Center for Experimental Medicine and Systems Biology

Division of Stem Cell Pathology
先進病態モデル研究分野

Professor	Yasuhiro Yamada, M.D., Ph.D.	教　授　博士(医学)	山　田　泰　広
Assistant Professor	Masaki Yagi, Ph.D.	助　教　博士(医科学)	八　木　正　樹
Assistant Professor	Sho Ohta, Ph.D.	助　教　博士(生命科学)	太　田　　翔

Epigenetic regulation plays a critical role for the cellular differentiation, the stable maintenance of cellular identity, and the reprogramming process. Accumulating evidence suggests that epigenetic abnormalities represented by abnormal DNA methylation have been involved in various diseases as well. We are interested in unveiling epigenetic regulation in the cellular differentiation, the maintenance of cellular identity, and the pathogenesis including age-related diseases such as cancer. Particularly, taking advantage of reprogramming technology to actively alter epigenetic regulation, we are investigating the role of epigenetic regulation on cancer development, maintenance, and progression. Finally, we will try to develop a novel approach targeting epigenetic regulation to treat cancer patients.

1. *In vivo* reprogramming drives *Kras*-induced cancer development

Hirofumi Shibata[1,2], Shingo Komura[1], Yosuke Yamada[1,3], Nao Sankoda[4], Akito Tanaka[1], Tomoyo Ukai[1], Mio Kabata[1], Satoko Sakurai[1], Bunya Kuze[2], Knut Woltjen[1,5], Hironori Haga[3], Yatsuji Ito[2], Yoshiya Kawaguchi[4], Takuya Yamamoto[1,6], and Yasuhiro Yamada[1,6]: [1]Department of Life Science Frontiers, Center for iPS Cell Research and Application (CiRA), Kyoto University, [2]Department of Otolaryngology, Gifu University Graduate School of Medicine, [3]Department of Diagnostic Pathology, Kyoto University Hospital, [4]Department of Clinical Application, Center for iPS Cell Research and Application (CiRA), Kyoto University, [5]Hakubi Center for Advanced Research, [6]AMED-CREST, AMED

Induced pluripotent stem cells (iPSCs) can be established from somatic cells by the transient expression of pluripotency-related transcription factors (TFs). Given that epigenetic regulation plays a central role in cell fate determination and maintenance, this technology allows for the reorganization of epigenetic regulation from the differentiated somatic cell state into the pluripotent state without affecting genetic information. Recent studies revealed the stepwise epigenetic changes during iPSC derivation. In early-stage reprogramming, the faithful shutdown of the somatic program occurs through the silencing of somatic cell-specific enhancers, which is associated with a loss of original cell identity.

We examined the effect of in vivo reprogramming on Kras-induced cancer development. We show that the transient expression of reprogramming factors (1-3 days) in pancreatic acinar cells results in the transient repression of acinar cell enhancers, which are similarly observed in pancreatitis. We next demonstrate that Kras and p53 mutations are insufficient to induce ERK signaling in the pancreas. Notably, the transient expression of reprogramming factors in Kras mutant mice is sufficient to induce the robust and persistent activation of ERK signaling in acinar cells and rapid formation of pancreatic ductal adenocarcinoma. In contrast, the forced expression of acinar cell-related

transcription factors inhibits the pancreatitis-induced activation of ERK signaling and development of precancerous lesions in Kras-mutated acinar cells. These results underscore a crucial role of dedifferentiation-associated epigenetic regulations in the initiation of pancreatic cancers.

2. Platforms of *in vivo* genome editing with inducible *Cas9* for advanced cancer modeling

Norihide Jo[1], Yuko Sogabe[1], Yosuke Yamada[1], Tomoyo Ukai[1,2], Harunobu Kagawa[1], Kanae Mitsunaga[1], Knut Woltjen[1,5], Yasuhiro Yamada[1,2,6]

CRISPR/Cas9 has become a common-place genome editing tool due to its efficiency, specificity and convenience, and its application *in vivo* has been extensively explored. Indeed, constitutive mutant rodents have been successfully generated from zygote or ES cells transduced with sgRNA and Cas 9, and are widely used in a variety of experiments. On the other hand, few technological advances have been made for conditional *in vivo* genome editing with CRISPR/Cas9. One approach is to externally deliver CRISPR/Cas9 directly into the animal's body by various methods such as virus and electroporation; however, low mutation efficiency in the target organ and variation in the resultant mutants may preclude the use of this system for *in vivo* experiments. Another approach is *Cas9*-transgenic animals, in which either Cre/loxP-conditional or doxycycline (Dox)-inducible *Cas9* are used to alleviate the challenges of delivery.

To make genome editing with *Cas9*-transgenic animals further accessible, we devised new platforms for efficient genome editing at multiple loci in a Dox-inducible manner *in vivo*. Loading up to four sgRNAs simultaneously into the targeting vector was feasible by employing a site-specific multi-segment cloning strategy. A recombinase-mediated cassette exchange (RMCE)-based platform exhibited efficient genome editing upon Dox administration while displaying minimal levels of unintended insertions and deletions (indels) in the absence of Dox both *in vitro* and *in vivo*. Finally, we used this platform to model cancers with complex genome editing including both activating and inactivating mutations in an inducible manner. Here, we devised two doxycycline (Dox)-inducible *Cas9* platforms that efficiently conduct conditional genome editing at multiple loci *in vitro* and *in vivo*. In these platforms, we took advantage of a site-specific multi-segment cloning strategy for the rapid and easy integration of multiple sgRNAs. We found that a platform containing *rtTA* at the *Rosa26* locus and *TRE-Cas9* together with multiple sgRNAs at the *Col1a1* locus exhibited a higher efficiency of inducible insertions and deletions (indels) with minimal leaky editing. Using this platform, we succeeded to model Wilms' tumor and the progression of intestinal adenomas with multiple mutations including an activating mutation with a large genomic deletion. Collectively, the established platform should make complicated disease modeling in mouse easily attainable, extending the range of *in vivo* experiments in various biological fields including cancer research.

Publications

1. Shibata H, Komura S, Yamada Y, Sankoda N, Tanaka A, Ukai T, Kabata M, Sakurai S, Kuze B, Woltjen K, Haga H, Ito Y, Kawaguchi Y, Yamamoto T, *Yamada Y. In vivo reprogramming drives Kras-induced cancer development. *Nature Commun.* 2018 9(1): 2081
2. Jo N, Sogabe Y, Yamada Y, Ukai T, Kagawa H, Mitsunaga K, Woltjen K, *Yamada Y. Platforms of in vivo genome editing with inducible Cas9 for advanced cancer modeling. *Cancer Sci.* 2018 Dec 26. doi: 10.1111/cas.13924.
3. Sogabe Y, Seno H, Yamamoto T, *Yamada Y. Unveiling epigenetic regulation in cancer, aging, and rejuvenation with in vivo reprogramming technology. *Cancer Sci.* 2018 Sep; 109(9): 2641-2650. doi: 10.1111/cas.13731.
4. Yamada Y, *Yamada Y. The causal relationship between epigenetic abnormality and cancer development: in vivo reprogramming and its future application. *Proc Jpn Acad Ser B Phys Biol Sci.* 2018; 94(6): 235-247. doi: 10.2183/pjab.94.016.
5. Ikeda T, Hikichi T, Miura H, Shibata H, Mitsunaga K, Yamada Y, Woltjen K, Miyamoto K, Hiratani I, Yamada Y, Hotta A, Yamamoto T, Okita K, Masui S. Srf destabilizes cellular identity by suppressing cell-type-specific gene expression programs. *Nature Commun.*, 2018 Apr 11; 9(1): 1387. doi: 10.1038/s41467-018-03748-1.
6. Hino K, Zhao C, Horigome K, Nishio M, Okanishi Y, Nagata S, Komura S, Yamada Y, Toguchida J, Ohta A, Ikeya M*. An mTOR Signaling Modulator Suppressed Heterotopic Ossification of Fibrodysplasia Ossificans Progressiva. *Stem Cell Reports.* 2018 Nov 1. pii: S2213-6711(18) 30430-2. doi: 10.1016/j.stemcr.2018.10.007.

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 fatal diseases such as septic shock and autoimmune diseases. For this reason, immune system must strictly control TLR responses to avoid disruption of homeostasis. However, molecular mechanisms involved in TLR regulation are not fully elucidated. We have previously shown that TLRs are regulated by various TLR associating molecules including MD-2, PRAT4A and Unc93B1. Our goal is to uncover molecular mechanism that is indispensable for appropriate TLR responses using genetically engineered mice.

1. Targeting the nucleic acids-sensing TLRs for therapeutic intervention in autoimmune diseases

Yuji Motoi[1], Ryutaro Fukui[1], Takuma Shibata[1], Kensuke Miyake[1,2]: [1]Division of Innate Immunity, Department of Microbiology and Immunology, [2]Laboratory of Innate Immunity, Center for Experimental Medicine and Systems Biology, The Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minatoku, TOKYO1208-8639, Japan.

TLR7 senses microbial-derived RNA in endolysosome, but can also erroneously respond to self-derived RNA. In fact, it has been reported that TLR7-dependent signaling promote autoimmune diseases. Thus, TLR7 can be therapeutic target. Although antibodies (Abs) are powerful tools for therapeutic intervention, TLR7 has been excluded from targets for Ab-mediated intervention because of its lack of cell surface expression. Despite this expectation, we found an anti-TLR7 Ab dose-dependently inhibits TLR7 responses in dendritic cells, macrophages and B cells. For this reason, we evaluated the therapeutic effect of anti-TLR7 Ab in *Unc93b1*$^{D34A/D34A}$ mice that cause thrombocytopenia, splenomegaly and chronic active hepatitis due to TLR7 hyper-responsiveness, and found that thrombocytopenia in *Unc93b1*$^{D34A/D34A}$ mice was significantly improved by the treatment with anti-TLR7 mAb. Furthermore, splenomegaly and hepatitis in mice treated with the anti-TLR7 mAb were also significantly remedy compared with control antibody.

On basis of these results, we established anti-human TLR7 Ab for blocking human TLR7 responses in vitro. Moreover, we generated human TLR7 transgenic (huTLR7 Tg) mice. We plan to use HuTLR7 Tg mice to evaluate the effects of anti-human TLR7 Ab *in vivo*.

In addition, TLR8 also recognize mouse TLR7 ligands in human and is involved in exacerbation of Rheumatoid Arthritis. Thus, in case of human disease, the anti-human TLR8 Ab that inhibits human TLR8 responses might work in clinical application. For this reason, we also constructed both anti-human TLR8 Abs and human TLR8 transgenic mice to verify our hypothesis.

2. TLR3-mTORC2 axis is required for response against HSV-1 infection

Ryota Sato[1*], Akihisa Kato[2,3], Takahiko Chimura[4], Shin-Ichiroh Saitoh[1], Takuma Shibata[1], Ryutaro Fukui[1], Jun Arii[2,3], Tsuneo Ikenoue[5], Toshiya Manabe[4], Yasushi Kawaguchi[2,3], Kensuke Miyake[1,6]: [1]Division of Innate Immunity, Department of Microbiology and Immunology, [2]Division of Molecular Virology, Department of Microbiology and Immunology, [3]Department of Infectious Disease Control, International Research Center for Infectious Diseases, [4]Division of Neuronal Network, Department of Basic Medical Sciences, [5]Division of Clinical Genome Research, Advanced Clinical Research Center, [6]Laboratory of Innate Immunity, Center for Experimental Medicine and Systems Biology, Institute of Medical Science, The University of Tokyo, Tokyo, 108-8639, Japan.

Toll-like receptor 3 (TLR3) is a double-stranded RNA (dsRNA) sensor indispensable for defense against Herpes Simplex Virus (HSV-1) infection in the brain. We show here that TLR3 was required for innate immune responses to HSV-1 in neurons and astrocytes. In HSV-1 infection, TLR3 recruited the mammalian target of rapamycin complex 2 (mTORC2) that lead to induction of chemokines and trafficking of TLR3 to the cell periphery. TLR3 trafficking enabled activation of molecules required for type I interferon (type I IFN) induction including mTORC1. Intracranial HSV-1 infection in mice was exacerbated by impairing TLR3 responses with an mTOR inhibitor, and significantly rescued by potentiating TLR3 responses with an agonistic anti-TLR3 antibody. These results suggest that the TLR3-mTORC2 axis might be a therapeutic target to combat herpes simplex encephalitis.

3. Requirement of glycosylation machinery in Toll-like receptor responses revealed by CRISPR/Cas9 screening

Ryota Sato[1], Takuma Shibata[1], Kiyoshi Yamaguchi[3], Yoichi Furukawa[3], Eigo Shimizu[4], Rui Yamaguchi[4], Seiya Imoto[5], Satoru Miyano[4], Kensuke Miyake[1,2]: [1]Division of Innate Immunity, Department of Microbiology and Immunology, [2]Laboratory of Innate Immunity, Center for Experimental Medicine and Systems Biology, [3]Division of Clinical Genome Research, Advanced Clinical Research Center, [4]Laboratory of DNA Information Analysis, Human Genome Center & [5]Division of Health Medical Data Science, Human Intelligence Center, The Institute of Medical Science, The University of Tokyo, Minato-ku, Tokyo 108-8639, Japan

The Toll family of receptors sense microbial products and activate a defense response. The molecular machinery required for the Toll-like receptor (TLR) response is not yet fully understood. In the present study, we used a CRISPR/CAS9 screening system to study TLR responses. We employed a cell line expressing TLR with an NF-κB-driven GFP reporter. The cell line was transduced with a guide RNA library and stimulated with TLR ligands. The cells impaired in GFP induction were sorted, and gRNAs were sequenced. Identified genes were ranked according to the count of sequence reads and the number of gRNA target sites. The screening system worked correctly, as molecules that were already known to be required for the TLR response were identified by the screening. Furthermore, this system revealed that the oligosaccharide transferase complex (OSTC) mediating co-translational glycosylation was required for TLR5, 7, and 9 responses. Protein expression of TLR5, but not an irrelevant molecule (CD44), was abolished by the lack of OSTC, suggesting the essential role of glycosylation in TLR5 protein stability. These results demonstrate that the screening system established here is able to reveal molecular mechanisms underlying the TLR response.

Publications

Sato, R. Kato, A. Chimura, T. Saitoh, SI. Shibata, T. Murakami, Y. Fukui, R. Liu, K. Zhang, Y. Arii, J. Sun-Wada, GH. Wada, Y. Ikenoue, T. Barber, GN. Manabe, T. Kawaguchi, Y. and Miyake, K. Combating herpesvirus encephalitis by potentiating a TLR3-mTORC2 axis. Nature Immunology. 19: 1071-1082, 2018

Center for Experimental Medicine and Systems Biology

Laboratory of Reproductive Systems Biology
生殖システム研究分野

Project Professor Masahito Ikawa, Ph.D.
Associate Professor Manabu Ozawa, Ph.D.

特任教授 博士(薬学) 伊川正人
准教授 博士(農学) 小沢学

In the "post-genome project era," genetically modified animals play a key role in basic molecular biological investigations and act as models of human disease. Our laboratory studies the mechanisms underlying the mammalian reproductive system in gene-manipulated mice. We are the first group in the world to generate transgenic mice expressing GFP throughout the body (Green mice). We also established the ES cells that give green fluorescent spermatozoa to trace their movement and acrosome reaction during fertilization. Another tool invented in our laboratory is the placenta-specific gene manipulation system using lentiviral (LV) vectors. Using these techniques, we are trying to elucidate the mechanism underlying gametogenesis, fertilization, implantation, and placentation. Our recent interest is using the CRISPR/Cas9 system as a genome-editing tool. The combination of GWAS studies with genome editing will pave the way to understand and control human fertility problems.

1. PTBP 1 contributes to spermatogenesis through regulation of proliferation in spermatogonia

Manami Senoo[1], Takashi Takijiri[1,2], Nobuaki Yoshida[2], Manabu Ozawa and Masahito Ikawa[3]: [1]Graduate School of Frontier Sciences, The University of Tokyo, [2]Laboratory of Developmental Genetics, Center for Experimental Medicine and Systems Biology, The Institute of Medical Science, The University of Tokyo, [3]Research Institute for Microbial Diseases, Osaka University.

Polypyrimidine tract-binding protein 1 (PTBP1) is a highly conserved RNA-binding protein that is a well-known regulator of alternative splicing. Testicular tissue is one of the richest tissues with respect to the number of alternative splicing mRNA isoforms, but the molecular role(s) of PTBP1 in the regulation of these isoforms during spermatogenesis is still unclear. Here, we developed a germ cell-specific *Ptbp1* conditional knockout (cKO) mouse model by using the Cre-loxP system to investigate the role of PTBP1 in spermatogenesis. Testis weight in *Ptbp1* cKO mice was comparable to that in age-matched controls until 3 weeks of age; at ≥ 2 months old, testis weight was significantly lighter in cKO mice than in age-matched controls. Sperm count in *Ptbp1* cKO mice at 2 months old was comparable to that in controls, whereas sperm count significantly decreased at 6 months old. Seminiferous tubules that exhibited degeneration in spermatogenic function were more evident in the 2-month-old *Ptbp1* cKO mice than in controls. In addition, the early neonatal proliferation of spermatogonia, during postnatal days 1-5, was significantly retarded in *Ptbp1* cKO mice compared with that in controls. An *in vitro* spermatogonia culture model (germline stem cells) revealed that hydroxytamoxifen-induced deletion of PTBP1 from germline stem cells caused severe proliferation arrest accompanied by an increase of apoptotic cell death. These data suggest that PTBP1 contributes to spermatogenesis through regulation of spermatogonia prolif-

eration.

2. Seminal vesicle secretory protein 7, PATE4, is not required for sperm function but for copulatory plug formation to ensure fecundity

Taichi Noda[3], Yoshitaka Fujihara[3], Takafumi Matsumura[3,4], Seiya Oura[3,4], Sumire Kobayashi[3,4], Masahito Ikawa[3,4]: [4]Graduate School of Pharmaceutical Sciences, Osaka University.

Seminal vesicle secretions (SVSs), together with spermatozoa, are ejaculated into the female reproductive tract. SVS7, also known as PATE4, is one of the major SVS proteins found in the seminal vesicle, copulatory plug, and uterine fluid after copulation. Here, we generated *Pate4* knockout (−/−) mice and examined the detailed function of PATE4 on male fecundity. The morphology and weight of *Pate4* −/− seminal vesicles were comparable to the control. Although *Pate4* −/− cauda epididymal spermatozoa have no overt defects during in vitro fertilization, *Pate4* −/− males were subfertile. We found that the copulatory plugs were smaller in the vagina of females mated with *Pate4* −/− males, leading to semen leakage and a decreased sperm count in the uterus. When the females mated with *Pate4* −/− males were immediately re-caged with *Pate4* +/+ males, the females had subsequent productive matings. When the cauda epididymal spermatozoa were injected into the uterus and plugged artificially [artificial insemination (AI)], *Pate4* −/− spermatozoa could efficiently fertilize eggs as compared to wild-type spermatozoa. We finally examined the effect of SVSs on AI, and observed no difference in fertilization rates between *Pate4* +/+ and *Pate4* −/− SVSs. In conclusion, PATE4 is a novel factor in forming the copulatory plug that inhibits sequential matings and maintains spermatozoa in the uterus to ensure male fecundity.

3. Polypyrimidine tract binding protein (Ptbp1) expression by Sertoli cells is essential for sperm production

Manabu Ozawa, Masahito Ikawa[3], Nobuaki Yoshida[2]

Alternative splicing (AS), by which multiple forms of protein from a single gene are translated, is an important mechanisms for regulating proper development or homeostasis of specific type of cells in mammals. Testis shows more AS than any other tissue except bran. Although AS regulation in testicular germ cells during spermatogenesis is becoming to be uncovered, its role in Sertoli cells, testicular somatic cells and play an essential role to support spermatogenesis through the life period, is almost unknown. In the present study, we showed that expression of polypyrimidine tract binding protein (PTBP1), known as one of key factors to regulate AS, in Sertoli cells is essential for spermatogenesis. We show that PTPB1 is abundantly expressed by Sertoli cells and spermatogonia in the testis, and less evident or none in spermatocyte or spermatid. Eighty-three percent (five out of six) of Sertoli cell specific Ptbp1 KO male (Ptbp1 cKO) were infertile, and sperm counts from epididymis at 8 to 9-week-old was almost 50-fold lower compared to the Control (0.5×10^6 vs 24.2×10^6, $P<0.01$). Immunohistochemical analysis revealed that Ptbp1 cKO shows an increase of apoptosis of germ cells but not of Sertoli cells, and the presence of giant multinucleated cells in the lumen of seminiferous tubules. Furthermore, aberrant expression of Connexin43, a key protein of gap junction contributing blood-testis-barrier, and abnormal detachment of Sertoli cells from basal membrane were frequently observed in the Ptbp1 cKO. The data suggests that Ptbp1 plays an important role to regulate germ cell differentiation into spermatid. NGS analysis for determining transcriptome and splicome of Ptbp1 cKO Sertoli cells are currently ongoing.

Publications

1. Noda T, Fujihara Y, Matsumura T, Oura S, Kobayashi S, Ikawa M. Seminal vesicle secretory protein 7, PATE4, is not required for sperm function but for copulatory plug formation to ensure fecundity. *Biol Reprod.* 2018 Nov 18.
2. Fujihara Y, Oji A, Kojima-Kita K, Larasati T, Ikawa M. Co-expression of sperm membrane proteins CMTM2A and CMTM2B is essential for ADAM3 localization and male fertility in mice. *J Cell Sci.* 2018 Oct 8; 131(19).
3. Abbasi F, Miyata H, Shimada K, Morohoshi A, Nozawa K, Matsumura T, Xu Z, Pratiwi P, Ikawa M. RSPH6A is required for sperm flagellum formation and male fertility in mice. *J Cell Sci.* 2018 Oct 11; 131(19).
4. Oura S, Miyata H, Noda T, Shimada K, Matsumura T, Morohoshi A, Isotani A, Ikawa M. Chimeric analysis with newly established EGFP/DsRed2-tagged ES cells identify HYDIN as essential for spermiogenesis in mice. *Exp Anim.* 2018 Aug 9.
5. Nozawa K, Satouh Y, Fujimoto T, Oji A, Ikawa

M. Sperm-borne phospholipase C zeta-1 ensures monospermic fertilization in mice. *Sci Rep.* 2018 Jan 22; 8(1): 1315.
6. Senoo M, Takijiri T, Yoshida N, Ozawa M, Ikawa M. PTBP1 contributes to spermatogenesis through regulation of proliferation in spermatogonia. *J Reprod Dev.* 2018 Nov 12.
7. Sasanuma H, Ozawa M, Yoshida N. RNA binding protein Ptbp1 is essential for BCR-mediated antibody production. *Int Immunol.* 2018 Nov 23.

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

Associate Professor　Susumu Nakae, Ph.D.

准教授　博士(農学)　　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.

IL-25 enhances Th17 cell-mediated contact dermatitis by promoting IL-1β production by dermal dendritic cells

Suto H[1], Nambu A[2], Morita H[3], Yamaguchi S[2], Numata T[2], Yoshizaki T[2], Shimura E[1,2], Arae K[3,4], Asada Y[5], Motomura K[3], Kaneko M[6,7], Abe T[7], Matsuda A[5], Iwakura Y[8], Okumura K[1], Saito H[3], Matsumoto K[3], Sudo K[9],, and Nakae S[2,10]: [1]Atopy Research Center, Juntendo University, Tokyo, [2]Laboratory of Systems Biology, Center for Experimental Medicine and Systems Biology, The Institute of Medical Science, The University of Tokyo, Tokyo, [3]Department of Allergy and Clinical Immunology, National Research Institute for Child Health and Development, Tokyo, [4]Department of Immunology, Faculty of Health Science, Kyorin University, Tokyo, [5]Department of Ophthalmology, Juntendo University School of Medicine, Tokyo, [6]Animal Resource Development Unit and [7]Genetic Engineering Team, RIKEN Center for Life Science Technologies, Kobe, [8]Center for Experimental Animal Models, Institute for Biomedical Sciences, Tokyo University of Science, Chiba, [9]Animal Research Center, Tokyo, Medical University, Tokyo, [10]Precursory Research for Embryonic Science and Technology, Japan Science and Technology Agency, Saitama.

As well as thymic stromal lymphopoietin (TSLP) and interleukin-33 (IL-33), IL-25 is known to induce Th2 cytokine production by various cell types—including Th2 cells, Th9 cells, invariant NKT cells and group 2 innate lymphoid cells—involved in Th 2-type immune responses. Since both Th2-type and Th17-type cells/cytokines are crucial for contact hypersensitivity (CHS), IL-25 may contribute to this by enhancing Th2-type immune responses. However, the precise role of IL-25 in the pathogenesis of CHS is poorly understood. In contrast to TSLP, we found that IL-25 was not essential for skin DC migration or hapten-specific Th cell differentiation in the sensitization phase of CHS. Unexpectedly, mast cell- and non-immune cell-derived IL-25 was important for hapten-specific Th17 cell-, rather than Th2 cell-, mediated inflammation in the elicitation phase of CHS by enhancing Th17-related, but not Th2-related, cytokines in the skin. In particular, IL-1 β produced by dermal dendritic cells in response to IL-25 was crucial for hapten-specific Th17 cell activation, contributing to induction of local inflammation in the elicitation phase of CHS. Our results identify a novel IL-25 inflammatory pathway involved in induction of Th17, but not Th2, cell-mediated CHS.

IL-31 is crucial for induction of pruritus, but not inflammation, in contact hypersensitivity.

Ayako Takamori[1,2], Aya Nambu[1], Keiko Sato[1], Sachiko Yamaguchi[1], Kenshiro Matsuda[3], Takafumi Numata[1,4], Takeru Sugawara[1], Takamichi Yoshizaki[1,5], Ken Arae[6], Hideaki Morita[6], Kenji Matsumoto[6], Katsuko Sudo[7], Ko Okumura[2], Jiro Kitaura[2], Hiroshi Matsuda[3] & Susumu Nakae[1,8]: [1]Laboratory of Systems Biology, Center for Experimental Medicine and Systems Biology, The Institute of Medical Science, The University of Tokyo, Tokyo, 108-8639, Japan, [2]Atopy Research Center, Juntendo University, Tokyo 113-8412, Japan, [3]Laboratory of Veterinary Molecular Pathology and Therapeutics, Division of Animal Life Science, Institute of Agriculture, Tokyo University of Agriculture and Technology, Tokyo 183-8509, Japan, [4]Department of Dermatology, Tokyo Medical University, Tokyo 160-0023, Japan, [5]Department of Cardiovascular Surgery, Saitama Medical Center, Jichi Medical University, Saitama 330-8503, Japan, [6]Department of Allergy and Clinical Immunology, National Research Institute for Child Health and Development, Tokyo 157-8535, Japan, [7]Animal Research Center, Tokyo Medical University, Tokyo 160-8402, Japan, [8]Precursory Research for Embryonic Science and Technology, Japan Science and Technology Agency, Saitama 332-0012, Japan.

IL-31, which is a member of the IL-6 family of cytokines, is produced mainly by activated $CD4^+$ T cells, in particular activated Th2 cells, suggesting a contribution to development of type-2 immune responses. IL-31 was reported to be increased in specimens from patients with atopic dermatitis, and IL-31-transgenic mice develop atopic dermatitis-like skin inflammation, which is involved in the pathogenesis of atopic dermatitis. However, the role of IL-31 in development of contact dermatitis/contact hypersensitivity (CHS), which is mediated by hapten-specific T cells, including Th2 cells, is not fully understood. Therefore, we investigated this using IL-31-deficient ($Il31^{-/-}$) mice, which we newly generated. We demonstrated that the mice showed normal migration and maturation of skin dendritic cells and induction of hapten-specific T cells in the sensitization phase of FITC-induced CHS, and normal induction of local inflammation in the elicitation phase of FITC- and DNFB-induced CHS. On the other hand, those mice showed reduced scratching frequency and duration during FITC- and/or DNFB-induced CHS. Our findings suggest that IL-31 is responsible for pruritus, but not induction of local skin inflammation, during CHS induced by FITC and DNFB.

Chitin promotes antigen-specific Th2 cell-mediated murine asthma through induction of IL-33-mediated IL-1β production by DCs

Ken Arae[1,2], Hideaki Morita[2], Hirotoshi Unno[2], Kenichiro Motomura[2], Sumika Toyama[2], Naoko Okada[2], Tatsukuni Ohno[3], Masato Tamari[2], Keisuke Orimo[2], Yuko Mishima[1], Hajime Suto[4], Ko Okumura[4], Katsuko Sudo[5], Hiroshi Miyazawa[6], Haruhiko Taguchi[1], Hirohisa Saito[2], Kenji Matsumoto[2], and Susumu Nakae[7,8]: [1]Department of Immunology, [6]Department of Medical technology, Faculty of Health Sciences, Kyorin University, Tokyo, 181-8612, Japan, [2]Department of Allergy and Clinical Immunology, National Research Institute for Child Health and Development, Tokyo, 157-8535, Japan, [3]Department of Molecular Immunology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, 113-8510, Japan, [4]Atopy Research Center, Juntendo University, Tokyo, 113-0033 Japan, [5]Animal Research Center, Tokyo Medical University, Tokyo, 160-8402, Japan, [7]Laboratory of Systems Biology, Center for Experimental Medicine and Systems Biology, The Institute of Medical Science, The University of Tokyo, Tokyo, 108-8639, Japan, [8]Precursory Research for Embryonic Science and Technology (PREST), Japan Science and Technology Agency, Saitama, Japan.

Chitin, which is a major component of house dust mites (HDM), fungi, crustaceans, etc., can activate immune cells, suggesting that it contributes to development of allergic disorders such as asthma. Although the pathophysiological sensitization route of asthmatic patients to allergens is considered via the respiratory tract, the roles of intranasally-administered chitin in development of asthma remain unclear. After ovalbumin (OVA) challenge, development of airway inflammation was profoundly exacerbated in mice sensitized with OVA in the presence of chitin. The exacerbation was dependent on IL-33, but not IL-25, thymic stromal lymphopoietin or IL-17A. Chitin enhanced IL-33-dependent IL-1β production by dendritic cells (DCs). Furthermore, chitin- and IL-33-stimulated DC-derived IL-1β promoted OVA-specific Th2 cell activation, resulting in aggravation of OVA-induced airway inflammation. These findings indicate the adjuvant activity of chitin via a new mechanism and provide important clues for development of therapeutics for allergic disorders caused by HDM, fungi and crustaceans.

IL-36α is involved in hapten-specific T-cell induction, but not local inflammation, during contact hypersensitivity

Takafumi Numata[1,2], Takamichi Yoshizaki[1,3], Sachiko Yamaguchi[a], Eri Shimura[1], Yoichiro Iwakura[4], Kazutoshi Harada[2], Katsuko Sudo[5], Ryoji Tsuboi[2], and Susumu Nakae[1]: [1]Laboratory of Systems Biology, Center for Experimental Medicine and Systems Biology, The Institute of Medical

Science, The University of Tokyo, Tokyo, 108-8639, Japan, [2]Department of Dermatology, Tokyo Medical University, Tokyo, 160-0023 Japan, [3]Department of Cardiovascular Surgery, Saitama Medical Center, Jichi Medical University, Saitama 330-8503, Japan, [4]Center for Experimental Animal Models, Institute for Biomedical Sciences, Tokyo University of Science, Chiba, 278-0022, Japan, [5]Animal Research Center, Tokyo Medical University, Tokyo 160-8402, Japan.

Levels of IL36α are known to be increased in specimens from patients with atopic dermatitis and psoriasis. In addition, it has been reported that IL-36α is crucial for development of imiquimod-induced psoriatic dermatitis in mice. On the other hand, the role of IL-36α in induction of allergic contact dermatitis/contact hypersensitivity (ACD/CHS) is poorly understood. We found that IL-36α was produced in keratinocytes of mice during imiquimod-induced psoriatic dermatitis, but it was hardly detectable in the skin of mice during either fluorescein isothiocyanate (FITC)- or 1-fluoro-2, 4-dinitrobenzene (DNFB)-induced CHS. Although IL-36α can enhance activation of dendritic cells (DCs) and T cells, in CHS, IL-36α was not essential for DC migration from the skin to draining LNs, but it was required for induction or activation of hapten-specific T cells such as Th/Tc1 or Th17 cells. However, local inflammation, assessed by measurement of ear skin thickness, was comparable between wild-type and IL-36α-deficient mice during both FITC- and DNFB-induced CHS. These observations indicate that IL-36α is involved in induction and/or activation of hapten-specific T-cell subsets in the sensitization phase of CHS, but not essential for induction of local inflammation in the elicitation phase.

The roles of IL-17C in T cell-dependent and -independent inflammatory diseases

Sachiko Yamaguchi[1,7], Aya Nambu[1], Takafumi Numata[1,2], Takamichi Yoshizaki[1,3], Seiko Narushima[1], Eri Shimura[1,4], Yoshihisa Hiraishi[1], Ken Arae[5,6], Hideaki Morita[6], Kenji Matsumoto[6], Ichiro Hisatome[7], Katsuko Sudo[8], & Susumu Nakae[1,9]: [1]Laboratory of Systems Biology, Center for Experimental Medicine and Systems Biology, The Institute of Medical Science, The University of Tokyo, Tokyo, 108-8639, Japan, [2]Department of Dermatology, Tokyo Medical University, Tokyo, 160-0023, Japan, [3]Department of Cardiovascular Surgery, Saitama Medical Center, Jichi Medical University, Saitama, 330-8503, Japan, [4]Department of Chemistry, Juntendo University School of Medicine, Chiba, 270-1695, Japan, [5]Department of Immunology, Faculty of Health Sciences, Kyorin University, Tokyo, 181-8612, Japan, [6]Department of Allergy and Clinical Immunology, National Research Institute for Child Health and Development, Tokyo, 157-8535, Japan, [7]Division of Regenerative Medicine and Therapeutics, Tottori University Graduate School of Medical Science, Yonago, 683-8503, Japan, [8]Animal Research Center, Tokyo Medical University, Tokyo 160-8402, Japan, [9]Precursory Research for Embryonic Science and Technology, Japan Science and Technology Agency, Saitama, 332-0012, Japan.

IL-17C, which is a member of the IL-17 family of cytokines, is preferentially produced by epithelial cells in the lung, skin and colon, suggesting that IL-17C may be involved in not only host defense but also inflammatory diseases in those tissues. In support of that, IL-17C was demonstrated to contribute to development of T cell-dependent imiquimod-induced psoriatic dermatitis and T cell-independent dextran sodium sulfate-induced acute colitis using mice deficient in IL-17C and/or IL-17RE, which is a component of the receptor for IL-17C. However, the roles of IL-17C in other inflammatory diseases remain poorly understood. Therefore, we investigated the contributions of IL-17C to development of certain disease models using *Il17c*$^{-/-}$ mice, which we newly generated. Those mice showed normal development of T cell-dependent inflammatory diseases such as FITC- and DNFB-induced contact dermatitis/contact hypersensitivity (CHS) and concanavalin A-induced hepatitis, and T cell-independent inflammatory diseases such as bleomycin-induced pulmonary fibrosis, papain-induced airway eosinophilia and LPS-induced airway neutrophilia. On the other hand, those mice were highly resistant to LPS-induced endotoxin shock, indicating that IL-17C is crucial for protection against that immunological reaction. Therefore, IL-17C neutralization may represent a novel therapeutic approach for sepsis, in addition to psoriasis and acute colitis.

IL-33, IL-25 and TSLP contribute to development of fungal-associated protease-induced innate-type airway inflammation

Yoshihisa Hiraishi[1,2], Sachiko Yamaguchi[1], Takamichi Yoshizaki[1,5], Aya Nambu[1], Eri Shimura[1,3], Ayako Takamori S[1], Seiko Narushima[1], Wakako Nakanishi[1], Yosuke Asada[1,4], Takafumi Numata[1], Maho Suzukawa[6], Yasuhiro Yamauchi[2], Akira Matsuda[4], Ken Arae[7], Hideaki Morita[7], Tomoaki Hoshino[8], Hajime Suto[3], Ko Okumura[3], Kenji Matsumoto[7], Hirohisa Saito[7], Katsuko Sudo[9], Motoyasu Iikura[10], Takahide Nagase[2], & Susumu Nakae[1,11]: [1]Laboratory of Systems Biology, Center for Experimental Medicine and Systems Biology, The Institute of Medical Science, The University of Tokyo, Tokyo 108-8639, Japan, [2]Department of Respiratory Medicine, Graduate School of Medicine, The University of Tokyo, Tokyo 113-8655,

Japan, [3]Atopy Research Center, Juntendo University School of Medicine, Tokyo 113-8412, Japan, [4]Department of Ophthalmology, Juntendo University School of Medicine, Tokyo 113-8412, Japan, [5]Department of Cardiovascular Surgery, Saitama Medical Center, Jichi Medical University, Saitama 330-8503, Japan, [6]National Hospital Organization Tokyo National Hospital, Tokyo 204-8585, Japan, [7]Department of Allergy and Clinical Immunology, National Research Institute for Child Health and Development, Tokyo 157-8535, Japan, [8]The 1st Department of Medicine, Kurume University School of Medicine, Fukuoka 830-0011, Japan, [9]Animal Research Center, Tokyo Medical University, Tokyo 160-8402, Japan, [10]Department of Respiratory Medicine, National Center for Global Health and Medicine, Tokyo 162-8655, Japan, [11]Precursory Research for Embryonic Science and Technology (PRESTO), Japan Science and Technology Agency, Saitama 332-0012, Japan.

Certain proteases derived from house dust mites and plants are considered to trigger initiation of allergic airway inflammation by disrupting tight junctions between epithelial cells. It is known that inhalation of proteases such as house dust mite-derived Der p1 and/or papaya-derived papain caused airway eosinophilia in naïve mice and even in *Rag*-deficient mice that lack acquired immune cells such as T, B and NKT cells. In contrast, little is known regarding the possible involvement of proteases derived from *Aspergillus* species (fungal-associated proteases; FAP), which are ubiquitous saprophytic fungi in the environment, in the development of allergic airway eosinophilia. Here, we found that inhalation of FAP by naïve mice led to airway eosinophilia that was dependent on protease-activated receptor-2 (PAR2), but not TLR2 and TLR4. Those findings suggest that the protease activity of FAP, but not endotoxins in FAP, are important in the setting. In addition, development of that eosinophilia was mediated by innate immune cells (ILCs) such as innate lymphoid cells, but not by acquired immune cells such as T, B and NKT cells. Whereas IL-33, IL-25 and thymic stromal lymphopoietin (TSLP) are involved in induction of FAP-induced ILC-mediated airway eosinophilia, IL-33—rather than IL-25 and/or TSLP—was critical for the eosinophilia in our model. Our findings improve our understanding of the molecular mechanisms involved in induction of airway inflammation by FAP.

Publications

1. Sugita, K,. Steer, CA,. Martinez-Gonzalez, I,. Altunbulakli, C,. Morita, H,. Castro-Giner, F,. Kubo, T,. Wawrzyniak, P,. Rückert, B,. Sudo, K,. Nakae, S,. Matsumoto, K,. O'Mahony, L,. Akdis, M,. Takei, F,. Akdis, CA. Type 2 innate lymphoid cells disrupt bronchial epithelial barrier integrity by targeting tight junctions through IL-13 in asthmatic patients. J Allergy Clin Immunol. 141(1): 300-310, 2018.
2. Tamari, M,. Orimo, K,. Motomura, K,. Arae, K,. Matsuda, A,. Nakae, S,. Saito, H,. Morita, H,. Matsumoto, K. The optimal age for epicutaneous sensitization following tape-stripping in BALB/c mice. Allergol Int. 67(3): 380-387, 2018.
3. Suto, H,. Nambu, A,. Morita, H,. Yamaguchi, S,. Numata, T,. Yoshizaki, T,. Shimura, E,. Arae, K,. Asada, Y,. Motomura, K,. Kaneko, M,. Abe, T,. Matsuda, A,. Iwakura, Y,. Okumura, K,. Saito, H,. Matsumoto, K,. Sudo, K,. Nakae, S. IL-25 enhances T_H17 cell-mediated contact dermatitis by promoting IL-1β production by dermal dendritic cells. J Allergy Clin Immunol. 142 (5): 1500-1509, 2018.
4. Takamori, A,. Nambu, A,. Sato, K,. Yamaguchi, S,. Matsuda, K,. Numata, T,. Sugawara, T,. Yoshizaki, T,. Arae, K,. Morita, H,. Matsumoto, K,. Sudo, K,. Okumura, K,. Kitaura, J,. Matsuda, H,. Nakae, S. IL-31 is crucial for induction of pruritus, but not inflammation, in contact hypersensitivity. Sci Rep. 8(1): 6639, 2018.
5. Luo, Q,. Fan, Y,. Lin, L,. Wei, J,. Li, Z,. Li, Y,. Nakae, S,. Lin, W,. Chen, Q. Interleukin-33 Protects Ischemic Brain Injury by Regulating Specific Microglial Activities. Neuroscience. 385: 75-89, 2018.
6. Tang, C,. Kakuta, S,. Shimizu, K,. Kadoki, M,. Kamiya, T,. Shimazu, T,. Kubo, S,. Saijo, S,. Ishigame, H,. Nakae, S,. Iwakura, Y. Suppression of IL-17F, but not of IL-17A, provides protection against colitis by inducing T_{reg} cells through modification of the intestinal microbiota. Nat Immunol. 19(7): 755-765, 2018.
7. Arae, K,. Morita, H,. Unno, H,. Motomura, K,. Toyama, S,. Okada, N,. Ohno, T,. Tamari, M,. Orimo, K,. Mishima, Y,. Suto, H,. Okumura, K,. Sudo, K,. Miyazawa, H,. Taguchi, H,. Saito, H,. Matsumoto, K,. Nakae, S. Chitin promotes antigen-specific Th2 cell-mediated murine asthma through induction of IL-33-mediated IL-1β production by DCs. Sci Rep. 8(1): 11721, 2018.
8. Matsumoto, Y,. Yokoi, H,. Kimura, T,. Matsumoto, Y,. Kawada, M,. Arae, K,. Nakae, S,. Ikeda, T,. Matsumoto, K,. Sakurai, H,. Saito, K. Gastrin-releasing peptide is involved in the establishment of allergic rhinitis in mice. Laryngoscope. 128(11): E377-E384, 2018.
9. Osada, Y,. Horie, Y,. Nakae, S,. Sudo, K,. Kanazawa, T. STAT6 and IL-10 are required for

the anti-arthritic effects of Schistosoma mansoni via different mechanisms. Clin Exp Immunol. 195(1): 109-120, 2018.
10. Numata, T,. Yoshizaki, T,. Yamaguchi, S,. Shimura, E,. Iwakura, Y,. Harada, K,. Sudo, K,. Tsuboi, R,. Nakae, S. IL-36α is involved in hapten-specific T-cell induction, but not local inflammation, during contact hypersensitivity. Biochem Biophys Res Commun. 506(3): 429-436, 2018.
11. Yamaguchi, S,. Nambu, A,. Numata, T,. Yoshizaki, T,. Narushima, S,. Shimura, E,. Hiraishi, Y,. Arae, K,. Morita, H,. Matsumoto, K,. Hisatome, I,. Sudo, K,. Nakae, S. The roles of IL-17C in T cell-dependent and -independent inflammatory diseases. Sci Rep. 8(1): 15750, 2018.
12. Hiraishi, Y,. Yamaguchi, S,. Yoshizaki, T,. Nambu, A,. Shimura, E,. Takamori, A,. Narushima, S,. Nakanishi, W,. Asada, Y,. Numata, T,. Suzukawa, M,. Yamauchi, Y,. Matsuda, A,. Arae, K,. Morita, H,. Hoshino, T,. Suto, H,. Okumura, K,. Matsumoto, K,. Saito, H,. Sudo, K,. Iikura, M,. Nagase, T,. Nakae, S. IL-33, IL-25 and TSLP contribute to development of fungal-associated protease-induced innate-type airway inflammation. Sci Rep. 8(1): 18052, 2018.