Center for Experimental Medicine
Laboratory of Cell Biology

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: human T cell leukemia virus type I transgenic mouse model and IL-1 receptor antagonist knockout mouse model

Shinobu Saijo, Reiko Horai, Akiko Nakajima, Noriyuki Fujikado, Katsuko Sudo, Hiroaki Yamamoto[1], Masahide Asano, and Yoichiro Iwakura:
[1]Kohno Clinical Medicine Research Institute

Rheumatoid arthritis (RA) is one of the most serious medical problems world-wide with approximately 1% of the people in the world affected. The disease is autoimmune in nature and characterized by chronic inflammation of the synovial tissues in multiple joints that leads to joint destruction. High levels of autoantibodies in the serum and augmentation of proinflammatory cytokine expression in the joints are characteristics 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 developed originally. One is the human T cell leukemia virus type I (HTLV-I) transgenic (Tg) mouse model (Iwakura et al., 1991) and the other is IL-1 receptor antagonist (Ra) knockout (KO) mouse model (Horai et al., 1998). Both of these models develop autoimmunity and chronic inflammatory arthropathy closely resembling RA in humans.

To elucidate roles of IL-1 in the development of RA, effects of IL-1 deficiency were examined using HTLV-I Tg mouse model as well as type II collagen (IIC)-induced arthritis (CIA) model. The development of arthritis was markedly suppressed in IL-1$\alpha/\beta^{-/-}$ mice in both models. Deficiency of either IL-1α or IL-1β was enough to suppress the disease. However, HTLV-I-Tg mice eventually developed arthritis, suggesting functional substitution of the defect with other cytokines. Antibody production after immunization with IIC was normal in IL-1$\alpha/\beta^{-/-}$ mice in CIA model, while autoantibody levels against IgG and IIC were decreased in IL-1$\alpha/\beta^{-/-}$ HTLV-I Tg mice. In IL-1$\alpha/\beta^{-/-}$ mice, T cell proliferating response against IIC was greatly reduced in both CIA and HTLV-I models, suggesting inefficiency of T cell activation. Furthermore, we found that expression of CD40 ligand (CD40L) and OX40 on T cells was greatly reduced in IL-1$\alpha/\beta^{-/-}$ mice. These observations suggest that T cell activation by IL-1 is important for the development of autoimmunity and arthritis in these mice.

Then, we have analyzed effects of genetic background on the development of arthritis using IL-1Ra KO mice that spontaneously develop RA-like chronic inflammatory arthropathy. We found that the disease was observed in all the IL-1Ra KO mice on the BALB/c background, however, these mice on the C57BL/6 background developed arthritis in lower incidence and older age. We have generated congenic mice in which only MHC class II locus was changed, and incidence of arthritis was compared. Although the development of arthritis on a BALB.B (H-2b) background was slightly delayed compared with a BALB/c (H-2d) background, it was clearly shown that the MHC locus was not responsible for the development of arthritis. We are now further analyzing the pathogenesis of the development of autoimmunity in IL-1Ra KO mice by generating bone marrow chimeras and by intercrossing with cytokine deficient mice.

2. Studies on the roles of IL-1 in the development of autoimmune diseases

Susumu Nakae, Aya Nambu, Yutaka Komiyama, Reiko Horai, Katsuko Sudo, Nobuo Sakaguchi[2], Masahide Asano, and Yoichiro Iwakura:[2]Department of Immunology, Kumamoto University School of Medicine

IL-1 is a proinflammatory cytokine that plays pleiotropic roles in host defense mechanisms. IL-1 is consisted of two molecules, IL-1α and IL-1β, and IL-1 Ra is a natural inhibitor of these molecules. Although adjuvant effects of exogenously administered IL-1 in humoral immune response is well known, roles of endogenous IL-1 and functional discrimination between IL-1α and IL-1β have not been elucidated completely. This year, we investigated the role of IL-1 in humoral immune response using gene-targeted mice. Both primary and secondary Ab production against T-dependent antigen, sheep red blood cells (SRBC), was significantly reduced in IL-1α/β$^{-/-}$ mice, and enhanced in IL-1Ra$^{-/-}$ mice. The intrinsic functions of B cells such as Ab production against type1 T-independent antigen, trinitrophenyl (TNP)-LPS, and proliferative responses against mitogenic stimuli were normal in IL-1α/β$^{-/-}$ mice. Proliferative response of T cells and cytokine production upon stimulation with anti-CD3 mAb were also normal, as was the phagocytotic ability of antigen-presenting cells (APCs). However, SRBC-specific proliferative response and cytokine production of T cells through the interaction with APCs were markedly impaired in IL-1α/β$^{-/-}$ mice, and enhanced in IL-1Ra$^{-/-}$ mice. Moreover, we show that SRBC-specific Ab production was reduced in IL-1β$^{-/-}$ mice, but not in IL-1α$^{-/-}$ mice. These results show that endogenous IL-1β, but not IL-1α, is involved in T cell-dependent Ab production, and IL-1 promotes the antigen-specific T cell helper function through the T cell-APC interaction.

Then, we investigated roles of IL-1 in the activation of T cells using an OVA-specific T cell receptor Tg mouse, DO11.10. We found that IL-1$^{-/-}$ APCs did not fully activate DO11.10 T cells, while IL-1Rα$^{-/-}$ APCs enhanced the reaction, indicating IL-1 promotes T cell-priming through T-APC interaction. The function of IL-1 was CD28-CD80/CD86-independent. We found that CD40L and OX40 expression on T cells were affected by the mutation, and the reduced antigen-specific B cell response in IL-1$^{-/-}$ mice was recovered by the treatment with agonistic anti-CD40 mAb both *in vitro* and *in vivo*. These observations indicate that IL-1 enhances T cell-dependent Ab production by augmenting CD40L and OX40 expression on T cells. We are also analyzing roles of IL-1 in the development of contact hypersensitivity, delayed-type hypersensitivity, airway hypersensitivity, graft-vs-host reaction, and autoimmune bowel disease.

3. Studies on the roles of IL-1 during stress response and in maintaining homeostasis of the body

Taizo Matsuki, Dai Chida, Kyoko Kagiwada, Satoru Ishii, and Yoichiro Iwakura

It is known that IL-1 plays important roles not only in the immune system but also in the central nervous system. We previously reported that IL-1 is crucial for the development of fever upon inflammation and glucocorticoid secretion upon stress response (Horai et al., 1998). We are now investigating the signal transduction mechanisms in the brain during stress response using specific cytokine gene knockout mice and cytokine receptor transgenic mice.

We also fond that the growth and the appetite were suppressed in IL-1Ra-deficient mice. Furthermore, we found that lipid metabolism was abnormal in these mice, causing leanness in these mice. We are now analyzing the mechanism regulating lipid metabolism.

4. Importance of IL-1 and TNF-α in physiological bone development

Hisataka Yasuda, Young-Mi Lee, Atsuko Minamida, and Yoichioro Iwakura

We previously molecular cloned osteoclastogenesis-inhibitory factor (OCIF) (also called OPG), osteoclast differentiation factor (ODF; also called OPGL, TRANCE, and RANKL), and receptor activator of NF-κB (RANK), all of which are important for regulating osteoclast differentiation and activation. ODF/RANKL is a member of the membrane-associated TNF ligand family and it induces osteoclast differentiation from progenitor cells co-treated with M-CSF in the absence of osteoblasts/stromal cells and osteotropic factors. ODF/RANKL is a long-sought ligand expressed on osteoblasts/stromal cells in response to osteotropic factors, and it mediates an essential signal to osteoclast progenitors for their differentiation into active osteoclasts. OCIF/OPG is a secreted member of the TNFR family, and it inhibits osteoclastogenesis *in vitro* and *in vivo*. RANK is the signaling receptor essential for ODF/RANKL-mediated osteoclastogenesis, and that OCIF/OPG acts as a decoy receptor for ODF/RANKL to compete against RANK. The discovery of ODF/RANKL, OCIF/OPG, and RANK opens a new era in the investigation of the regulation of osteoclast differentiation/function. Even though molecular mechanism of osteoclast differentiation and activation is almost clarified, factors (e.g. cytokines and hormones) regulating the expression of ODF/RANKL, OCIF/OPG, and RANK *in vivo* are not well studied.

Inflammatory cytokines such as IL-1 and TNF α play a major role in bone resorption in pathological conditions (e.g. rheumatoid arthritis and periodontal diseases). IL-1 and TNF α also regulate the expression of ODF/RANKL and OCIF/OPG *in vitro*. However, the roles of these cytokines in bone development in physiological conditions are unknown. In addition, the relationship between inflammatory cytokines and ODF/RANKL or OCIF/OPG is not known in physiological conditions. Previous studies demonstrated that no obvious abnormality in bone in IL-1 receptor type I (IL-1R1) KO mice and TNFα receptor type I (TNFR1) KO mice whose genetic background were C57BL/6 x 129/SV. We addressed the role of IL-1 and TNF α in physiological bone remodeling using IL-1αβ, double KO mice, TNF- α KO mice, and IL-1αβ, TNFα triple KO mice, all of which were backcrossed to BALB/cA strain mice for 8 generations. Measurement of Bone Mineral Density (BMD) of femur with dual energy X-ray absorptiometry revealed significant increases in 8-week old mice with each genotype. Radiographs showed massive increase in bone density especially in the epiphysis and metaphysis of femur of these KO mice. Histological analysis also showed that marked increase of bone volume in trabecular bone of these KO mice. The thickness of cortical bone also was increased in these KO mice. The morphology of the growth plate and the columnar organization of chondrocytes are normal, but cartilaginous remnants were markedly observed in the cortical bone of these KO mice, which suggests a decrease in osteoclastic activity in resorption of bone and cartridge. Taken together these results indicate that IL-1 and TNF α may have an important role in physiological bone development and their effects seem to be synergistic. The mechanism by which IL-1 or TNFα KO mice have increased bone mass is under investigation.

5. Studies on AIDS models: mechanisms of T cell depletion and roles of chemokine receptor

Byung-Il Choi, Motohiko Kadoki, Jiro Yasuda, Tamaki Miyao, Masakazu Kamata[3], Yoko Aida[3], and Yoichiro Iwakura:[3]RIKEN Tsukuba Institute

We have been studying pathogenesis of AIDS using transgenic HIV infection models. We have produced Tg mice carrying the reverse transcriptase-deficient HIV genome and showed that these mice were a good model for healthy carriers in which viral gene expression was induced by various stimuli. In this study, we have produced another Tg mice carrying the Vpr gene, an accessory protein gene of HIV, with CD4 enhancer/promoter in order to investigate its pathogenicity *in vivo*, since Vpr is known to affect viral replication as well as cell growth, differentiation and apoptosis *in vitro*. Interestingly, apoptotic death of T lymphocytes was enhanced in those mice, causing marked reduction of T cells in lymphatic organs and peripheral blood. Involvement of Bcl-x, Bax and Caspase-1, but not of the Fas-Fas ligand system, was suggested in the apoptotic processes. These observations suggest that Vpr is involved in the pathogenesis of T cell depletion in HIV-infected people.

We are also studying roles of chemokines in the development of AIDS, because some of them provide co-receptors for HIV. For this, we have generated KO mice for CCR8 and conditional KO mice using Cre-lox P system for CXCR4 gene, because CXCR4 null mice are embryonic lethal. Both of these mice were born healthy, and we are now analyzing effects on the immune system using various disease models and effects on HIV replication by crossing with HIV Tg mice.

Recently, it has been reported that over expression of hu-CRM1 in murine cells rescued Rev function. So we are now generating mice transgenic for hu-CRM1 to overcome inefficiency of unspliced HIV-1 RNA transport in murine cells. We are planning to intercross these mice with HIV Tg mice to analyze the pathogenicity of the virus, and also with hu-CD4 and co-receptor transgenic mice to examine efficiency of viral replication in this animal.

6. Inhibition of B16 melanoma experimental metastasis by interferon-γ through direct inhibition of cell proliferation and activation of anti-tumor host mechanisms

Shigeru Kakuta, Yoh-ichi Tagawa, Shinwa Shibata, Masanobu Nanno[4], and Yoichiro Iwakura: [4]Yakult Central Institute for Microbiological Research

IFN-γ has pleiotropic activities other than the antivirus action, including cell growth inhibition, NK cell and cytotoxic T lymphocyte activation, and angiogenesis inhibitory activity, and these activities are supposed to be involved in its anti-tumor activity. It is not elucidated completely, however, which activity is mainly involved in the tumor suppression *in vivo*. In this study, we analyzed inhibitory mechanisms of endogenous IFN-γ against B16 melanoma experimental metastasis. Tumor deposits to the lungs and liver were increased and the life span was shorter in IFN-$\gamma^{-/-}$ mice, indicating crucial roles of IFN-γ in the anti-tumor mechanisms. Interestingly, tumor deposits were not increased in IFN-γ receptor $(R)^{-/-}$ mice. Furthermore, only low levels of tumor-specific CTL development and activation of NK cells were observed, indicating that anti-metastatic effects of IFN-γ is not mediated by host cells. The survival period of B16 melanoma-bearing IFN-$\gamma R^{-/-}$ mice was, however, shorter than wild-type mice. These observations suggest that IFN-γ prevents B16 melanoma experimental metastasis by directly inhibiting the

cell growth, although anti-tumor host functions may also be involved in later phase.

7. Cloning of a novel 2',5'-oligoadenylate synthetase-like molecule, Oasl5 in mice

Shinwa Shibata, Shigeru Kakuta, Kazushige Hamada[5], Yoshihiro Sokawa[5] and Yoichiro Iwakura: [5]Department of Biotechnology, Kyoto Institute of Technology

The 2′,5′-oligoadenylate synthetase (2-5OAS) is a enzyme that catalyzes synthesis of 2′,5′-oligoadenylates (2-5A) in a dsRNA-dependent manner, and known as a major component of the IFN-induced host defense mechanisms against microbial infections. Here, we report the presence of a novel 2-5OAS-like molecule, termed Oasl5, in mice. The size of *Oasl5* cDNA was about 2 kb and encoded a protein consisting of 362 aa. The amino acid sequence showed 76% similarity to the mouse 2-5OAS, however, several motifs being important for the enzyme activity were not conserved. The *Oasl5* mRNA was most significantly expressed in the brain, and relatively weak expression was found in other organs such as the spleen, kidney, ovary and testis. It was also expressed in embryonic stem (ES) cells. The *Oasl5* mRNA expression in ES cells was elevated 5-fold after treatment with IFN and about 2-fold in the brain when stimulated with IFN inducer, polyinosinic-polycytidylic acid (poly[I:C]). *In situ* hybridization analysis revealed that *Oasl5* is expressed in neurons in the central nervous system in adult mice. When *Oasl5* was expressed in *E. coli*, it yielded 42 kDa protein that binds to dsRNA, but it did not show oligoadenylate synthetase activity. These findings suggest a novel function of Oasl5, which are independent of oligoadenylate synthetase activity, in the brain and developing embryos.

8. Reprogramming of somatic stem cell by aggregation with mouse embryo

Chie Soeta and Yoichiro Iwakura

Successful production of cloned animals by nuclear transplantation has demonstrated that maternal cytoplasmic factors are capable of reinitialize differentiated somatic cells into undifferentiated state. Moreover, it was shown recently that adult somatic stem cells (haematopoetic stem cells, neural stem cells, etc.) had still high developmental potential. These stem cells could differentiate into all the three embryonic germ layers when they formed chimeras with normal blastocysts, suggesting that microenvironment is important for the cells to differentiate. We are now trying to find conditions that induce reprogramming of the lineage-specific somatic stem cells.

Publications

Takeda, K., Hayakawa, Y., Smyth, M. J., Kayagaki, N., Yamaguchi, N., Kakuta, S., Iwakura, Y., Yagita, H., and Okumura, K. Involvement of tumor necrosis factor-related apoptosis-inducing ligand in surveillance of tumor metastasis by liver natural killer cells. *Nature Medicine*, **7**, 94-100 (2001).

Fukamauchi, F., Aihara, O., Wang, Y. J., Akasaka, K., Takeda, Y., Horie, M., Kawano, H., Sudo, K., Asano, M., Watababe, K., and Iwakura, Y. TAG-1-deficient mice have marked elevation of adenosine A1 receptors in the hippocampus. *Biochem. Biphys. Res. Commun.,* **281**, 220-226 (2001).

Seino, K., Fukao, K., Muramoto, K., Yanagisawa, K., Takada, Y., Kakuta, S., Iwakura, Y., Van Kaer, L., Takeda, K., Nakayama, T., Taniguchi, M., Bashuda, H., Yagita, H., and Okumura, K. Requirement for NKT cells in the induction of allograft tolerance. *Proc. Natl. Acad. U. S. A.,* **98**, 2577-2581 (2001).

Ishii, T., Serizawa, S., Kohda, A., Nakatani, H., Shiroishi, T., Okumura, K., Iwakura, Y., Nagawa, F., Tsuboi, A., and Sakano, H. Projection of three subsets of olfactory neurons expressing the odorant receptor transgene and the two cognate endogenous alleles. *Genes to Cell*, **6**, 71-78 (2001).

Naruse-Nakajima, C., Asano, M., and Iwakura, Y. Involvement of EphA2 in the formation of the tail notochord via interaction with ephrinA1. *Mecha. Develop.,* **102**, 95-105 (2001).

Ozaki, H., Watanabe, Y., Takahashi, K., Kitamura, K., Tanaka, A., Urase, K., Momoi., T., Sudo, K., Sakagami, J., Asano, M., Iwakura, Y., and Kawakami, K. *Six-4*, a putative *myogenin* gene regulator, is not essential for mouse embryonal development. *Mol. Cell. Biol.,* **21**, 3343-3350 (2001).

Norose, K., Mun, H. S., Aosai, F., Chen, M., Hata, H., Tagawa, Y., Iwakura, Y., Yano, A. Organ infectivity of *Toxoplasma gondii* in interferon-γ knockout mice. *J. Parasitol.,* **87**, 447-52 (2001).

Yoneto, T., Waki, S., Takai, T., Tagawa, Y., Iwakura, Y., Mizuguchi, J., Nariuchi, H., and Yoshimoto, T. A critical role of Fc receptor-mediated antibody-dependent phagocytosis in the host resistance to blood-stage *Plasmodium berghei* XAT infection. *J. Immunol.,* **166**, 6236-6241 (2001).

Hayakawa, Y., Takeda, K., Yagita, H., Kakuta, S., Iwakura, Y., Van Kaer, L., Saiki, I., and Okumura, K. Critical contribution of IFN-γ and NK cells, but not perforin-mediated cytotoxicity, to anti-metastatic effect of alpha-galactosylceramide. *Eur J*

Immunol., **31**, 1720-1727 (2001).

Shibata, S., Kakuta, S., Hamada, K., Sokawa, Y., and Iwakura, Y. Cloning of a novel 2', 5'-oligoadenylate synthetase-like molecule, Oasl5 in mice. *Gene*, **271**, 261-271 (2001).

Nakae, S., Asano, M., Horai, R., Sakaguchi, N., and Iwakura, Y. IL-1 enhances T cell-dependent antibody production through induction of CD40L and OX40 on T cells. *J. Immunol.*, **167**, 90-97 (2001).

Yasuda, J., Miyao, T., Kamata, M., Aida, Y., and Iwakura, Y. T cell apoptosis causes peripheral T cell depletion in mice transgenic for the HIV-1 *vpr*. *Virology*, **285**, 181-192 (2001).

Kotani, N., Asano, M., Iwakura, Y., and Takasaki, S. Knockout of mouse β1,4-galactosyltransferase-1 gene results in a dramatic shift of outer chain moieties of N-glycans from type 2 to type 1 chains in hepatic membrane and plasma glycoproteins. *Biochem J.*, **357**, 827-834 (2001).

Boutin, H., LeFeuvre, R. A., Horai, R., Asano, M., Iwakura, Y., and Rothwell, N. Role of IL-1α and IL-1β in ischaemic brain damage. *J. Neuroscience*, **21**, 5528-5534 (2001).

Morita, Y., Maravei, D. V., Bergeron, L., Wang, S., Perez, G. I., Tsutsumi, O., Taketani, Y., Asano, M., Horai, R., Korsmeyer, S. J., Iwakura, Y., Yuan, J., and Tilly, J. L. Caspase-2 deficiency prevents programmed germ cell death resulting from cytokine insufficiency but not meiotic defects caused by loss of ataxia telangiectasia-mutated (Atm) gene function. *Cell Death Differ.*, **8**, 614-620 (2001).

Sato, M., Chamoto, K., Tsuji, T., Iwakura, Y., Togashi, Y., Koda, T., and Nishimura, T. Th1 cytokine-conditioned bone marrow-derived dendritic cells can bypass the requirement for Th functions during the generation of CD8(+) CTL. *J. Immunol.*, **167**, 3687-3691 (2001).

Imaizumi, Y., Mizushima, H., Matsumoto, H., Dohi, K., Motsumoto, K., Ohtaki, H., Funahashi, H., Matsunaga, S., Horai, R., Asano, M., Iwakura, Y., and Shioda, S. Increased expression of interleukin-1β in mouse hippocampus after global cerebral ischemia. *Acta Histochem. Cytochem.*, **34**, 357-362 (2001).

Dal, J. H., Iwatani, Y., Ishida, T., Terunuma, H., Kasai, H., Iwakura, Y., Fujiwara, H., and Ito, M. Glycyrrhizin enhances interleukin-12 production in peritoneal macrophage. *Immunology*, **103**, 235-243 (2001).

Miura, T., Kudo, T., Matsuki, A., Sekikawa, K., Tagawa, Y., Iwakura, Y., Nakane, A. Effect of 6-Hydroxydopamine on host resistance against Listeria monocytogenes infection. *Infect. Immun.*, **69**, 7234-7241 (2001).

Le Feuvre, R. A., Brough, D., Iwakura, Y., Takeda. K., Rothwell, N. J. Priming of macrophages with LPS potentiates P2X7 mediated cell death via a caspase-1 dependent mechanism, independently of cytokine production. *J. Biol. Chem.*, **277**, 3210-3218 (2001).

Nakae, S., Naruse-Nakajima, C., Sudo, K., Horai, R., Asano, M., and Iwakura, Y. IL-1α, but not IL-1β, is required for contact-allergen-specific T cell activation during the sensitization phase in contact hypersensitivity. *Int. Immunol.*, **13**, 1471-1478 (2001).

Kyuwa, S., Kawamura, S., Shibata, S. Machii, K., Tagawa, Y., Iwakura, Y., and Urano, T. The severity of hepatic lesion after intraperitoneal JHMV infection in IFN-gamma deficient mice is parallel to viral replication in hepatocytes *in vitro*. *Adv Exp Med Biol.*, **494**, 95-99 (2001).

Moshkin, M. P., Tamagawa, A., Kolosova, I. E., Gerlinskaya, L. A., Iwakura, Y., and Endo, Y. Social behavior in male mice deficient in genes for interleukin-1. *Med. Immunolo.*, **3**, 449-457 (2001).

Yokoyama, H., Yasuda, J., Okamoto, H., and Iwakura, Y. Pathological changes of renal epithelial cells in mice transgenic for the TT virus ORF1 gene. *J. Gen. Virol.*, in press.

Saijo, S., Asano, M., Horai, R., Yamamoto, H., and Iwakura, Y. Suppression of autoimmune arthritis in IL-1-deficient mice in which T cell activation is impaired due to low levels of CD40L and OX40 expression on T cells. *Arth. Rheum.*, in press.

Nakae, S., Asano, M., Horai, R., and Iwakura, Y. Interleukin (IL)-1β, but not IL-1α, is required for T cell-dependent antibody production. *Immunology*, in press.

Kakuta, S., Tagawa, Y., Shibata, S., Nanno, M., and Iwakura, Y. Inhibition of B16 melanoma experimental metastasis by interferon-γ through direct inhibition of cell proliferation and activation of anti-tumor host mechanisms. *Immunology*, in press.

Kyuwa, S., Shibata, S., Tagawa, Y., Iwakura, Y., Machii, K., and Urano, T. Acute hepatic failure in IFN-γ-deficient BALB/c mice after murine coronavirus infection. *Virus Research*, in press.

Asahi, Y., Shimada, S., Watanabe, I., Yoshikawa, T., Sato, Y., Iwasaki, T., Hasegawa, H., Ohwaki, M., Iwakura, Y., Suzuki, Y., Aizawa, C., Sata, T., Kurata, T., and Tamura, S. Protection against influenza virus infection in poly Ig receptor-knockout mice immunized intranasally with adjuvant-combined vaccines. *J. Immunol.*, in press.

Yano, K., Nakagawa, N., Yasuda, H., Tsuda, E., and Higashio, K. Synovial cells from a patient with rheumatoid arthritis produce osteoclastogenesis inhibitory factor/osteoprotegerin: reciprocal regulation of the production by inflammatory cytokine and basic fibroblast growth factor. *J. Bone Miner. Metab.*, **19**, 365-372 (2001).

Quinn, J. M. W., Itoh, K., Udagawa, N., Hausler, K., Yasuda, H., Shima, N., Mizuno, A., Higashio, K., Takahashi, N., Suda, T., Martin, T. J., and Gillespie, T. Transforming growth factor β affects osteoclast differentiation via direct and indirect actions, *J. Bone Miner. Res.*, **16**, 1787-1794 (2001).

Itoh K, Udagawa N, Katagiri T, Iemura S, Ueno N, Yasuda H, Higashio K, Quinn JM, Gillesrie MT, Martin TJ, Suda T, Takahashi N. Bone morphogenetic protein 2 stimulates osteoclast differentiation and survival supported by recepter activator of nuclear factor-kappaB ligand. *endocrinology*, **142**, 3656-3662 (2001).

岩倉洋一郎．関節リウマチモデルマウス．現代医療(別冊)，33:1158-1166, 2001.

岩倉洋一郎(分担執筆)．発生工学手法によるヒト疾患モデルの作製．シリーズ21世紀の健康と医生物学(3)「からだを守る」，山岸秀夫・宮澤正顕　編集，昭和堂．137-165, 2001.

宝来玲子，岩倉洋一郎．IL-1raによる免疫応答制御．臨床免疫，科学評論社．35:320-327, 2001.

宝来玲子，岩倉洋一郎．IL-1受容体アンタゴニストノックアウトマウス．最新医学（特集）慢性関節リウマチー遺伝子異常とその修復，最新医学社．56:57-62, 2001.

宝来玲子，岩倉洋一郎．IL-1Raノックアウトマウスの関節炎．炎症と免疫，先端医学社．10:85-89, 2002.

保田尚孝．破骨細胞分化因子と炎症性骨破壊．最新医学，56:883-890, 2001.

保田尚孝．慢性関節リウマチに伴う骨粗鬆症におけるオスゲオプロテゲリンの意義．CLINICAL CALCIUM, 11:602-607, 2001.

Center for Experimental Medicine
Laboratory of Gene Expression & Regulation

Gene targeting technology has revealed many aspects of gene functions in vivo. Knock out mice offer the opportunity not only to analyze complex gene function in vivo, but also to present various human disease model where new therapeutic approach can be explored. To allow a more detailed dissection of gene function, we try to introduce a point mutation or to disrupt gene in certain lineages (or stages) by conditional gene targeting using Cre-loxP system. In the process of analyzing knock out mice, we have isolated spontaneous mutant mice which develop chylous ascites and edematous limbs. ES cells, which are used for gene targeting, are the only stem cells being cultured in vitro. To elucidate the moecular mechanism that regulates self-renewal and differentiation of pluripotent ES cells, we try to identify a factor(s) cooperating with Oct-3/4 which is a critical transcription factor for maintaining of undifferentiated state of ES cells. We are also studying the ethiopathogenesis of human systemic autoimmune disease such as systemic lupus erythematosus (SLE) in view of chromatin modifications, for instance, ADP-ribosylation in chromatin using a promyelocytic leukemia cell line HL-60.

1. Requirement of Fas expression in B cells for tolerance induction

Hidehiro Fukuyama[1,2], Masashi Adachi[1], Sachiko Suematsu[3], Keiko Miwa[1,2], Takashi Suda[4], Nobuaki Yoshida[5] and Shigekazu Nagata[1,2]:[1]Department of Genetics, Osaka University Medical School, [2]Core Research for Evolutional Science and Technology, JST, [3]Department of Molecular Immunology, Medical Institute of Bioregulation, Kyushu University, [4]Center for the Development of Molecular Target Drugs, Cancer Research Institute, Kanazawa University, [5]Laboratory of Gene Expression and Regulation, Center for Experimental Medicine, The Institute of Medical Science, The University of Tokyo

Fas is a death receptor belonging to the tumor necrosis factor receptor family and is expressed in various cell types, in particular, in lymphoid cells. A loss-of-function mutation in the Fas gene (*lpr* mutation) causes lymphadenopathy and splenomegaly, and accelerates autoimmune diseases in some strains of mice such as MRL. The Fas cDNA driven by murine *lck* distal promoter was used to establish the transgenic MRL-*lpr* mouse lines. The transgenic mice expressed the functional Fas in mature T cells and B cells. The lymphadenopathy and splenomegaly caused by accumulation of abnormal T cells in the *lpr* mice were rescued in the transgenic mice. The number of B cells in the periphery as well as the serum IgG level were significantly reduced, and the autoimmune symptoms and mortality were ameliorated. These results indicate that the mature B cells as well as T cells should undergo the Fas-medicated apoptosis to prevent the development of autoimmune diseases.

2. Requirement of Phospholipase Cδ4 for the Zona Pellucida-induced Acrosome Reaction

Kiyoko Fukami[6], Kazuki Nakao[7], Takafumi Inoue[8], Yuki Kataoka[5], Manabu Kurokawa[9], Rafael A. Fissore[9], Kenji Nakamura[7], Motoya Katsuki[7], Katsuhiko Mikoshiba[8,10], Nobuaki Yoshida[5] and Tadaomi Takenawa[6]:[6]Department of Biochemistry, [7]Division of DNA Biology and Embryo Engineering, Center for Experimental Medicine, [8]Department of Neurobiology, The Institute of Medical Science, The University of Tokyo, [9]Department of Veterinary and Animal Sciences, Paige Laboratory, University of Massachusetts, U.S.A, [10]Brain Science Institute, Riken

Several phospholipase C (PLC) isoforms have been found in male and female mammalian gametes, and splicing isoforms of PLCδ4 are predominantly expressed in testis. Male mice in which the *PLCδ4* gene had been disrupted either produced few small litters or were sterile. *In vitro* fertilization studies showed that insemination with PLCδ4$^{-/-}$ sperm resulted in significantly fewer eggs becoming activated and that the calcium transients associated with fertilization were absent or delayed. PLCδ4$^{-/-}$ sperm were unable to initiate the acrosome reaction, an exocytotic event required for fertilization and induced by interaction with the egg coat , the zona pellucida. These data demonstrate that PLCδ4 functions in the acrosome reaction that is induced by the zona pellucida during mammalian fertilization.

3. Abnormalities of Synapses and Neurons in the Hippocampus of Neuropsin-Deficient Mice

Akio Hirata[11], Shigetaka Yoshida[11], Naoko Inoue[11], Kazumasa Matsumoto[11], Ayako Ninomiya[11], Manabu Taniguchi[12], Tomohiro Matsuyama[13], Keiko Kato[11], Hisashi Iizasa[14], Yuki Kataoka[5], Nobuaki Yoshida[5] and Sadao Shiosaka[11]:[11]Division of Structural Cell Biology, Nara Institute of Science and Technology, NAIST, [12]Department of Anatomy and Neuroscience, Osaka University Medical School, [13]Fifth Department of Internal Medicine, Hyogo College of Medicine, [14]Department of Pharmaceutics, Kyoritsu College of Pharmacy

We produced null-mutant mice of neuropsin, an extracellular matrix serine protease, to examine the neural functions of this protein particularly in the hippocampus. Golgi-Cox impregnation and Nissl staining revealed morphological change of cell soma in the mutant mice compared to wild-type mice. However, Golgi-Cox impregnation revealed no apparent change in the dendritic arborization and spine density. Quantitative electronmicroscopic analysis revealed that number of asymmetrical synapses were significantly decreased in the stratum radiatum, the major terminal field of Schaffer-collaterals, whereas free boutons still holding synaptic vesicles but with no synaptic specialization were increased in number in the same microscopic fields. An increased number of parvalbumin-immunoreactive cells (known as fast spiking cells) in mutant was also observed. These results strongly suggest that neuropsin is involved in connectivity of a group of CA1 synapses and consequently in the hippocampal networking.

4. Chromatosome composed of histone H1 and nucleosome is an independent immune target from nucleosome in systemic lupus erythematosus

Yoshiyuki Kanai[5] and Tetsuo Kubota[15]:[15]School of Allied Science, Tokyo Medical and Dental University

Dissection of chromatin components as a part of autoimmune target is important work in etiopathogenesis of systemic lupus erythematosus(SLE). To this end, we challenged to establish a new strategy and have found new aspects as follows. As a chromatin source, we used the nuclei of cultured HL-60 cells of human promyelocytic leukemia cell origin. Nuclei digested with a micrococcal nuclease(MN) were solubilized with various concentrations of NaCl buffer in the presence of protease-inhibitor cocktail and 2-ME. Mononucleosomes thus produced were isolated by high-performance liquid chromatography(HPLC) using a column of Superdex 200. They were further digested with MN to cleave off linker DNA, followed by HPLC separation. Meanwhile, histone H1(H1) was purified from whole histones of HL-60 origin on cation-exchange chromatography. Mononucleosome(NS) and H1 were combined in nutral 250mM NaCl buffer to give chromatosome(CS). Autoantibodies to these antigens were assessed by ELISA as well as Western blot. Of 22 cases of SLE, 5 showed high titers against NS, CS and H1. To investigate immunological interrelationships of these antigens, two sera were selected for each antigen binding as a function of serum dilution. Interestingly, binding activities of both sera to CS were increased with dilution up to 1:800, whereas those against either NS or H1 were decreased according to dilution, suggesting that the CS expresses a specific epitope(s), or that the epitope could be modified by unknown factor(s) in the serum. Taken together, CS could develops a new aspect in SLE not only in etiopathogenesis but also in basic research in chromatin chemistry.

5. Novel purification method of nucleosomes from chromatin by high-performance liquid chromatography (HPLC)

Yoshiyuki Kanai

I previously developed a novel purification meth-

od of nucleosomes from medium in which cultured HL-60 cells, of human premyelocytic leukemia cell origin, were undergoing apoptosis. This time, I applied this method to cell chromatins. In brief, HL-60 cell chromatins were digested with micrococcal nuclease for 45 min.. Chromatins rendered insoluble by this treatment were suspended in 0.25M NaCl-containing neutral Tris buffer. Soluble fraction obtained by spinning the suspension contained mononucleosome. Further purification has been achieved by HPLC. Purity was ascertained by both SDS-PAGE and agarose gel electrophoresis. This method is the first-ever in terms of speed of purification/isolation. Stability in liquid of nucleosomes obtained by the conventional method is said to be of two weeks. By contrast, that of the present method is of eight weeks as far as judged by both immunogenicity and HPLC. Nucleosome thus obtained is guaranteed to serve a good antigen for detecting anti-nucleosome antibodies in various kinds of disease.

6. Genetic analysis of lymphatic development and functions in mammals

Hirotake Ichise, Takeshi Kuraishi, Akiko Hori and Nobuaki Yoshida

The lymphatics are thought to be responsible for edematous condition in patients, especially in those suffering from primary lymphedema. Resent studies show that lymphangiogenesis, as well as angiogenesis, also plays some roles on tumor metastasis. However, the lymphatic development in mammals has been unknown from lack of useful mutant animal that has obvious lymphatic abnormality. In order to understand the mechanism of lymphatic development and functions, we are generating "reporter" strains by transgenesis or targeted mutagenesis, using marker genes (lacZ, GFP, PLAP) under transcriptional regulation of the lymphatic endothelial-specific genes, such as LYVE-1, prox-1 and VEGFR-3. We have also started on the conditonal knockout analyses of potential growth factor/receptor and transcriptional factors in lymphangiogenesis. We are also under investigation of an original spontaneous mutant mouse line developing chylous ascites and lymphedema that are thought to be due to lymphatic abnormality. The blood flow is found not only in blood vessels but also in lymphatic vessels in the homozygous mutants. The peripheral capillary-lacteal shunt at the intestinal villi is one of the cause for blood flow observed in lymphatics of the homozygous mutant mice.

We are trying forward genetic approaches to find the candidate for this mutation.

7. cDNA cloning of ROX that binds to the *Rex-1* gene promoter

Mitsuharu Sato, Yuhki Nakatake and Nobuaki Yoshida

The mechanism that establishes pluripotent, undifferentiated state in mouse embryonic stem (ES) cells is not fully understood. Although many genes have been identified and shown to be specific in ES cells, it is still unclear how undifferentiated state in ES cells is maintained.

One of the POU-family transcription factors, Oct-3/4 is known to be an essential factor for the maintenance of undifferentiated ES cells. Oct-3/4 functions as a transcriptional activator in ES cells but it does not in differentiated cells. This prompted us to investigate what was required for Oct-3/4 to function in undifferentiated cells. It was suggested that E1A-like factor in ES cells cooperated with Oct-3/4, but the nature of E1A-like factor is yet to be identified.

To identify the factor(s) that cooperates with Oct-3/4, we focused on the new DNA binding activity found in *Rex-1* gene promoter. *Rex-1* gene, which is expressed only in undifferentiated cells, has an octamer-binding motif in its promoter and the expression of *Rex-1* was shown to be regulated by Oct-3/4. Recently, a new DNA binding activity, termed Rox, was identified in *Rex-1* promoter and Rox binds to the sequence adjacent to that of Oct-3/4 binding site. To clone the cDNA of Rox, we are starting protein purification using DNA affinity chromatography. The yeast one-hybrid screening was also started to identify Rox DNA binding component. We obtained several positive clones in the yeast one-hybrid screening and we are analyzing the character of these clones.

Publications

Fukuyama, H., Adachi, M., Suematsu, S., Miwa, K., Suda, T., Yoshida, N. and Nagata, S. Requirement of Fas expression in B cells for tolerance induction. Eur. J. Immunol., in press.

Fukami, K., Nakao, K., Inoue, T., Kataoka, Y,. Kurokawa, M., Rafael A. Fissore, Nakamura, K., Katsuki, M., Mikoshiba, K., Yoshida, N., and Takenawa, T. Requirement of Phospholipase C_4 for the Zona Pellucida-induced Acrosome Reaction. Science, 292:920-923, 2001

Hirata, A., Yoshida, S., Inoue, N., Matsumoto, K., Ninomiya, A.,Taniguchi, M., Matsuyama, T., Kato, K., Iizasa, H., Kataoka, Y., Yoshida, N. and Shiosaka, S. Abnormalities of Synapses and Neu-

rons in the Hippocampus of Neuropsin-Deficient Mice. Mol. Cell. Neuroscience, 17: 600-610, 2001
Kanai, Y., Q. Lu and Kubota, T. Histone H3 dimer as a preferential target of anti-dsDNA/nucleosome autoantibodies. Lupus, 10: S6, 2001
Kanai, Y., and Kubota, T. Anti-chromatin autoantibodies in systemic lupus erythematosus. Autoimmunity Rev, in press.
吉田進昭：サイトカイン遺伝子ノックアウトマウスの作製．臨床検査，医学書院．45:47-54, 2001
吉田進昭：再生医療臨床展開をめざしたマウスES細胞研究概論．分子細胞治療，先端医学社．印刷中
橋本博史他：厚生労働省厚生科学特定疾患・難治性血管炎に関する調査研究報告．日本臨床免疫学会誌，336-346, 2001
金井芳之：抗DNA抗体と抗ヌクレオソーム抗体．組織培養工学，ニューサイエンス社．27:10-14, 2001
金井芳之：ヌクレオソームと自己免疫．腎臓，印刷中
金井芳之：クロマトソーム、ヌクレオソームとSLE．臨床免疫，科学評論社．印刷中