Batf*. These results indicate that excess IL-1 signaling can overcome the requirement of IL-6 in the differentiation of Th17 cells by inducing IL-21 and Th17 cell-specific transcription factors.

## 4. Dendritic cell immunoreceptor (DCIR), an inhibitory type of C-type lectin receptor, is important for the homeostasis of the immune system and bone metabolism

**Tomonori Kaifu, Rikio Yabe, Takumi Maruhashi, Akimasa Seno, Guangyu Ma, and Yoichiro Iwakura**

A balance between positive and negative signals through cellular receptors with opposing functions is important for the homeostasis of the immune system and bone metabolism. The homeostasis of the immune system is maintained by counterbalancing signals between activation and inhibitory receptors, whereas bone homeostasis is maintained by an equilibrium between bone resorption by osteoclasts and bone formation by osteoblasts. Disturbance of the balance between these opposite signals may cause harmful effects. In fact, continuous activation of the immune system causes inflammation, resulting in tissue damage and loss of mineral components due to excess activation of osteoclasts.

DCIR is a member of the C-type lectin with an extracellular carbohydrate recognition domain (CRD) with an atypical $Ca^{2+}$-dependent EPS motif and an intracellular cytoplasmic region with an immunoreceptor tyrosine-based inhibitory motif (ITIM). Gene disruption of this molecule in mice causes spontaneously development of autoimmune disorders such as enthesitis and sialadenitis due to over expansion of dendritic cells. In this study, we further examined effects of DCIR deficiency on disease development and the physiology. We found that DCIR-deficient mice exacerbated experimental autoimmune encephalomyelitis, a mouse model for multiple sclerosis, and T cell proliferation of these mice was enhanced upon stimulation with

myelin oligodendrocyte glycoprotein. Interestingly, DCIR-deficient mice developed ankylosing spondylitis-like joint disorder, suggesting that DCIR is involved in the regulation of bone metabolism. Given that defective DCIR is prone to autoimmune diseases and bone-related disorders, DCIR could be a potential therapeutic target that terminates excessive activation of immune responses through ITIM-mediated negative signaling. We are now trying to identify the receptors for this molecule.

## 5. HIDE1 deficient mice normally respond to collagen-induced arthritis and experimental autoimmune encephalomyelitis

**Kenji Shimizu, Toshimasa Kusaka, and Yoichiro Iwakura**

Previously, we searched for candidate genes responsible for the development of autoimmunity and arthritis using two mouse lines with spontaneous autoimmune arthritis, i.e. HTLV-I-*tax* Tg mice and *Il1rn*$^{-/-}$ mice. Among these genes, high expression genes of immature dendritic cell 1 (HIDE1) was found as a gene that is highly activated in these arthritic mice. HIDE1 is a type 1 membrane protein with an immunoglobulin-like domain in its extracellular region and no known motif in its intracellular region. This gene was expressed in dendritic cells, macrophages and neutrophil, but not in T cells and B cells.

Because HIDE1 is a member of the immunoglobulin super family and highly expressed in DCs under inflammatory conditions, we investigated the physiological functions of this molecule in the development of arthritis by generating HIDE1 deficient mice. These mice were fertile, were born at a normal mendelian ratio, and showed no obvious phenotypic abnormalities. We also found no apparent abnormalities in the cell populations of the spleen, lymph nodes, thymus, and bone marrow. We examined the susceptibility of HIDE1 deficient mice to collagen-induced arthritis. Contrary to our expectation, incidence and severity scores were normal. To further investigate the involvement of HIDE1 in other diseases, we induced experimental autoimmune encephalomyelitis and contact hypersensitivity. However, no difference was found in the incidence and severity of inflammation between wild type mice and HIDE1 deficient mice. Thus, we conclude that HIDE1 is dispensable for the development of inflammatory diseases.

## 6. Roles of IL-17A and IL-17F in host defense against bacteria, inflammatory colitis, and intestinal polyp formation

**Ce Tang, Motohiko Kadoki, Tomonori Kamiya, Yamato Sasaki, Shigeru Kakuta, and Yoichiro Iwakura**

IL-17A is a cytokine produced by Th17 cells and plays important roles in the development of allergic and autoimmune diseases. IL-17A also plays important roles in the host defense mechanism against bacteria and fungi. We showed that IL-17F, the highest homologous member to IL-17A in the IL-17 family, plays only marginal roles in the development of allergic or autoimmune diseases, but is equally important with IL-17A in the host defense against mucoepithelial bacterial infection, such as *Staphylococcus* and *Citrobacter*. Now, we are investigating the effects of IL-17F and IL-17A on naïve CD4$^{+}$ T cells and on Th1/Th17-type immune responses during *Myocobactia* infection. We are also analyzing the role of IL-17F in the induction of CD45RB$^{high}$CD4 T cell-induced colitis and the development of polyps in Apc$^{Min}$ mice.

## 7. Attempts to generate HIV-1 susceptible mice for AIDS research

**Takuya Tada, Motohiko Kadoki, and Yoichiro Iwakura**

Attempts to produce mouse models for AIDS have been hampered by species-specific host range barriers in mice. To overcome these barriers, we have generated Tg mice carrying the HIV-1 genome and showed that bacterium-derived LPS induces the activation of HIV-1 through the induction of TNF and IL-1 in splenocytes, while LPS directly activates HIV-1 gene expression in macrophages. In parallel with these studies, we also attempted to generate HIV-1 susceptible mice in which corresponding human genes responsible for the host range barriers were introduced into the mouse genome. Accordingly, we have generated Tg mice carrying the human CD4, CXCR4, CCR5, Cyclin T1, and CRM1 genes, all of which are suggested to be involved in the host range barrier. Although the initial viral infection steps, such as viral adsorption and uncoating, and viral transcription came to proceed efficiently in these Tg mice, these mice were still refractory to the infection of HIV-1, suggesting the involvement of additional host factors in the host range barrier. Regarding this, we previously showed that the efficiency of nuclear import of HIV-1 preintegration complex (PIC) was lower in

mouse cells and that this was due to inefficient nuclear entry of HIV-1 integrase (IN). Because we previously found that lens epithelium-derived growth factor transcription coactivator p75 (LEDGF/p75), a host factor that binds IN, is involved in the nuclear transport of IN, we examined if Tg mice carrying the human (h) LEDGF/p75 can overcome the host range barrier in mice. Nuclear translocation of GFP-IN was monitored using embryonic fibroblasts (MEF) of the Tg mice. We found that GFP-IN efficiently localized in the nucleus of hLEDGF/p75 Tg MEF compared with non-Tg mice. These results suggest that LEDGF/p75 is a critical host factor for HIV- IN nuclear import. The Tg mice carrying the human CD4, CXCR4, CCR5, Cyclin T1, CRM1, and LEDGF genes should give a better susceptibility to HIV-1 infection.

### 8. The role of glucocorticoids in pregnancy, parturition, lactation, and nurturing in melanocortin receptor 2-deficient mice

**Dai Chida[2,3], Keiko Miyoshi[4], Tsuyoshi Sato[5], Tetsuya Yoda[5], Takefumi Kikusui[6], and Yoichiro Iwakura[2]: [2]Center for Experimental Medicine and Systems Biology, Institute of Medical Science, University of Tokyo, [3]Department of Immunology and Pathology, Research Institute, National Center for Global Health and Medicine, [4]Institute of Health Biosciences, The University of Tokushima Graduate School, [5]Department of Oral and Maxillofacial Surgery, Saitama Medical University, [6]Companion Animal Research, Azabu University**

Maternal glucocorticoids are critical for fetal development, although overexpression can be deleterious. Previously, we established a mouse line deficient in melanocortin receptor 2 (MC2R). $MC2R^{-/-}$ mice have undetectable levels of corticosterone despite high levels of ACTH and defects resembling those in patients with familial glucocorticoid deficiency. In this study, we analyzed the role of glucocorticoids in pregnancy, parturition, lactation, and nurturing in $MC2R^{-/-}$ mice. $MC2R^{-/-}$ mice were fertile and produced normal litters when crossed with $MC2R^{+/+}$ mice. However, $MC2R^{-/-}$ females crossed with $MC2R^{-/-}$ males had no live births, and about 20% of the embryos at day 18.5 of pregnancy were of normal body size but were dead. $MC2R^{-/-}$ pregnant females crossed with $MC2R^{+/+}$ males had detectable serum corticosterone levels, suggesting transplacental passage of corticosterone from fetus to mother. $MC2R^{+/-}$ pups delivered from $MC2R^{-/-}$ dams crossed with $MC2R^{+/+}$ males thrived poorly with $MC2R^{-/-}$ dams, but grew to adulthood when transferred to a foster mother after birth, suggesting that $MC2R^{-/-}$ females are poor mothers or cannot nurse. Mammary glands were smaller in $MC2R^{-/-}$ females, with normal formation of alveoli and reduced expression of milk proteins. Myoepithelial cells, which force milk out of the alveoli, were fully developed and differentiated in $MC2R^{-/-}$ mammary glands. Pup retrieval behavior was intact in $MC2R^{-/-}$ mice. Exogenous corticosterone rescued mammary gland development in $MC2R^{-/-}$ females, and the pups of treated females grew to adulthood. Our results reveal the importance of glucocorticoids for fetal survival late in pregnancy, mammary gland development, and milk protein gene expression.

## Publications

### Original papers

1. Kautz-Neu, K., Kostka, S.L., Dinges, S., Iwakura, Y., Udey, M.C., and Von Stebut, E. IL-1 signaling is dispensable for protective immunity in Leishmania-resistant mice. *Exp. Dermatol*., 20, 76-78 (2011).
2. Shikama, Y., Kuroishi, T., Nagai, Y., Iwakura, Y., Shimauchi, H., Takada, H., Sugawara, S., and Endo, Y. Muramyldipeptide augments the actions of lipopolysacchairde in mice by stimulating macrophages to produe pro-IL-1B and by down-regulation of suppressor of cytokine signaling 1 (SOCS1). *Innate Immun*., 17, 54-59 (2011).
3. Hata, T., Takahashi, M., Hida, S., Kawaguchi, M., Kashima, Y., Usui, F., Morimoto, H., Nishiyama, A., Izawa, A., Koayama, J., Iwakura, Y., Taki, S., and Ikeda, U. Critical role of Th17 cells in inflammation and neovascularization after ischemia. *Cardiovascular Res*., 90, 364-372 (2011).
4. Cho, J.S., Zussman, J., Donegan, N.P., Ramos, R.I., Garcia, N.C., Uslan, D.Z., Iwakura, Y., Simon, S.I., Cheung, A.L., Modlin, R.L., Kim, J., and Miller, L.S. Noninvasive in vivo imaging to evaluate immune responses and antimicrobial therapy against *Staphylococcus aureus* and USA300 MRSA skin infection. *J. Invest. Dermatol*., 131, 907-915 (2011).
5. Murakami, M., Okuyama, Y., Ogura, H., Asano, S., Arima, Y., Tsuruoka, M., Harada, M., Kanamoto, M., Sawa, Y., Iwakura, Y., Takatsu, K., Kamimura, D., and Hirano, T.

Local microbleeding facilitates IL-6- and IL-17-dependent arthritis in the absence of tissue antigen recognition by activated T cells. *J. Exp. Med*., 208, 103-114 (2011).
6. Rivera, A., Hohl, T.M., Collins, N., Leiner, I., Gallegos, A., Saijo, S., Coward, J.W., Iwakura, Y., and Pamer, E.G. Dectin-1 diversifies *Aspergillus fumigatus*-specific T cell responses by inhibiting T helper type 1 CD4 T cell differentiation. *J. Exp. Med*., 208, 369-381 (2011).
7. Bhatia, S., Fei, M., Yarlagadda, M., Qi, Z., Akira, S., Saijo, S., Iwakura, Y., van Rooijen, N., Gibson, G.A., St Croix, C.M., Ray, A., and Ray, P. Rapid host defense against *Aspergillus fumigatus* involves alveolar macrophages with a predominance of alternatively activated phenotype. *PLoS One*, 6, e15943 (2011).
8. Andrews, D.M., Chow, M.T., Ma, Y., Cotterell, C.L., Watt, S.V., Anthony, D.A., Akira, S., Iwakura, Y., Trapani, J.A., Zitvogel, L., and Smyth, M.J. Homeostatic defects in interleukin 18-deficent mice contribute to protection against the lethal effects of endotoxin. *Immunol. Cell Biol*., 89, 739-746 (2011).
9. Tanaka, S., Kondo, H., Kanda, K., Ashino, T., Nakamachi, T., Sekikawa, K., Iwakura, Y., Shioda, S., Numazawa, S., and Yoshida, T. Involvement of interleukin-1 in lipololysaccharide-induced microglial activation and learning and memory deficits. *J. Neurosci., Res*., 31, 5699-5709 (2011).
10. Chida, D., Miyoshi, K., Sato, T., Yoda, T., Kikusui, T., and Iwakura, Y. The role of glucocorticoids in pregnancy, parturition, lactation, and nurturing in melanocortin receptor 2-deficient mice. *Endocrinology*, 152, 1652-1660 (2011).
11. Sharma, A.K., LaPar, D.J., Zhao, Y., Li, L., Lau, C.L., Kron, I.L., Iwakura, Y., Okusa, M. D., and Laubach, V.E. Natural killer T cell-derived IL-17 mediates lung ischemia-reperfusion injury. *Am. J. Respir. Crit. Care Med*., 183, 1539-1549 (2011).
12. Sharma, A.K., LaPar, D.J., Zhao, Y., Li, L., Lau, C.L., Kron, I.L., Iwakura, Y., Okusa, M. D., and Laubach, V.E. Natural killer T cell-derived IL-17 mediates lung ischemia-reperfusion injury. *Am. J. Respir. Crit. Care Med*., 183, 1539-1549 (2011).
13. Zhou, Y., Sonobe, Y., Akahori, T., Jin, S., Kawanokuchi, J., Noda, M., Iwakura, Y., Mizuno, T., and Suzumura, A. IL-9 promotes Th17 cell migration into the cetral nervous system via CC chemokine ligand-20 produced by astrocytes. *J. Immunol*., 186, 4415-4421 (2011).
14. Nakajima, K., Kanda, T., Takaishi, M., Miyoshi, K., Nakajima, H., Kamijima, R., Tarutani, M., Benson, J.M., Elloso, M.M., Iwakura, Y., DiGiovanni, J., and Sano, S. Distinct roles of IL-23 and IL-17 in the development of psoriasis-like lesions in a mouse model. *J. Immunol*., 186, 4481-4489 (2011).
15. Piao, J.H., Hasegawa, M., Heissig. B., Hattori, K., Takeda, K., Iwakura, Y., Okumura, K., Inohara, N., and Nakano, H. Tumor necrosis factor receptor-associated factor (Traf) 2 controls homeostasis of the colon to prevent spontaneous development of murine inflammatory bowel disease. *J. Biol. Chem*., 286, 17879-17888 (2011).
16. Fei, M., Bhatia, S., Oriss, T.B., Yarlagadda, M., Khare, A., Akira, S., Saijo, S., Iwakura, Y., Fallert Junecko, B.A., Reinhart, T.A., Foreman, O., Ray, P., Kolls, J., and Ray, A. TNF-α from inflammatory dendritic cells (DCs) regulates lung IL-17A/IL-5 levels and neutrophilia versus eosinophilia during persistent fungal infection. *Proc. Natl. Acad. Sci. USA*, 108, 5360-5365 (2011).
17. Besnard, A.G., Guillou, N., Tschopp, J., Erard, F., Couillin, I., Iwakura, Y., Quesniaux, V., Ryffel, B., and Togbe, D. NLRP3 inflammasome is required in murine asthma in the absence of aluminum adjuvant. *Allergy*, 66, 1047-1057 (2011).
18. Bernthal, N.M., Pribaz, J.R., Stavrakis, A.I., Billi, F., and Miller, J.S. Protective role of IL-1β against post-arthroplasty *Staphylococcus aureus* infection. *J. Orthopaedic Res*., 29, 1621-1626 (2011).
19. Madhur, M.S., Funt, S.A., Li, L., Vinh, A., Chen, W., Lob, H.E., Iwakura, Y., Blinder, Y., Rahman, A., Quyyumi, A.A., and Harrison, D.G. Role of interleukin 17 in inflammation, atherosclerosis, and vascular function in apolipoprotein E-deficient mice. *Arterioscler. Thromb. Vasc. Biol*., 31, 1565-1572 (2011).
20. Brereton, C.F., Sutton, C.E., Ross, P.J., Iwakura, Y., Pizza, M, Rappuoli, R., Lavelle, E.C., and Mills, K.H. *Escherichia coli* heat-labile enterotoxin promotes protective Th17 responses against infection by driving innate IL-1 and IL-23 production. *J. Immunol*., 186, 5896-5906 (2011).
21. Kamanaka, M., Huber, S., Zenewicz, L.A., Gagliani, N., Rathinam, C., O'Connor, W. Jr., Wan, Y.Y., Nakae, S., Iwakura, Y., Hao, L., and Flavell, R.A. Memory/effector (CD45 $RB^{lo}$) CD4 T cells are controlled directly by IL-10 and cause IL-22-dependent intestinal pathology. *J. Exp. Med*., 208, 1027-1040 (2011).
22. Kautz-Neu, K., Kostka, S.L., Dinges, S., Iwa-

kura, Y., Udey, M.C., and von Stebut, E.A role for leukocyte-derived IL-1RA in DC homeostasis revealed by increased susceptibility of IL-1RA-deficient mice to cutaneous leishmaniasis. *J. Invest. Dermatol*., 131, 1650-1659 (2011).
23. Qi, C., Cai, Y., Gunn, L., Ding, C., Li, B., Kloecker, G., Qian, K., Vasilakos, J., Saijo, S., Iwakura, Y., Yannelli, J.R., and Yan, J. Differntial pathways regulating innate and adaptive anti-tumor immune responses by particulate and soluble yeast-derived beta-glucans. *Blood*, 117, 6825-6836 (2011).
24. Shibata, K., Yamada, H., Sato, T., Dejima, T., Nakamura, M., Ikawa, T., Hara, H., Yamasaki, S., Kageyama, R., Iwakura, Y., Kawamoto, H., Toh, H., and Yoshikai, Y. Notch-Hes 1 pathway is required for the delopment of IL-17-producing γδ T cells. *Blood*, 118, 586-593 (2011).
25. Neveu, W.A., Bernardo, E., Allard, J.L., Nagaleekar, V., Wargo, M.J., Davis, R.J., Iwakura, Y., Whittaker, L.A., and Rincon, M. Fungal allergen β-glucans trigger p38 MAPK-mdediated IL-6 translation in lung epithelial cells. *Am. J. Respir. Cell Mol. Biol*., 45, 1133-1141 (2011).
26. Wakabayashi, C., Kiyama, Y., Kunugi, H., Manabe, T., and Iwakura, Y. Age-dependent regulation of depression-like behaviors through modulation of adrenergic receptor α, A subtype expression revealed by the analysis of interleukin-1 receptor antagonist knockout mice. *Neuroscience*, 192, 475-484 (2011).
27. Odobasic, D., Gan, P.Y., Summers, S.A., Semple, T.J., Muljadi, R.C., Iwakura, Y., Kitching, A.R., and Holdsworth, S.R. Interleukin-17A promotes early but attenuates established disease in crescentic glomerulonephritis in mice. *Am. J. Pathol*., 179, 1188-1198 (2011).
28. Esplugues, E., Huber, S., Gagliani, N., Hauser, A.E., Town, T., Wan, Y.Y., O'Connor, W., Rongvaux, A., Van Rooijen, N., Haberman, A.M., Iwakura, Y., Kuchroo, V. K., Kolls, J.K., Bluestone, J.A., Herold, K.C., and Flavell, R.A. Control of T(H)17 cells occurs in the small intestine. *Nature*, 475, 514-518 (2011).
29. Cantini, G., Pisati, F., Mastropietro, A., Frattini, V., Iwakura, Y., Finocchiaro, G., and Pellegatta, S.A critical role for regulatory T cells in driving cytokine profiles of Th17 cells and their modulation of glioma microenvironment. *Cancer Immunol. Immunotherapy*, 60, 1739-1750 (2011).
30. Hayakawa, Y., Sato-Matsushita, M., Takeda, K., Iwakura, Y., Tahara, H., and Irimura, T. Early activation and IFN-g production of tumor infiltrating mature CD27(high) NK cells. *Cancer Sci*., 102, 1967-1971 (2011).
31. Takahashi, H., Kouno, M., Nagao, K., Wada, N., Hata, T., Nishimoto, S., Iwakura, Y., Yoshimura, A., Yamada, T., Kuwana, M., Fujii, H., Koyasu, S., and Amagai, M. Desmoglein 3-specific $CD4^+$ T cells induce pemphigus vulgaris and interface dermatitis in mice. *J. Clin. Invest*., 121, 3677-3688 (2011).
32. Horie I, Abiru N, Sakamoto H, Iwakura Y, and Nagayama Y. Induciton of autoimmune thyroiditis by depletion of $CD4^+CD25^+$ regulatory T cells in thyroiditis-resistant IL-17, but not interferon-gamma receptor, knockout nonobese diabetic-H2h4 mice. *Endocrinology*, 152, 4448-4458 (2011).
33. Dejima, T., Shibata, K., Yamada, H., Hara, H., Iwakura, Y., Naito, S., and Yoshikai, Y.A protective role of naturally occuring IL-17A-producing γδT cells in the lung at the early stage of systemic cardidiasis in mice. *Infect. Immun*., 79, 4503-4510 (2011).
34. Nadeau, S., Filali, M., Zhang, J., Kerr, B.J., Rivest, S., Soulet, D., Iwakura, Y., de Rivero Vaccari, J.P., Keane, R.W., and Lacroix, S. Functional recovery after peripheral nerve injury is dependent on the proinflammatory cytokines IL-1β and TNF: implicaitons for neuropathic pain. *J. Neurosci*., 31, 12533-12542 (2011).
35. Itoh, S., Kimura, N., Axtell, R.C., Velotta, J. B., Gong, Y., Wang, X., Kajiwara, N., Nambu, A., Shimura, E., Adachi, H., Iwakura, Y., Saito, H., Okumura, K., Sudo, K., Steinman, L., Robbins, R.C., Nakae, S., and Fischbein, M.P. Interleukin-17 accelerates allograft rejection by suppressing regulatory T cell expansion. *Circulation*, 124, S187-196 (2011).
36. Yoshida, T., Yasumura, M., Uemura, T., Lee, S.J., Ra, M., Taguchi, R., Iwakura, Y., and Mishina, M. IL-1 receptor accessory protein-like 1 associated with mental retardation and autism mediates synapse formation by trans-synaptic interaction with protein tyrosine phosphatase d. *J. Neurosci*., 31, 13485-13499 (2011).
37. Yokoyama, K., Tezuka, T., Kotani, M., Nakazawa, T., Hoshina, N., Shimoda, Y., Kakuta, S., Sudo, K., Watanabe, K., Iwakura, Y., and Yamamoto, T. NYAP: a phosphoprotein family that links PI3K to WAVE1 signaling in neurons. *EMBO J*. 30, 4739-4754 (2011).
38. Xu, M., Morishima, N., Mizoguchi1, I., Chiba, Y., Fujita, K., Kuroda, M., Iwakura, Y., Cua, D.J., Yasutomo, K., Mizuguchi, J., and Yoshimoto, T. Regulation of the development of acute hepatitis by IL-23 through

IL-22 and IL-17 production. *Eur. J. Immunol*., 41, 2828-2839 (2011).
39. Kojima, M., Ashino, T., Yoshida, T., Iwakura, Y., and Degawa, M. Interleuikin-1 controls the constitute expression of the cyp7a1 gene by regulating the expression of cyp7a1 transcription regulators in the mouse liver. *Biol. Pharm. Bull*., 34, 1644-1647 (2011).
40. Satou, N., Ishihara, K., Hiratsuka, M., Tanaka, H., Endo, Y., Saito, S., Iwakura, Y., Leonard, W.J., and Hirasawa, N. Induction of thymic stromal lymphopoietin production by xylene and exacerbation of picryl chloride-induced allergic inflammation in mice. *Int. Arch. Allergy Immunol*., 157, 194-201 (2011).
41. Ashino, T., Sugiuchi, J., Uehara, J., Naito-Yamamoto, Y., Kenmotsu, S., Iwakura, Y., Shioda, S., Numazawa, S., and Yoshida, T. Auranofin protects against cocaine-induced hepatic injury through induction of heme oxygenase-1. *J. Toxicol. Sci*., 36, 635-643 (2011).
42. Yan, H., Kakuta, S., Nishihara, M., Sugi, M., Adachi, Y., Ohno, N., Iwakura, Y., and Tsuji, N.M. *Biosci. Biotechnol. Biochem*., 75, 2178-2183 (2011).
43. Oshiro, K., Kohama, H., Umemura, M., Uyttenhove, C., Inagaki-Ohara, K., Arakawa, T., Harada, M., Nakae, S., Iwakura, Y., Nishimaki, T., and Matsuzaki, G. Interleukin-17A is involved in enhancedment of tuor progression in murine intestine. *Immunobiology*, 217, 54-60 (2012).
44. Nakamura. Y., Terahara, M., Iwamoto, T., Yamada, K., Asano, M., Kakuta, S., Iwakura, Y., and Totsuka, M. Up-regulation of polymeric immunoglobulin receptor expression by the heat-inactivated potential probiotic Bifidocacterium bifidum OLB6378 in a mouse intestinal explant model. *Scand. J. Immunol*., 75, 176-183 (2012).
45. Seki, T., Kumagai, T., Kwansa-Bentum, B., Furushima-Shimogawara, R., Anyan, W. K., Miyazawa, Y., Iwakura, Y., and Ohta, N. IL-4/IL-13 suppress excessive neutrophil infiltration and hepatocyte damage during acute murine schistosomiasis japonica. *Infect. Immun*., 80, 159-168 (2012).
46. Nishimori, H., Maeda, Y., Teshima, T., Sugiyama, H., Kobayashi, K., Yamasuji, Y., Kadohisa, S., Uryu, H., Takeuchi, K., Tanaka, T., Yoshino, T., Iwakura, Y., and Tanimoto, M. Synthetic retinoid Am80 ameliorates chronic graft-versus-host disease by downregulating Th1 and Th17. *Blood*, 119, 285-295 (2012).
47. Nishimura, S., Manabe, I., Nagasaki, M., Kakuta, S., Iwakura, Y., Takayama, N., Ooehara, J., Otsu, M., Kamiya, A., Petrich, B., Urano, T., Kadono, T., Sato, S., Aiba, A., Yamashita, H., Sugiura, S., Kadowaki, T., Nakauchi, H., Eto, K., and Nagai, R. *In vivo* imaging visualizes discoid platelet aggregations without endothelium disruption and implicates contribution of inflammatory cytokine and integrin signaling. *Blood, in press*.
48. Danzaki, K., Matsui, Y., Ikesue, M., Ohta, D., Ito, K., Kanayama, M., Kurotaki, D., Morimoto, J., Iwakura, Y., Yagita, H., Tsutsui, H., and Uede, T. Interleukin-17A deficiency accelerates unstable atherosclerotic plaque formation in apolipoprotein E-deficeint mice. *Arterioscler. Thromb. Vasc. Biol*., 32, 273-280, (2012)
49. Kimizuka, Y., Kimura, S., Saga, T., Ishii, M., Hasegawa, N., Betsuyaku, T., Iwakura, Y., and Tateda, T. Roles of IL-17 in an experimental Legionella pneumonia model. *Infect. Immun*., in press.
50. Botelho, F.M., Bauer, C.M.T., Finch, D., Nikota, J.K., Zavitz, C.C.J., Kelly, A., Lambert, K.N., Piper, S., Foster, M.L., Goldring, J.J.P., Wedzicha, J.A., Bassett, J., Bramson, J., Iwakura, Y., Sleeman, M., Kolbeck, R., Coyle, A.J., Humbles, A.A., and Stämpfli, M. R. IL-1α/IL-1R1 expression in chronic obstructive pulmonary disease and mechanistic relevance to smoke-induced neutrophilia in mice. *PLoS ONE*, 6, e28457 (2011).
51. Liao, Y.H., Xia, N., Zhou, S.F., Tang, T.T., Yan, X.X., Lv, B.J., Nie, S.F., Wang, J., Iwakura, Y., Xiao, H., Yuan, J., Jevallee, H., Wei, F., Shi, G.P., and Cheng, X. Interleukin-17A contributes to myocardial ischemia/reperfusion injury by regulating cardiomyocyte apoptosis and neurtrophil infiltration. *J. Am. Coll. Cardiol*., 59, 420-942 (2012).
52. Washino, T., Moroda, M., Iwakura, Y., and Aosai, F. Toxoplasma gondii infection inhibits Th17-mediated spontaneous development of arthritis in IL-1 receptor atagonist-deficient mice. *Infect. Immun*., 2012 Jan 30. [Epub ahead of print].
53. Kojima, M., Ashino, T., Yoshida, T., Iwakura, Y., and Degawa, M. Involvement of interleukin-1 in lead nitrate-induced hypercholesterolemia in mice. *Biol. Pharm. Bull*., 35, 246-250 (2012).
54. Arima, Y., Harada, M., Kamimura, D., Park, J.-H., Kawano, F., Yull, F.E., Kawamoto, T., Iwakura, Y., Betz, U.A.K., Márquez, G., Blackwell, T.S., Ohira, Y., Hirano, T., and Murakami, M. Regional neural activation directly influences 1 immune signals in the CNS. *Cell*, 148, 447-457 (2012).
55. Shime, H., Matsumoto, M., Oshiumi, H., Tanaka, S., Nakane, A., Iwakura, Y., Tahara,

H., Inoue, N., and Seya, T. Toll-like receptor 3 signaling converts tumor-supporting myeloid cells to tumoricidal effectors. *Proc. Natl. Acad. Sci. USA*, 2012 Jan 19. [Epub ahead of print].
56. Monticone, S., Satoh, F., Giacchetti, G., Viola, A., Morimoto, R., Kudo, M., Iwakura, Y., Ono, Y., Turchi, F., Paci, E., Veglio, F., Boscaro, M., Rainey, W., Ito, S., and Mulatero, P. Effect of adrenocorticotropic hormone stimulation during adrenal vein sampling in rimary aldosteronism. *Hypertension*. 2012 Feb 13. [Epub ahead of print].
57. Yoshida, T., Shiroshima, T., Lee, S.-J., Yasumura, M., Uemura, T., Chen, X., Iwakura, Y., and Mishina, M. Interleukin-1 receptor accessory protein organizes neuronal synaptogenesis as a cell adhesion molecule. *J. Neurosci.*, in press.
58. Usui, F., Kimura, H., Ohshiro, T., Tatsumi, K., Kawashima, A., Nishiyama, A., Iwakura, Y., Ishibashi, S., and Takahashi, M. Interleukin-17 deficiency reduced vascular inflammation and development of atherosclerosis in western diet-induced ApoE-deficient mice. *Biochem. Biophys. Res. Commun.*, in press.
59. Ogawa, M., Yoshikawa, Y., Kobayashi, T., Mimuro, H., Fukumatsu, M., Kiga, K., Piao, Z., Ashida, H., Yoshida, M., Kakuta, S., Koyama, T., Goto, Y., Nagatake, T., Nagai, S., Kiyono, H., Kawalec, M., Reichhart, J.M., Sasakawa, C. A tecpr1-dependent selective autophagy pathway targets bacterial pathogens. Cell Host Microbe, 9, 376-389 (2011).

## Reviews

1. Drummond, R.A., Saijo, S., Iwakura, Y., and Brown, G.D. The Role of Syk/CARD9 coupled C-type lectins in antifungal immunity. *Eur. J. Immunol.*, 41, 276-281 (2011).
2. Ishigame, H., Nakae, S., and Iwakura, Y. The roles of IL-17A and IL-17F in mucosal infection and allergy. In "*Th17 cells in health and disease*", (ed. S. Jiang) (ISBN 978-1-4419-9370-0). Springer Science + Buisiness Media, LLC 2011, New York, NY, p. 269-297 (2011).
3. Iwakura, Y., Ishigame, H., Saijo, S., and Nakae, S. Functional specialization of IL-17 family members. *Immunity*, 34, 149-162 (2011).
4. Saijo, S., and Iwakura, Y. Dectin-1 and Dectin-2 in innate immunity against fungi. *Int. Immunol.*, 23, 467-472 (2011).
5. Iwakura, Y., Saijo, S., Nakae, S., Fujikado, N., Ishigame, H., and Murayama, M. Therapeutic targets for rheumatoid arthritis: lessons from animal models. *Arthritis Res. Ther.*, 14 (Suppl. 1), 018 (2012).

和文総説

1. 角田茂，岩倉洋一郎．遺伝子改変動物．新しい薬学事典．笠原忠，木津純子，諏訪俊男編集．朝倉書店．印刷中
2. 角田茂，西城忍．真菌免疫応答におけるC型レクチン受容体の役割．化学療法の領域 28(1)：59–66，2012．
3. 角田茂．リウマチ．論文ができてしまう！疾患モデルマウス表現型解析指南．山村研一，若菜茂晴編集．中山書店．pp. 316–321，451–454，2011．
4. 岩倉洋一郎．発刊にあたって．免疫疾患–疾患モデルの作製と利用(岩倉洋一郎編)．シリーズモデル動物利用マニュアル．エル・アイ・シー．2011．
5. 角田茂，岩倉洋一郎．IL-1．免疫疾患-疾患モデルの作製と利用(岩倉洋一郎編)．シリーズモデル動物利用マニュアル．エル・アイ・シー．215–222，2011．
6. 石亀晴道．IL-17．免疫疾患-疾患モデルの作製と利用(岩倉洋一郎編)．シリーズモデル動物利用マニュアル．エル・アイ・シー．295–303，2011．
7. 岩倉洋一郎．遺伝子改変マウス．"哺乳動物の分子生物学"を切り拓いた技術バイオテクノロジーの温故知新．実験医学．羊土社．29：1316–1320，2011．

*Center for Experimental Medicine and Systems Biology*

# Laboratory of Developmental Genetics
## システム疾患モデル研究センター　発生工学研究分野


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|---|---|---|---|---|---|
| Professor | Nobuaki Yoshida M.D., D.M.Sc. | | 教　授 | 医学博士 | 吉　田　進　昭 |
| Assistant Professor | Hirotake Ichise D.V.M., Ph.D. | | 助　教 | 獣医学博士 | 市　瀬　広　武 |
| Assistant Professor | Taeko Ichise Ph.D. | | 助　教 | 医学博士 | 市　瀬　多恵子 |
| Assistant Professor | Akinori Tokunaga Ph.D. | | 助　教 | 医学博士 | 徳　永　暁　憲 |
| Project Assistant Professor | Manabu Ozawa Ph.D. | | 特任助教 | 農学博士 | 小　沢　　学 |



*Gene targeting technology has revealed many aspects of gene functions in vivo. Knock out mice offer the opportunities of not only analyzing the complex gene functions in vivo, but also presenting various human disease models, where new therapeutic approaches can be explored. To allow more detailed dissection of gene function, we introduce a point mutation or disrupt genes in certain lineages (or stages) using Cre-loxP system, a method of conditional gene targeting. In the process of analyzing knock out mice, we have isolated spontaneous mutant mice which develop chylous ascites and edematous limbs. In order to understand the mechanism of lymphatic development and functions in more detail, we are also generating various knock-out/knock-in mouse lines including a conditional knock out mouse. In addition, we focus on analysis of neural development, aiming to understand the molecular mechanism of the maintenance of stemness and neural differentiation and to advance towards cell therapy of the damaged or degenerating nervous system. For this purpose, we are generating several conditional knock out mouse lines.*


### 1. Mastermind-like 1 (MamL1)/Mastermind-like 3 (MamL3) are essential for Notch signaling in vivo.


**Toshinao Oyama[1], Kenichi Harigaya[1], Nobuo Sasaki[2], Yoshiaki Okamura[2], Yumiko Saga[2], Katsuto Hozumi[3], Akiko Suganami[4], Yutaka Tamura[4], Takahiro Nagase[5], Hisashi Koga[5], Motoi Nishimura[6], Reiko Sakamoto, Mitsuharu Sato, Nobuaki Yoshida and Motoo Kitagawa[1]: [1]Department of Molecular and Tumor Pathology, Chiba University Graduate School of Medicine, [2]Division of Mammalian Development, National Institute of Genetics, [3]Department of Immunology, Tokai University School of Medicine, [4]Department of Bioinformatics, Chiba University Graduate School of Medicine, [5]Kazusa DNA Research Institute, [6]Department of Molecular Diagnosis, Chiba University Graduate School of Medicine.**


Mastermind (Mam) is one of the elements of Notch signaling, a system that plays a pivotal role in metazoan development. Mam proteins form transcriptionally activating complexes with the intracellular domains of Notch, which are generated in response to the ligand-receptor interaction, and CSL DNA-binding proteins. In mammals, three structurally divergent Mam isoforms (MamL1, MamL2 and MamL3) have been identified. There have also been indications that Mam interacts functionally with various other

transcription factors, including the p53 tumor suppressor, β-catenin and NF-κB. We have demonstrated previously that disruption of MamL1 causes partial deficiency of Notch signaling in vivo. However, MamL1-deficient mice did not recapitulate total loss of Notch signaling, suggesting that other members could compensate for the loss or that Notch signaling could proceed in the absence of Mam in certain contexts. Here, we report the generation of lines of mice null for MamL3. Although MamL3-null mice showed no apparent abnormalities, mice null for both MamL1 and MamL3 died during the early organogenic period with classic pan-Notch defects. Furthermore, expression of the lunatic fringe gene, which is strictly controlled by Notch signaling in the posterior presomitic mesoderm, was undetectable in this tissue of the double-null embryos. Neither of the single-null embryos exhibited any of these phenotypes. These various roles of the three Mam proteins could be due to their differential physical characteristics and/or their spatiotemporal distributions. These results indicate that engagement of Mam is essential for Notch signaling, and that the three Mam isoforms have distinct roles in vivo.

## 2. Polypyrimidine tract-binding protein regulates the cell cycle through IRES-dependent translation of CDK11p58 in mouse embryonic stem cells.

**Satona Ohno, Masaki Shibayama, Mitsuharu Sato, Akinori Tokunaga and Nobuaki Yoshida**

Polypyrimidine tract-binding protein (PTB/PTBP1/hnRNP I) is a member of the heterogeneous nuclear ribonucleoprotein family that binds specifically to pyrimidine-rich sequences of RNAs. Although PTB is a multifunctional protein involved in RNA processing and internal ribosome entry site (IRES)-dependent translation, the role of PTB in early mouse development is unclear. Ptb knockout mice exhibit embryonic lethality shortly after implantation and Ptb−/− embryonic stem (ES) cells have a severe proliferation defect that includes a prolonged G2/M phase. The present study shows that PTB promotes M phase progression by the direct repression of CDK11(p58) IRES activity in ES cells. The protein expression and IRES activity of CDK11(p58) in Ptb−/− ES cells is higher than that of wild-type ES cells, indicating that PTB is involved in the repression of CDK11(p58) expression through IRES-dependent translation in ES cells. Interestingly, CDK11(p58) IRES activity is activated by upstream of N-Ras (UNR) in 293T and NIH3T3 cells, whereas UNR is not present in the Cdk11 mRNA-protein complex in ES cells. In addition, PTB interacts directly with the IRES region of CDK11(p58) in ES cells. These results suggest that PTB regulates the precise expression of CDK11(p58) through direct interaction with CDK11(p58) IRES and promotes M phase progression in ES cells.

## 3. Obesity resistance and increased hepatic expression of catabolism-related mRNAs in Cnot3+/− mice.

**Masahiro Morita[7], Yuichi Oike[8,9], Takeshi Nagashima[10], Tsuyoshi Kadomatsu[9], Mitsuhisa Tabata[9], Toru Suzuki[7], Takahisa Nakamura[7], Nobuaki Yoshida, Mariko Okada[4] and Tadashi Yamamoto[7,11]: [7]Division of Oncology, Department of Cancer Biology, Institute of Medical Science, University of Tokyo, [8]PRESTO, Japan Science Technology Agency, [9]Department of Molecular Genetics, Graduate School of Medical Sciences, Kumamoto University, [10]Laboratory for Cellular System Modeling, RIKEN Research Center for Allergy and Immunology, [11]Cell Signal Unit, Okinawa Institute of Science and Technology.**

Obesity is a life-threatening factor and is often associated with dysregulation of gene expression. Here, we show that the CNOT3 subunit of the CCR4-NOT deadenylase complex is critical to metabolic regulation. *Cnot3*$^{+/-}$ mice are lean with hepatic and adipose tissues containing reduced levels of lipids, and show increased metabolic rates and enhanced glucose tolerance. *Cnot3*$^{+/-}$ mice remain lean and sensitive to insulin even on a high-fat diet. Furthermore, introduction of *Cnot3* haplodeficiency in *ob/ob* mice ameliorated the obese phenotype. Hepatic expression of most mRNAs is not altered in *Cnot3*$^{+/-}$ vis-à-vis wild-type mice. However, the levels of specific mRNAs, such as those coding for energy metabolism-related PDK4 and IGFBP 1, are increased in *Cnot3*$^{+/-}$ hepatocytes, having poly(A) tails that are longer than those seen in control cells. We provide evidence that CNOT3 is involved in recruitment of the CCR4-NOT deadenylase to the 3′ end of specific mRNAs. Finally, as CNOT3 levels in the liver and white adipose tissues decrease upon fasting, we propose that CNOT3 responds to feeding conditions to regulate deadenylation-specific mRNAs and energy metabolism.

## 4. Unc93B1 restricts systemic lethal inflammation by orchestrating TLR7- and TLR9-trafficking.

**Ryutaro Fukui[12], Shin-Ichiroh Saitoh[12], Atsuo Kanno[12], Masahiro Onji[12], Takuma Shibata[12,13],**

**Akihiko Ito[14], Morikazu Onji[15], Mitsuru Matsumoto[16], Shizuo Akira[17,18], Nobuaki Yoshida and Kensuke Miyake[12,13]: [12]Division of Infectious Genetics, Department of Microbiology and Immunology, [13]Laboratory of Innate Immunit. Laboratory, [14]Department of Pathology, Faculty of Medicine, Kinki University, [15]Department of Gastroenterology and Metabology, Ehime University, [16]Division of Molecular Immunology, Institute for Enzyme Research, University of Tokushima, [17]Laboratory of Host Defense, World Premier International Immunology Frontier Research Center, [18]Department of Host Defense, Research Institute for Microbial Diseases, Osaka University.**

Toll-like receptor-7 (TLR7) and 9, innate immune sensors for microbial RNA or DNA, have been implicated in autoimmunity. Upon activation, TLR7 and 9 are transported from the endoplasmic reticulum (ER) to endolysosomes for nucleic acid sensing by an ER-resident protein, Unc93B1. Little is known, however, about a role for sensor transportation in controlling autoimmunity. TLR9 competes with TLR7 for Unc93B1-dependent trafficking and predominates over TLR7. TLR9 skewing is actively maintained by Unc93B1 and reversed to TLR7 if Unc93B1 loses preferential binding via a D34A mutation. We here demonstrate that mice harboring a D34A mutation showed TLR7-dependent, systemic lethal inflammation. $CD4^+$ T cells showed marked differentiation toward T helper 1 (Th1) or Th17 cell subsets. B cell depletion abolished T cell differentiation and systemic inflammation. Thus, Unc93B1 controls homeostatic TLR7 activation by balancing TLR9 to TLR7 trafficking.

## 5. Fbxl10/Kdm2b deficiency accelerates neural progenitor cell death and leads to exencephaly.

**Tsuyoshi Fukuda, Akinori Tokunaga, Reiko Sakamoto and Nobuaki Yoshida**

Histone methylation is the important transcription regulatory system that affects mammalian development and cell differentiation. Alterations in epigenetic gene regulation are associated with disease. Fbxl10 (F-box and leucine-rich repeat protein 10) is a JmjC domain-containing histone demethylase. Although Fbxl10 has been implicated in cell cycle regulation, cell death, senescence, and tumorigenesis, these functions are controversial and its physiological function is unclear. To determine the in vivo function of Fbxl10, in this study, we generated a homozygous mutation in the mouse Fbxl10 gene. About half of Fbxl10-deficient mice exhibit failure of neural tube closure, resulting in exencephaly and die shortly after birth. Fbxl10 deficiency also causes retinal coloboma and a curled tail with low penetrances. Fbxl10 mRNA is specifically expressed in the cranial neural folds at E8.5 embryos, and apoptosis increased in the neuroepithelium and mesenchyme of Fbxl10-deficient E9.5 embryos, consistent with neural tube defects found in Fbxl10-deficient mice. Depletion of Fbxl10 induced the increased expression of p19ARF, an inducer of apoptosis, in E8.5 embryos and mouse embryonic fibroblast cells. In addition, the number of mitotic neural progenitor cells is significantly increased in the mutant E14.5 brain. Our findings suggest that the Fbxl10 gene makes important contributions to embryonic neural development by regulating cell proliferation and cell death in mice.

## 6. Understanding the mechanism of blood-lymphatic vascular separation

**Taeko Ichise, Nobuaki Yoshida, and Hirotake Ichise**

The lymphatic vasculature constitutes a highly specialized vascular system that is essential for fluid homeostasis, lipid absorption from the intestine, and immune cell trafficking. During embryonic development, lymphatic vessels originate from blood vessels, but become separated from blood vessels. Lymphatic and blood vessels are connected only in a few sites where lymph goes back to blood circulation. Recent studies have demonstrated that hematopoietic cells play an important role in blood-lymphatic vascular separation; however, hematopoietic cell types responsible for the separation have been debated, and mechanisms underlying the separation process remain largely unknown.

To gain an understanding of the vascular partitioning, we searched for the affected gene in a spontaneous mouse mutant exhibiting blood-filled lymphatic vessels, and identified a null mutation of the *Plcg2* gene, which encodes phospholipase Cγ2 (PLCγ2), by positional candidate cloning. Aberrant separation of blood and lymphatic vessels was observed in lethally irradiated wild-type mice reconstituted with PLCγ2-null bone marrow cells, as well as PLCγ2-null mice. These findings indicate that PLCγ2-expressing hematopoietic cells play an essential role in initiating and maintaining the separation of the blood and lymphatic vasculature.

We are presently using genetically engineered mouse models to address the issues of which type of hematopoietic cells is essential for the vascular separation, and how PLCγ2 is involved in the intracellular signalling which mediates

the separation.

## 7. Studies on the role of RNA splicing in neural development

**Akinori Tokunaga, Takayuki Shibasaki, Reiko Sakamoto, Mamiko Hamano, Nobuaki Yoshida**

We focus on analysis of neural development, aiming to understand the molecular mechanism of the maintenance of stemness and neural differentiation and to advance towards cell therapy of the damaged or degenerating nervous system.

### i) Analysis of PTB conditional knockout mouse.

PTB is a member of the heterogeneous nuclear ribonucleoprotein family that binds specifically to pyrimidine-rich sequences of RNAs. PTB is a multifunctional protein involved in RNA processing and internal ribosome entry site (IRES)-dependent translation, and preferentially expressed in neural stem cells (NSCs) in the central nervous system. Although RBPs are indispensable for the normal functions and cell migration of neurons, little is known about the role of RBPs in neural stem cells (NSCs). In vitro functional analysis of PTB in vertebrate neural cell have revealed that PTB has important roles on many alternative splicing events during neural cell differentiation. But it is still unknown whether self-renew and/or cell fate of NSCs are regulated by PTB in vivo. To explore the role of PTB in the early development of mouse brain, we inactivated the gene by employing a Nestin promoter-driven Cre-mediated conditional gene targeting system. We found that most mutant mice die by 10 weeks and almost all mutant mice developed a characteristic dome-like appearance of their heads. Histological analyses of PTB mutant brains revealed that these mice develop severe hydrocephalus. The cell polarity and adherens junction (AJ) of the apical ventricular surface in dorsal cortex were lost in a pacthy distribuion at E15.5. By E16.5, Tbr2-positive neural progenitor cells and neurons were observed in ventricular zone (VZ) of all AJ-negative spots, which leads depletion of VZ by E18.5. Thus postnatal maturation of ependymal cells with ciliary tufts from radial glia cells (RGCs) was disturbed, which may compromises cerebrospinal fluid dynamics and results in hydrocephalus. Our findings suggest that PTB is important for self-renew and/or cell fate of RGCs, through involving in maintenance of cell polarity and AJ in the dorsal neuroepithelium of lateral ventricles.

### ii) Analysis of PTB/nPTB conditional knockout mouse.

Neural polypyrimidine-tract-binding protein (nPTB), which is identified as a homologue of PTB, is one of RNA binding protein involved in the heterogeneous nuclear ribonucleoprotein (hnRNP) family. It is common knowledge that many genes are regulated by alternative splicing during neural development, and previous studies suggested that alternative splicing could contribute to the gene expression and functional diversity of isoforms. nPTB is expressed predominantly in the nervous system, muscle and testis. During neural development, expression of PTB is decreased along with differentiation of neural stem cells into neurons. In contrast, expression level of nPTB that was repressed by PTB is accordingly increased. Thus, tissue-specific RNA binding protein, nPTB may play an important role in neural development. But, functional differences between PTB/nPTB and meaning of developmental stage associated expression change from PTB to nPTB remain to be elucidated. In our study, we are generating nPTB conditional knockout mice to shed light on a role of nPTB in vivo. And now we are also analyzing the function of PTB/nPTB in vitro by using knockdown experiment via a formation of embryoid body (EB) from ES cells.

To address the function of PTB/nPTB, we generate and will analyze PTB/nPTB double knockout mouse.

## 8. Studies on epigenetic gene regulation in neural development

**Akinori Tokunaga, Eri Kawakami, Reiko Sakamoto, Nobuaki Yoshida**

The polycomb repressive complex (PRC) 1/2 proteins play a critical role in regulation of gene expression through modification of histone and chromatin structure. Histone modification is the important transcription regulatory system that affects mammalian development and cell differentiation. Alterations in epigenetic gene regulation are associated with disease. We aim to determine the mechanisms of epigenetic gene regulation and to understand their roles in neural development, and are focused on the regulatory genes: Fbxl10/11 and RYBP (Ring1A and YY1 binding protein).

We generated a homozygous mutation in the mouse Fbxl10 gene. About half of Fbxl10-deficient mice exhibit failure of neural tube closure, resulting in exencephaly and die shortly after birth. Fbxl10 deficiency also causes retinal coloboma and a curled tail with low pene-

trances. Fbxl10 mRNA is specifically expressed in the cranial neural folds at E8.5 embryos, and apoptosis increased in the neuroepithelium and mesenchyme of Fbxl10-deficient E9.5 embryos, consistent with neural tube defects found in Fbxl10-deficient mice. Depletion of Fbxl10 induced the increased expression of p19ARF, an inducer of apoptosis, in E8.5 embryos and mouse embryonic fibroblast cells. In addition, the number of mitotic neural progenitor cells is significantly increased in the mutant E14.5 brain. Our findings suggest that the Fbxl10 gene makes important contributions to embryonic neural development by regulating cell proliferation and cell death in mice. Fbxl11 is more broadly expressed, we are generating Fbxl10/11 double knockout mouse.

### 9. Functional analysis of histone demethylase Fbxl10 on male gamete development

**Manabu Ozawa, Reiko Sakamoto and Nobuaki Yoshida**

Histone methylation is one of the important epigenetic modifications of genome to orchestrate appropriate spatiotemporal gene expression for normal tissue development or differentiation. Fbxl10 is a histone dymethylase and catalyzes tri-methylated H3K4 and di-methylated H3K36, either of which modification is believed to repress adjacent gene expressions.

Gametogenesis is also under control of epigenetic modification, and status of DNA or histone modifications changes drastically during the development. Some previous studies using epigenetic gene-null mice showed infertile phenotypes. These results make us hypothesize that Fbxl10 null mice show abnormal gametogenesis. To test the hypothesis, we, in this year, have determined spermatogenesis in testis of the gene knockout mice (KO mice). KO mice at younger age (< 6month-old) have comparable number of sperms in epididymis as wild type control, whereas aged male (>1 year old) has lower number of sperms (almost 4-folds less than control). Layers of testicular germ cells in seminiferous tube are quite poor and spermatocytes after first meiosis are almost missing in the aged null mice. Layer of germ cells in seminiferous tube in the younger mice looks similar to the control, whereas distribution patterns of immature or meiotic germ cells are distinct from the control. We are now determining molecular mechanisms which cause the abnormal phenotype in the testis of Fbxl10 null mice.

## Publications

1. Oyama, T., Harigaya, K., Sasaki, N., Okamura, Y., Saga, Y., Hozumi, K., Suganami, A., Tamura, Y., Nagase, T., Koga, H., Nishimura, M., Sakamoto, R., Sato, M., Yoshida, N., Kitagawa, M.: Mastermind-like 1 (MamL1)/ Mastermind-like 3 (MamL3) are essential for Notch signaling in vivo. Development, 138: 5235-5246, 2011.
2. Ohno, S., Shibayama, M., Sato, M., Tokunaga, A. and Yoshida, N.: Polypyrimidine tractbinding protein regulates the cell cycle through IRES-dependent translation of CDK11p58 in mouse embryonic stem cells. Cell Cycle, 10: 3706-3713, 2011.
3. Morita, M., Oike, Y., Nagashima, T., Kadomatsu, T., Tabata, M., uzuki, T., Nakamura, T., Yoshida, N., Okada, M. and Yamamoto, T. : Obesity resistance and increased hepatic expression of catabolism-related mRNAs in Cnot3+/− mice. EMBO J. 30, 4678-4691, 2011.
4. Fukui, R., Saitoh, S-I., Kanno, A., Onji, M., Takuma Shibata, T., Itoh, A., Yoshid, N. and Kensuke Miyake.: Unc93B1 restricts systemic lethal inflammation by orchestrating TLR7- and TLR9-trafficking. Immunity, 35: 69, 2011.
5. Fukuda, T., Tokunaga, A., Sakamoto, R. and Yoshida, N.: Fbxl10/Kdm2b deficiency accelerates neural progenitor cell death and leads to exencephaly. Mol. Cell. Neurosci., 46: 614, 2011.

*Center for Experimental Medicine and Systems Biology*

# Laboratory of Innate Immunity
## 自然免疫研究分野

Professor Kensuke Miyake, M.D., Ph.D.

教 授 医学博士 三 宅 健 介

*Pathogen sensors, such as Toll-like receptor (TLR), play sentinel roles in detecting pathogenic ligands during infection and induce both innate and acquired immune responses. Meanwhile, excessive TLR responses are strongly associated with septic shock and autoimmune diseases. Immune system must strictly control TLR responses to avoid disruption of homeostasis, although molecular mechanisms involved in regulating those responses remain unclear. We have previously shown that TLR associating molecules, including MD-2, PRAT4A and Unc93B1, are essential for controlling TLR responses. To correctly understand coordinated TLR responses, our goal is to elucidate unknown molecular mechanism which is indispensable for appropriate TLR responses using genetically engineered mice.*

### 1. Roles of PRAT4A (PRotein Associated with Toll-like receptor 4) in immune modulation.

**Takuma Shibata[1,2], Yuji Motoi[1], Nobuaki Yoshida[2] and Kensuke Miyake[1,2]: [1]Division of Infectious Genetics, [2]Center for Experimental Medicine and Systems Biology, The Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minatoku, TOKYO 1208-8639, Japan.**

TLRs induce complex inflammatory responses that functions to protect the host from invading pathogens. But, in excessive or persistent inflammatory responses, hosts suffer the disadvantage of septic shock, delayed tissue repair and autoimmune diseases. To control the extent and dilated inflammation by TLRs, hosts evolved the multiple regulatory mechanisms, called tolerance.

We have previously reported that a novel TLR associating protein 'PRotein Associated with TLR4 (PRAT4A)', an endoplasmic reticulum resident protein, controls maturation and intracellular trafficking of multiple TLRs. PRAT4 A deficient Macrophages/Dendritic cells (DCs), with abnormal TLR distribution, showed impaired immune responses to TLR2/4/7/9 ligands, except to TLR3 ligand. As mRNA level of PRAT4A significantly decreased after stimulation by various TLR ligands in physiological state, PRAT4A deficiency presumably mimicked the tolerant state. These facts raised the possibility that PRAT4A play a central role in tolerance suppressing excessive inflammation by TLRs. Overexpression studies may be helpful in evaluating the tolerant effect of PRAT4A.

Furthermore, using established anti-PRAT4A monoclonal antibodies, we found secreted PRAT 4A which was detected in the serum for wild mice and the culture supernatant of Macrophages. There is another possibility that PRAT4 A is not only a TLR associating chaperone but also another immune modulator.

To further address PRAT4A function, we constructed Cre/loxP conditional transgenic mice by targeting ROSA26 locus which enables us to analyze cell type-specific overexpression studies *in vitro* and *in vivo*. Now we start to analyze conventional and conditional PRAT4A transgenic lines.

## 2. The meaning of intracellular localization of TLRs.

**Takuma Shibata[1,2], Yuji Motoi[1], Kensuke Miyake[1,2]: [1]Division of Infectious Genetics, [2]Center for Experimental Medicine and Systems Biology, The Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minatoku, TOKYO 1208-8639, Japan.**

TLR4/MD-2, a sensor for LPS, delivers the MyD88-dependent signal from the cell surface, then traffics to endolysosomes, and delivers the TRIF/TICAM-1-dependent signal. Both signals are thought to be dependent on cell surface TLR 4/MD-2. Although TLR4/MD-2 is located also in the recycling endosomes, the Golgi apparatus, or the endoplasmic reticulum (ER), little is known about what role for intracellular TLR4/MD-2 may play in LPS responses. Here we studied intracellular LPS-sensing in macrophages.

PRAT4A (protein associated with TLR4 A) is a cochaperone for the general chaperone gp96 and is required for cell surface expression of TLR4/MD-2. Cell surface TLR4/MD-2 was undetectable on either PRAT4A$^{-/-}$ thioglycollate-elicited peritoneal macrophages (P-Macs) or bone marrow derived macrophages (BM-Macs). LPS responses were all abolished in PRAT4A$^{-/-}$ P-Macs, whereas some LPS responses remained detectable in PRAT4A$^{-/-}$ BM-Macs. Of note, LPS responses in PRAT4A$^{-/-}$ BM-Macs were not necessarily dependent on TRIF / TICAM-1-signaling. PRAT4A$^{-/-}$ BM-Macs showed unimpaired production of both TRIF/TICAM-1-dependent chemokine RANTES (CCL5) and of MyD88-dependent chemokine MCP-1 (CCL2). Moreover, upregulation of the costimulatory molecules CD40 and CD86 was not altered. In contrast, TRIF/TICAM-1-dependent production of type I interferon was profoundly impaired. In response to heat-killed *E. coli* bacteria, BM-Macs also required PRAT4A independent TLR4/MD-2 for production of MCP-1 (CCL2) and RANTES (CCL5) and for upregulation of CD40 and CD86, indicating that intracellular TLR4/MD-2 is able to sense phagocytosed bacteria and activate immune responses. These results demonstrate that intracellular TLR4/MD-2 is responsible for a unique set of LPS responses.

## 3. Finding a novel tetraspanin protein involved in the negative regulation of multiple TLR responses.

**Takuma Shibata[1,2], Nobuaki Yoshida[2] and Kensuke Miyake[1,2]**

Multiple TLRs work in concert to sense a pathogen and mount defense responses. Little is known, however, about a mechanism coordinating multiple TLRs responses.

Under the screening of TLR2 regulating molecules, we found a novel tetraspanin protein which co-precipitated with TLR2 in immunoprecipitation assay.

Conditional knock out and Conditional transgenic mice using ROSA26 locus of this novel tetraspanin protein have been generated to allow rigorous assessment of its function. Further study is under way to reveal a role of this gene in immune responses.

## 4. Characterization of cleaved forms of TLR7 and TLR9

**Takuma Shibata[1,2], Atsuo Kanno[1], Masahiro Onji[1], Yuji Motoi[1], Ryutaro Fukui[1], Shin-Ichiroh Saitoh[1], Nobuaki Yoshida[2] and Kensuke Miyake[1,2]**

Vertebrates have TLR3, 7, 8 and 9 as sensors of microbial nucleic acids, however it is suggested that TLR7/9 responses strongly associate with autoimmune diseases owing to inappropriate recognition of self nucleic acid. In endolysosome, TLR7 and TLR9 recognize a single-stranded RNA and an unmethylated CpG motif in microbial DNA, respectively. TLR7/9 ordinarily reside in Endoplasmic Reticulum, and ligand stimulation enhance the trafficking of TLR7/9 to endolysosome. Such strict regulation of TLR7/9 subcellular localization seems to have a role for blocking self nucleic acid recognition. Previous reports indicated novel posttranscriptional modification in TLR7/9 that ectodomains of TLR7/9 were cleaved in endolysosome. It seemed that ectodomain cleavage in TLR7/9 represents another strategy to restrict excessive TLR7/9 activation.

Recently, we originally identified cleavage sites in TLR7 and TLR9. To clarify the meaning of ectodomain cleavage in TLR7/9, we constructed knock-in mice which load TLR7/9 without cleavage site. Now we start to analyze those Knock-in mice to reveal the meaning of cleavage in TLR7/9.

## 5. Roles for Unc93 homolog B1-dependent TLR7/9 balance in vivo

**Atsuo Kanno[1], Ryutaro Fukui[1], Shin-Ichiroh Saitoh[1], Takuma Shibata[1,2], Yuji Motoi[1], Nobuaki Yoshida[2] and Kensuke Miyake[1,2]**

Nucleic acid sensing Toll-like receptor 7 (TLR 7) and TLR9 recognize microbial RNA and

DNA, respectively. These TLRs potentially recognize self-derived nucleic acid and have been shown to have a role in autoimmune diseases. For maintenance of homeostasis, it is important to keep the responsiveness of the nucleic acid-sensing TLRs under the tight control.

Unc93 homolog B1 (Unc93B1) is reported to be indispensable for TLR7/9 responses. We have previously found that the alanine substitution for the 34$^{th}$ aspartic acid (D34A) of Unc93B1 enhanced TLR7 response but downregulated TLR9 response. These results suggest that TLR7 and TLR9 are reciprocally linked by Unc93B1, and the TLR7/TLR9 balance is biased towards TLR9 in the steady state.

To further clarify a role for Unc93B1-dependent TLR7/TLR9 balance *in vivo*, we started to generate knock-in mice harboring various types of mutations around D34 locus in the Unc93B1 gene which showed more accelerated phenotypes than D34A mutant mice. So far, we have finished germ line transmission for this knock in mice.

### 6. Identification of regulatory molecules for TLR responses and constructing genetically altered mice.

**Takuma Shibata[1,2], Yuji Motoi[1], Atsuo Kanno[1], Natsuko Yamakawa[1], Mabel Chan[1], Xiaobing Li[1], Yusuke Murakami[1], Hirotsugu Hatai[1], Ryutaroh Fukui[1], Shin-ichiroh Saitoh[1], Nobuaki Yoshida[2] and Kensuke Miyake[1,2]**

Except above projects, we try functional clonings and co-immunoprecipitation assay to comprehensively identify regulatory molecules associating with TLR responses. After simple screenings in cell lines, we constructed conditional knock out mice to reveal the physiological function of found novel molecules *in vivo*. So far, we found more than 20 candidate genes, and continued or finished to construct conditional knock out mice.

## Publications

Shibata, T., Motoi, Y., Tanimura, N., Yamakawa, N., Akashi-Takamura, S., and Miyake, K. Intracellular TLR4/MD-2 in macrophages senses Gram-negative bacteria and induces a unique set of LPS-dependent genes. Int Immunol 23: 503-10, 2011.

「モデル動物利用マニュアル」〈疾患モデルの作製と利用　免疫疾患〉岩倉洋一郎編集．Life Science Information Center．481−489，2011．

*Center for Experimental Medicine and Systems Biology*

# Laboratory of Cancer Cell signaling
## 発癌機構研究分野

Professor Tadashi Yamamoto, Ph.D

教 授 理学博士 山 本 雅

*Protein-tyrosine kinases are important not only for the development of malignant tumors but also for the regulation of growth and function of normal cells. We are interested in characterizing signal transduction downstream of protein-tyrosine kinases that are relevant to cancer development and to neuronal function.*

### Roles of Fyn kinase signaling in the brain development

**Naosuke Hoshina, Toru Suzuki*, Tadashi Yamamoto (*Division of Oncology)**

During the course of Fyn substrate screening, we have identified Protocadherin17 (PCDH17) as a potential target of the Fyn tyrosine kinase. Although several protocadherin members are involved in cancer progression, its physiological roles are largely unknown. To analyze physiological roles of specific protocadherin members *in vivo*, we focused on PCDH17. PCDH17 was specifically expressed in the brain, with high expression in the basal ganglia including striatum, globus pallidus, and substantia nigra. Developmentally, PCDH17 was highly expressed at the stage of synaptogenesis. Furthermore, PCDH17 and its family member, PCDH10 exhibited totally complementary expression patterns in the basal ganglia. At synapses, PCDH17 was localized in perisynaptic regions and showed calcium-dependent homophilic binding, but not heterophilic binding to PCDH10. To elucidate the function of PCDH17 *in vivo*, we generated PCDH17 knockout mice. With these mice, we are now studying the roles of PCDH17 in the basal ganglia circuitry using morphological, electrophysiological and behavioral approaches.

Expression levels of cadherin family proteins have been implicated in the progression of various cancers. Generally, tumors expressing lower levels of cadherin tended to exhibit higher invasive potentials. Recent reports suggested that silencing of PCDH17 or its closely related family member PCDH10 is involved in cancer malignancy. Therefore, we would like to examine whether mice lacking expression of PCDH17 are prone to develop tumors and/or whether deficiency of PCDH17 could promote tumor progression by introducing *pcdh17* homo-deficiency into cancer-prone mice harboring oncogenically mutated K-*ras*.

Database screening of the possible target of Src-family kinases identified a yet uncharacterized protein which we tentatively termed Bst. Bst specifically expressed in the brain and is well tyrosine phosphorylated in the basal ganglia. Physiological studies that include analysis of *bst* targeted mice are in progress.

*Center for Experimental Medicine and Systems Biology*

# Laboratory of Systems Biology
## システムズバイオロジー研究分野

Project Associate Professor Susumu Nakae, Ph.D.

特任准教授 農学博士 中 江 進

*Gene-modified mice are considered to be powerful tools for understanding of pathophysiological function of the targeted gene(s) in vivo. Our research focus is the understanding of pathogenesis of rejection and immune disorders such as allergy and autoimmunity using gene-modified mice.*

### Interleukin-17 accelerates allograft rejection by suppressing regulatory T cell expansion.

**Satoshi Itoh[1], Naoyuki Kimura[1], Robert C. Axtell[2], Jeffrey B. Velotta[1], Yong Gonquon[1], Xi Wang[1], Naoki Kajiwara[3,4], Aya Nambu[3,5], Eri Shimura[3], Hideo Adachi[6], Yoichiro Iwakura[7], Hirohisa Saito[4], Ko Okumura[3], Katsuko Sudo[8], Lawrence Steinman[2], Robert C. Robbins[1], Susumu Nakae[5,9], Michael P. Fischbein[1]: [1]Department of Cardiothoracic Surgery and [2]Department of Neurology and Neurological Sciences, Stanford University School of Medicine, USA, [3]Atopy Research Center, Juntendo University, [4]Department of Allergy and Immunology, National Research Institute for Child Health & Development, [5]Laboratory of Systems Biology and [7]Laboratory of Molecular Pathogenesis, Center for Experimental Medicine and Systems Biology, and [9]Frontier Research Initiative, Institute of Medical Science, University of Tokyo, [6]Department of Cardiovaschlar Surgery, Jichi Medical University, Saitama Medical Center.**

Interleukin-17 (IL-17), which is predominantly produced by Th17 cells distinct from Th1 or Th2 cells, participates in the pathogenesis of infectious, autoimmune and allergic disorders. However, the precise role in allograft rejection remains uncertain. In the present study, we investigated the role of IL-17 in acute allograft rejection using IL-17-deficient mice. Donor hearts from FVB mice were heterotopically transplanted into either C57BL/6J-IL-17-deficient (IL-$17^{-/-}$) or -wild-type (WT) mice. Allograft survival was significantly prolonged in IL-$17^{-/-}$ recipient mice due to reduced local inflammation accompanied by decreased inflammatory cell recruitment and cytokine/chemokine expression. IL-$17^{-/-}$ recipient mice exhibited decreased IL-6 production and reciprocally enhanced regulatory T cell (Treg) expansion, suggesting a contribution of Tregs to prolonged allograft survival. Indeed, allografts transplanted into anti-CD25 mAb-treated IL-$17^{-/-}$ recipient mice (Treg-depleted) developed acute rejection similar to WT recipient mice. Surprisingly, we found that gamma delta T cells rather than $CD4^+$ and $CD8^+$ T cells were key IL-17 producers in the allografts. In support, equivalent allograft rejection was observed in Rag-$2^{-/-}$ recipient mice engrafted with either WT or IL-$17^{-/-}$ $CD4^+$ and $CD8^+$ T cells. Finally, hearts transplanted into γδ T cell deficient mice resulted in decreased allograft rejection compared to WT control. In summary, during heart transplantation, (1) IL-17 is crucial for acceleration of acute rejection, (2)

IL-17-deficiency enhances Treg expansion, and (3) γδ T cells rather than $CD4^+$ and $CD8^+$ T cells are a potential source of IL-17. IL-17-neutralization may provide a potential target for novel therapeutic treatment for cardiac allograft rejection.

### Interleukin-16 deficiency suppresses the development of chronic rejection in murine cardiac transplantation model

**Satoshi Itoh[1], Naoyuki Kimura[1], Susumu Nakae[2], Robert C. Axtell[3], Jeffrey B. Velotta[1], Owen P. Palmer[1], Ernst Jan Bos[1], Xi Wang[1], Denis R. Merk[1], Claude M. Nagamine[4], Hideo Adachi[5], Hardy Kornfeld[6], Robert C. Robbins[1] and Michael P. Fischbein[1]: [1]Department of Cardiothoracic Surgery, [3]Department of Neurology and Neurological Sciences, [4]Department of Comparative Medicine, Stanford University School of Medicine, USA, [2]Frontier Research Initiative, Institute of Medical Science, University of Tokyo, [5]Department of Cardiovascular Surgery, Jichi Medical University, Saitama Medical Center, [6]Department of Pulmonary, Allergy & Critical Care Medicine, University of Massachusetts Medical School, USA.**

Interleukin-16 (IL-16), originally identified as a T cell chemoattractant factor, promotes the recruitment of various cells expressing CD4, a receptor for IL-16. The precise role of IL-16 in transplant rejection remains unknown. Therefore, the goal of the present study is to determine the contribution of IL-16 to the development of chronic rejection in heart transplants. Utilizing a murine heterotopic heart transplant model, C-H-$2^{bm12}$KhEg (H-$2^{bm12}$) donor hearts were transplanted into either (a) IL-16-deficient (IL-$16^{-/-}$) C57BL/6J or (b) wild type control recipients (MHC class II mismatch). Grafts were harvested 52 days after transplantation and then quantified morphometrically for GCAD. Graft infiltrating cells were detected 10 days and 52 days after transplantation, by flow cytometry and by immunohistochemistry, respectively. Intragraft cytokine and chemokine profiles were assessed with luminex technique. GCAD, or chronic rejection was significantly attenuated in donor hearts transplanted into IL-$16^{-/-}$ recipient mice compared with wild-type controls. More specifically, donor hearts transplanted into IL-$16^{-/-}$ recipients had a significant reduction in coronary artery luminal occlusion, intima-to-media ratio, and percentage of diseased vessels. GCAD reduction was associated with decreased donor organ inflammation, as well as donor organ cytokine (IL-1β and IL-6) and chemokine (MCP-1 and KC) protein expression. In summary, IL-16-deficiency resulted in the reduction of chronic rejection, graft inflammatory cell recruitment, and allograft inflammatory cytokine and chemokine production. Therefore, IL-16 neutralization may provide a potential target for novel therapeutic treatment for cardiac allograft rejection.

### N-acetylglucosaminyltransferase V-deficiency increases susceptibility to murine malaria

**Akiko Shibui[1,2], Junko Doi[3], Mohammed E.M. Tolba[1,4], Chiharu Shiraishi[2], Yoshitaka Sato[2], Shumpei Ishikawa[5], Junichi Watanabe[1], Sadao Nogami[3], Susumu Nakae[6,7], Sumio Sugano[1] and Nobumichi Hozumi[2,8]: [1]Department of Medical Genomics, Graduate School of Frontier Sciences, University of Tokyo, [2]Research Institute for Biological Sciences, Tokyo University of Science, [3]Department of Veterinary Medicine, Nihon University, [4]Department of Parasitology, Assiut University, Egypt, [5]Department of Pathology, Graduate School of Medicine, University of Tokyo, [6]Frontier Research Initiative, and [7]Laboratory of Systems Biology, Center for Experimental Medicine and Systems Biology, Institute of Medical Science, University of Tokyo, [8]Faculty of Health Sciences, Ryotokuji University.**

It is considered that several glycoproteins on erythrocytes in mammalian species are involved in malaria parasite infection. To elucidate the role of N-glycans on malaria parasite infection, we induced experimental murine malaria infection (using *Plasmodium berghei* ANKA) in mice deficient in N-acetylglucosaminyltransferase V (Mgat5), which is one of the enzymes involved in β1,6-GlcNAc N-glycan biosynthesis. After infection, *Mgat5*$^{-/-}$ mice showed severe body weight loss and parasitemia compared with wild-type mice. The *Mgat5*$^{-/-}$ mice, but not wild-type mice, also showed severe pathology accompanied by marked infiltration of plasma cells into the lungs and liver. These results suggest that β1,6 GlcNAc N-glycans on/in host erythrocytes may interfere with invasion of the parasites and progression to severe malaria.

## Publications

1. Ohno, T., Oboki, K., Morita, H., Kajiwara, N., Arae, K., Tanaka, S., Ikeda, M., Iikura,

M., Akiyama, T., Inoue, J., Matsumoto, K., Sudo, K., Azuma, M., Okumura, K., Kamradt, T., Saito, H. and Nakae, S. Paracrine IL-33 stimulation enhances lipopolysaccharide-mediated macrophage activation. PLoS One. 6: e18404, 2011.
2. Suzukawa, M., Nagase, H., Ogahara, I., Han, K., Tashimo, H., Shibui, A., Koketsu, R., Nakae, S., Yamaguchi, M. and Ohta, K. Leptin enhances survival and induces migration, degranulation and cytokine synthesis of human basophils. J Immunol. 186: 5254-5260, 2011.
3. Meguro, A., Ozaki, K., Hatanaka, K., Oh, I., Sudo, K., Ohmori, T., Matsu, H., Tatara, R., Sato, K., Sakata, Y., Nakae, S., Leonard, W.J., Ozawa, K. Lack of IL-21 signal attenuates graft-versus-leukemia effect in the absence of CD8 T-cells. Bone Marrow Transplant. 46: 1557-1565, 2011.
4. Shibui, A., Nambu, A., Shimura, E., Yamaguchi, S., Shiraishi, C., Sato, Y., Okumura, K., Sugano, S., Hozumi, N and Nakae, S. Alteration of immune responses by N-acetylglucosaminyltransferase V during allergic airway inflammation. Allergol Int. 60: 345-354, 2011.
5. Arae, K., Oboki, K., Ohno, T, Hirata, M., Nakae, S., Taguchi, T., Saito, H. and Nakajima, T. Cimetidine Enhances Antigen-Specific IgE and Th2 Cytokine Production. Allergol Int. 60: 339-344, 2011.
6. Kamanaka, M., Zenewicz, L.A., Huber, S., Gagliani, N., Rathinam, C., O'Connor W Jr., Wang, Y.Y., Nakae, S., Iwakura, Y., Hao, L. and Flavell, R.A. Memory/effector (CD45 $RB^{lo}$) CD4 T cells are controlled directly by IL-10 and cause IL-22 dependent intestinal pathology. J Exp Med. 208: 1027-1040, 2011.
7. Itoh, S., Kimura, N., Axtell, R.C., Velotta, J. B., Gong, Y., Wang, X., Kajiwara, N., Nambu, A., Shimura, E., Adachi, H., Iwakura, Y., Saito, H., Okumura, K., Sudo, K., Steinman, L., Robbins, R.C., Nakae, S.* and Fischbein, M.P.* Interleukin-17 accelerates allograft rejection by suppressing regulatory T cell expansion. Circulation. 124: S187-96, 2011. *equally corresponding authors
8. Shibui, A., Doi, J., Tolba, M.E.M., Shiraishi, C., Sato, Y., Ishikawa, S., Watanabe, J., Nogami, S., Nakae, S., Sugano, S. and Hozumi, N. N-acetylglucosaminyltransferase V-deficiency increases susceptibility to murine malaria. Exp Parasitol. 129: 318-321, 2011.
9. Kimura, N., Itoh, S., Nakae, S., Axtell, R.C., Velotta, J.B., Bos, E.J., Merk, D.R., Gong, Y., Okamura, H., Nagamine, C.M., Adachi, H., Cruikshank, W.W., Kornfeld, H., Robbins, R. C. and Fischbein, M.P. Interleukin-16 deficiency suppresses the development of chronic rejection in murine cardiac transplantation model. J Heart Lung Transplant. 30: 1409-1417, 2011.
10. Otsuka, A., Kubo, M., Honda, T., Egawa, G., Nakajima, S., Tanizaki, H., Kim, B., Matsuoka, S., Watanabe, T., Nakae, S., Miyachi, Y., and Kabashima, K. Requirement of interaction between mast cells and skin dendritic cells to establish contact hypersensitivity. PLoS One. 6: e25538, 2011.
11. Oshiro, K., Kohama, H., Umemura, M., Uyttenhove, C., Inagaki-Ohara, K., Arakawa, T., Harada, M., Nakae, S., Iwakura, Y., Nishimaki, T. and Matsuzaki, G. Interleukin-17A is involved in enhancement of tumor progression in murine intestine. Immunobiology. 217: 54-60, 2012.
12. Sawaguchi, M., Tanaka, S., Nakatani, Y., Harada, Y., Mukai, K., Matsunaga, Y., Ishiwata, K., Oboki, K., Kambayashi, T., Watanabe, N., Karasuyama, H., Nakae, S., Inoue, H. and Kubo, M. Role of mast cells and basophils in IgE responses and in allergic airway hyperresponsiveness. J Immunol. 188: 1809-1818, 2012.
13. Morita, H., Arae, K., Ohno, T., Kajiwara, N., Oboki, K., Matsuda, A., Suto, H., Okumura, K., Sudo, K., Takahashi, T., Matsumoto, K. and Nakae, S. ST2 requires Th2-, but not Th17-, type airway inflammation in epicutaneously antigen-sensitized mice. Allergol Int. in press.
14. Kimura, N., Nakae, S., Itoh, S., Merk, D.R., Wang, X., Gong, Y., Okamura, H., Chang, P. A., Adachi, H., Robbins, R.C., and Fischbein, M.P. Potential role of γδ T cell-derived IL-17 in acute cardiac allograft rejection. Ann Thorac Surg. in press.
15. Bonilla, W.V., Fröhlich, A., Senn, K., Kallert, S., Fernandez, M., Johnson, S., Kreutzfeldt, M., Hegazy, A.N., Schrick, C., Fallon, P.G., Klemenz, R., Nakae, S., Adler, H., Merkler, D., Löhning, M. and Pinschewer, D.D. The alarmin interleukin-33 drives protective antiviral $CD8^+$ T cell responses. Science. in press.
16. Iwakura, Y., Ishigame, H., Saijo, S. and Nakae, S. Functional specialization of interleukin-17 family members. Immunity. 34: 149-162, 2011.
17. Oboki, K., Nakae, S., Matsumoto, K. and Saito, H. IL-33 and airway inflammation. Allergy Asthma Immunol Res. 3: 81-88, 2011.
18. 志村絵理，中江　進．IL−17と接触過敏症．アレルギーの臨床．31：202−207，2011．
19. 南部あや，中江　進．IL−17と疾患．化学と生物．49：454−460，2011．
20. 南部あや，中江　進．(トピックス)M2マク

ロファージの分化機構の発見．実験医学．29：2657–2658，2011．
21. 中江　進．インターロイキン17：肺及び皮膚免疫における役割．表面．49：96–109，2011．
22. 中江　進．Th17細胞依存的なアレルギー疾患の発症機構の解明．アレルギア．40：83–86，2011．