Department of Microbiology and Immunology

Division of Infectious Genetics
感染遺伝学分野

Professor	Kensuke Miyake, M.D., Ph.D.	教　授	医学博士	三　宅　健　介
Associate Professor	Shin-Ichiroh Saitoh, Ph.D.	准教授	博士(医学)	齋　藤　伸一郎
Project Associate Professor	Ryutaro Fukui, Ph.D.	特任准教授	博士(医学)	福　井　竜太郎
Assistant Professor	Takuma Shibata, Ph.D.	助　教	博士(医学)	柴　田　琢　磨
Assistant Professor	Ryota Sato, Ph.D.	助　教	博士(医学)	佐　藤　亮　太

Immune cells express multiple Toll-like receptors (TLRs) that are simultaneously activated by various pathogen-derived products from microorganisms and viruses. Recent reports have demonstrated that imbalances in TLR responses can result in the development of autoimmune diseases. Nucleic acid(NA) -sensing TLRs detect not only bacterial and viral NAs, but also host-derived NAs. To prevent excessive immune responses to host-derived NA, there may exist regulatory mechanisms that control TLR expression, localization, and function. Based on this hypothesis, it is believed that TLRs are involved not only in autoimmune diseases, but also in the pathogenesis of a variety of other diseases. Our research endeavors to uncover the regulatory mechanisms that control TLR-mediated recognition of pathogenic ligands, as well as the identification of endogenous ligands. Our research goal is to clarify the pathogenic mechanisms of histiocytosis and autoimmune diseases that are thought to be mediated by TLRs.

1. Endosomal abnormalities in dendritic cells cause autoimmune liver diseases

Shin-Ichiroh Saitoh[1], Kenichi Harada[5], Yoshiko Mori Saitoh[1], Ge-Hong Sun-Wada[6], Tamami Denda[3], Yasunori Ota[3], Hiroshi Sagara[4], Yuji Watanabe[4], Yoh Wada[7], and Kensuke Miyake[1, 2]

[1]Division of Infectious Genetics, Department of Microbiology and Immunology. [2]Laboratory of Innate Immunity, [3]Department of Pathology, Research Hospital, [4]Medical Proteomics Laboratory, The Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minatoku, Tokyo 108-8639, Japan. [5]Department of Human Pathology, Kanazawa University School of Medicine, Kanazawa, 920-8640, Japan. [6]Department of Biochemistry, Faculty of Pharmaceutical Sciences, Doshisha Women's College, Kohdo, Kyotanabe, Kyoto 610-0395, Japan. [7]Division of Biological Science, Institute of Scientific and Industrial Research, Osaka University, 8-1 Mihogaoka, Ibaraki, Osaka 567-0047, Japan.

Autoimmune hepatitis (AIH) and primary biliary cholangitis (PBC) are autoimmune liver diseases with unknown etiologies. Although T cells are thought to drive these liver diseases, little is known about the underlying mechanism of T cell activation in these liver diseases. Since antigen presentation is regulated by endosome maturation which Rab7a controls, we investigated the changes in the immune response that occur upon blocking endosome maturation by Rab7a deficiency in dendritic cells (DCs). As a result, DC-specific Rab7a-deficient mice developed AIH and PBC. Failure to suppress Vps34-dependent endosome fusion due to Rab7a deficiency markedly enhanced cross-presentation by forming giant endosomes and altering MHC class Ⅰ transport. MHC class Ⅰ was accumulated in the giant endosomes. Hyperactivated CD8+ T cells caused fibrosis around portal veins and central veins, a hallmark of AIH. Female mice had a

worse condition of PBC. αβ T cell ablation protected the mice against AIH, but not PBC, where cytotoxic γδ T cells were localized around the bile duct. αβ- and γδ-T cell deficiency in the mice ameliorated both AIH and PBC. This study revealed that endosomal abnormalities in DCs strongly enhanced cross-presentation and γδ T cell activation resulting in autoimmune liver diseases. CD8+ T cells and γδ T cells are potential therapeutic targets for AIH and PBC.

2. Nucleosides drive histiocytosis in SLC29A3 disorders by activating TLR7

Takuma Shibata[1], Ryota Sato[1], Masato Taoka[2], Shin-Ichiroh Saitoh[1], Mayumi Komine[3], Kiyoshi Yamaguchi[4], Susumu Goyama[5], Yuji Motoi[1], Jiro Kitaura[6], Kumi Izawa[6], Yoshio Yamauchi[2], Yumiko Tsukamoto[7], Takeshi Ichinohe[8], Etsuko Fujita[3], Ryosuke Hiranuma[1], Ryutaro Fukui[1], Yoichi Furukawa[4], Toshio Kitamura[9], Toshiyuki Takai[10], Arinobu Tojo[11], Mamitaro Ohtsuki[3], Umeharu Ohto[12], Toshiyuki Shimizu[12], Manabu Ozawa[13], Nobuaki Yoshida[13], Toshiaki Isobe[2], Eicke Latz[14], Kojiro Mukai[15], Tomohiko Taguchi[15], Kensuke Miyake[1]*

[1]Division of Innate Immunity, Department of Microbiology and Immunology, The Institute of Medical Science, The University of Tokyo; Tokyo 108-8639, Japan. [2]Department of Chemistry, Graduate School of Science, Tokyo Metropolitan University; Tokyo 192-0397, Japan. [3]Department of Dermatology, Jichi Medical University; Tochigi 329-0498, Japan. [4]Division of Clinical Genome Research, The Institute of Medical Science, The University of Tokyo; Tokyo 108-8639, Japan. [5]Division of Molecular Oncology, Department of Computational Biology and Medical Sciences, Graduate School of Frontier Sciences, The University of Tokyo; Tokyo 108-8639, Japan. [6]Atopy Research Center, Juntendo University Graduate School of Medicine; Tokyo 113-8421, Japan. [7]Department of Mycobacteriology, Leprosy Research Center, National Institute of Infectious Diseases; Tokyo 189-0002, Japan. [8]Division of Viral Infection, Department of Infectious Disease Control, International Research Center for Infectious Diseases, The Institute of Medical Science, The University of Tokyo; Tokyo 108-8639, Japan. [9]Division of Cellular Therapy, The Institute of Medical Science, The University of Tokyo; Tokyo 108-8639, Japan. [10]Department of Experimental Immunology, Institute of Development, Aging and Cancer, Tohoku University; Sendai 980-8575, Japan. [11]Department of Hematology and Oncology, Research Hospital, The Institute of Medical Science, The University of Tokyo; Tokyo 108-8639, Japan. [12]Graduate School of Pharmaceutical Sciences, The University of Tokyo; Tokyo 113-0033, Japan. [13]Laboratory of Developmental Genetics, Center for Experimental Medicine and Systems Biology, The Institute of Medical Science, The University of Tokyo; Tokyo 108-8639, Japan. [14]Institute of Innate Immunity, University Hospital Bonn, University of Bonn; 53127 Bonn, Germany. [15]Laboratory of Organelle Pathophysiology, Department of Integrative Life Sciences, Graduate School of Life Sciences, Tohoku University; Sendai 980-8577, Japan.

Loss-of-function mutations in *SLC29A3* cause lysosomal nucleoside storage and histiocytosis: phagocyte accumulation in multiple organs. However, little is known about the mechanism by which lysosomal nucleoside storage drives histiocytosis. Herein, histiocytosis in *Slc29a3*$^{-/-}$ mice was shown to depend on Toll-like receptor 7 (TLR7), which senses a combination of nucleosides and oligoribonucleotides (ORNs). TLR7 increased phagocyte numbers by driving the proliferation of Ly6C^{hi} immature monocytes and their maturation into Ly6C^{low} phagocytes in *Slc29a3*$^{-/-}$ mice. Downstream of TLR7, FcRγ and DAP10 were required for monocyte proliferation. Histiocytosis is accompanied by inflammation in SLC29A3 disorders. However, TLR7 in nucleoside-laden splenic macrophages failed to activate inflammatory responses. Enhanced production of pro-inflammatory cytokines was observed only after stimulation with ssRNAs, which would increase lysosomal ORNs. Patient-derived monocytes harboring the G208R *SLC29A3* mutation showed enhanced survival and proliferation in a TLR8 antagonist-sensitive manner. These results demonstrated that non-inflammatory TLR7/8 responses to lysosomal nucleoside stress drive SLC29A3 disorders.

3. Anti-TLR7 antibody protects against lupus nephritis in NZBWF1 mice by targeting B cells and patrolling monocytes

Reika Tanaka[1], Yusuke Murakami[1, 2], Ryutaro Fukui[1], Kiyoshi Yamaguchi[3], Yoichi Furukawa[3], Naomi Yamashita[2], Kensuke Miyake[1, 4]

: [1]Division of Innate Immunity, Department of Microbiology and Immunology, The Institute of Medical Science, The University of Tokyo. [2]Department of Pharmacotherapy, Research Institute of Pharmaceutical Sciences, Musashino University. [3]Division of Clinical Genome Research, Advanced Clinical Research Center, The Institute of Medical Science, The University of Tokyo. [4] Laboratory of Innate Immunity, Center for Experimental Medicine and Systems Biology, The Institute of Medical Science, The University of Tokyo.

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by autoantibody production and multiple organ damage. We found that inhibition of Toll-like receptor 7 (TLR7) rescues NZBWF1 mice from lethal nephritis by reducing the activation of B cells and monocytes. Immunohistochemistry analysis of the kidneys revealed that Ly6C-negative/

FcγRIV-positive patrolling monocytes (PatMCs) infiltrated into glomeruli. To clarify the role for PatMCs in nephritis, we focused on the molecules expressing in PatMCs. We performed RNA-sequencing and antibody array screening by comparing PatMC and Ly6C-positive classical monocytes, with or without TLR7 inhibition. In results, expression of several lupus-related molecules, for example, IL-10, PD-L2, and PECAM-1 were induced by TLR7 signaling. We are analyzing the mechanisms of these molecules as further study.

Publications

Leibler, C., John, S., Elsner, R. A., Thomas, K. B., Smita, S., Joachim, S., Levack, R. C., Callahan, D. J., Gordon, R. A., Bastacky, S., Fukui, R., Miyake, K., Gingras, S., Nickerson, K. M., and Shlomchik, M. J. Genetic dissection of TLR9 reveals complex regulatory and cryptic proinflammatory roles in mouse lupus. Nat Immunol 23:1457, 2022.

Liu, X., Sato, N., Yabushita, T., Li, J., Jia, Y., Tamura, M., Asada, S., Fujino, T., Fukushima, T., Yonezawa, T., Tanaka, Y., Fukuyama, T., Tsuchiya, A., Shikata, S., Iwamura, H., Kinouchi, C., Komatsu, K., Yamasaki, S., Shibata, T., Sasaki, A. T., Schibler, J., Wunderlich, M., O'Brien, E., Mizukawa, B., Mulloy, J. C., Sugiura, Y., Takizawa, H., Miyake, K., Kitamura, T., and Goyama, S. IMPDH inhibition activates TLR-VCAM1 pathway and suppresses the development of MLL-fusion leukemia. EMBO Mol Med 15:e15631, 2023.

Maeda, F., Kato, A., Takeshima, K., Shibazaki, M., Sato, R., Shibata, T., Miyake, K., Kozuka-Hata, H., Oyama, M., Shimizu, E., Imoto, S., Miyano, S., Adachi, S., Natsume, T., Takeuchi, K., Maruzuru, Y., Koyanagi, N., Jun, A., and Yasushi, K. Role of the Orphan Transporter SLC35E1 in the Nuclear Egress of Herpes Simplex Virus 1. J Virol 96:e0030622, 2022.

Miyake, K., Shibata, T., Fukui, R., Sato, R., Saitoh, SI., Murakami, Y. Nucleic Acid Sensing by Toll-Like Receptors in the Endosomal Compartment. Front Immunol. 13:941931, 2022.

Sakaniwa, K., Fujimura, A., Shibata, T., Shigematsu, H., Ekimoto, T., Yamamoto, M., Ikeguchi, M., Miyake, K., Ohto, U., and Shimizu, T. TLR3 forms a laterally aligned multimeric complex along double-stranded RNA for efficient signal transduction. Nat Commun 14:164, 2023.

Shibata, T., Sato, R., Taoka, M., Saitoh, SI., Komine, M., Yamaguchi, K., Goyama, S., Motoi, Y., Kitaura, J., Izawa, K., Yamauchi, Y., Tsukamoto, Y., Ichinohe, T., Fujita, E., Hiranuma, R., Fukui, R., Furukawa, Y., Kitamura, T., Takai, T., Tojo, A., Ohtsuki, M., Ohto, U., Shimizu, T., Ozawa, M., Yoshida, N., Isobe, T., Latz, E., Mukai, K., Taguchi, T., Miyake, K. TLR7/8 stress response drives histiocytosis in SLC29A3 disorders. BioRχiv, doi: https://doi.org/10.1101/2022.10.27.513971, 2022