International Research and Development Center for Mucosal Vaccine

Division of Mucosal Barriology
粘膜バリア学分野

Visiting Professor　Koji Hase, Ph.D.

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The goal of our research is to explore antigen uptake receptors on specialized epithelial M cells to identify potential targets for mucosal vaccine delivery. Thus, this division aims to develop novel mucosal vaccines by taking advantage of the conjugation of M-cell-receptor ligands with various vaccine antigens.

1. Botulinum toxin A complex exploits intestinal M cells to enter the host and exert neurotoxicity.

Takuhiro Matsumoto, Yo Sugawara, Masahiro Yutani, Sho Amatsu, Hideo Yagita, Tomoko Kohda, Yutaka Nakamura, Shinji Fukuda, Koji Hase, Hiroshi Ohno and Yukako Fujinaga

To cause food-borne botulism, botulinum neurotoxin (BoNT) in the gastrointestinal lumen must traverse the intestinal epithelial barrier. However, the mechanism by which BoNT crosses the intestinal epithelial barrier remains unclear. BoNTs are produced along with one or more non-toxic components, with which they form progenitor toxin complexes (PTCs). Here we show that serotype A1 L-PTC, which has high oral toxicity and makes the predominant contribution to causing illness, breaches the intestinal epithelial barrier from microfold (M) cells via an interaction between haemagglutinin (HA), one of the non-toxic components, and glycoprotein 2 (GP2). HA strongly binds to GP 2 expressed on M cells, which do not have thick mucus layers. Susceptibility to orally administered L-PTC is dramatically reduced in M-cell-depleted mice and GP2-deficient ($Gp2^{-/-}$) mice. Our finding provides the basis for the development of novel antitoxin therapeutics and delivery systems for oral biologics.

2. The microbiota regulates type 2 immunity through RORγt$^+$ T cells

Caspar Ohnmacht, Joo-Hong Park, Sascha Cording, James B. Wing, Koji Atarashi, Yuuki Obata, Valérie Gaboriau-Routhiau, Rute Marques, Maria Fedoseeva, Meinrad Busslinger, Nadine Cerf-Bensussan, Ivo G. Boneca, David Voehringer, Koji Hase, Kenya Honda, Shimon Sakaguchi, Gérard Eberl

Changes to the symbiotic microbiota early in life, or the absence of it, can lead to altered type 2 immunity, including predisposing individuals to developing allergy. While it is unclear how the microbiota regulates type 2 immunity, it is a strong inducer of pro-inflammatory T helper (Th) 17 cells and regulatory T cells (Tregs) in the intestine. Here, we report that microbiota-induced Tregs express the nuclear hormone receptor RORγt, and differentiate along a pathway that also leads to Th17 cells and is regulated by the vitamin A metabolite retinoic acid. RORγt$^+$ Tregs, and more generally RORγt$^+$ T cells, inhibit the generation of Gata3$^+$ T cells, which include Th2 cells and the other major population of intestinal Tregs. In the absence of RORγt$^+$ Tregs, Th2-driven worm expulsion is more

efficient while Th2-associated pathology is exacerbated. Thus, the microbiota regulates type 2 responses through the induction of "type 3" RORγt$^+$ Tregs and Th17 cells, and acts as a key factor in balancing immune responses at mucosal surfaces.

Publications

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International Research and Development Center for Mucosal Vaccine

Division of Innate Immune Regulation
自然免疫制御分野

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Innate immunity is the 'gateway' of immune response. By controlling innate immunity, it is thought that the whole immunity is controllable. Our major focus is the elucidation and understanding of molecular function of the innate immune cells in small intestine for the development of mucosal vaccine against infectious diseases and mucosal immune therapy for inflammatory bowel diseases, food allergy and cancer.

1. Development of next-generation vaccine targeting on DCs in small intestinal lamina propira (LP)

Naoki Takemura[1], Kouta Matsunaga[1], Yasuo Ouchi[1], Kouji Kobiyama[2], Taiki Aoshi[2], Ken J Ishii[2], Shintaro Sato[3], Shizuo Akira[4], Hiroshi Kiyono[3], Satoshi Uematsu[1]: [1]Division of Innate Immune regulation, International Research and Development Center for Mucosal Vaccines, The Institute of Medical Science, The University of Tokyo. [2]Laboratory of Adjuvant Innovation, National Institute of Biomedical Innovation. [3]Division of Mucosal Immunology, Department of Microbiology and Immunology, The Institute of Medical Science, The University of Tokyo. [4]Laboratory of Host Defense, WPI Immunology Frontier Research Center, Osaka University.

$CD103^+$ DCs are the major conventional DC population in the intestinal lamina propria (LP). Our previous report showed that low density cells in the LP could be classified into four subsets based on the difference in CD11c/CD11b expression patterns: $CD11c^{hi}CD11b^{lo}$ DCs, $CD11c^{hi}CD11b^{hi}$ DCs, $CD11c^{int}CD11b^{int}$ macrophages and $CD11c^{int}CD11b^{hi}$ eosinophils. The $CD11c^{hi}CD11b^{hi}$ DCs, which are $CD103^+$, specifically express Toll-like receptor (TLR) 5 and TLR9, and induce the differentiation of naïve B cells into IgA^+ plasma cells. These DCs also mediate the differentiation of antigen (Ag)-specific Th17 and Th1 cells in response to flagellin. Intraperitoneal injection of activated Ag-loaded $CD11c^{hi}$ $CD11b^{hi}$ DCs induce Ag-specific IgG in serum and IgA in stool samples, Th1 and Th17 responses and strong cytotoxic T lymphocytes (CTL) activity. Thus, $CD11c^{hi}CD11b^{hi}$ DCs are suitable targets for oral vaccines in the intestine. $CD11c^{hi}CD11b^{hi}$ LPDCs but not conventional DCs in other tissues specifically express Raldh2, which catalyzes the conversion of retinal to retinoic acid. Recent report showed that Raldh2 is essential for the induction of IgA. We found that GM-CSF, essential differentiation factor for LPDC can induce Raldh2 in conventional DCs in spleens (SP). Intraperitoneal injection of Ag-loaded conventional SPDCs treated with GM-CSF induced Ag-specific IgA similar to $CD11c^{hi}$ $CD11b^{hi}$ LPDCs. This evidence showed that the reagent which can induce Raldh2 can be a adjuvant to induce IgA class switching. We started to screen microbial components to induce Raldh2 in conventional DCs.

2. Analysis of resident macrophages in small intestinal LP

Yasuo Ouchi[1], Naoki Takemura[1], Kouta Matsunaga[1], Shintaro Sato[2], Yasumasa Kimura[3], Shizuo Akira[4], Hiroshi Kiyono[2], Satoshi Uematsu[1]: [1]Division of Innate Immune regulation, International Research and Development Center for Mucosal Vaccines, The Institute of Medical Science, The University of Tokyo. [2]Division of Mucosal Immunology, Department of Microbiology and Immunology, The Institute of Medical Science, The University of Tokyo. [3]Division of Systems Immunology, International Research and Development Center for Mucosal Vaccines, The Institute of Medical Science, The University of Tokyo. [4]Laboratory of Host Defense, WPI Immunology Frontier Research Center, Osaka University.

$CD11c^{int}CD11b^{int}$ cells in small intestinal LP are resident macrophages. They specifically express chemokine receptor CX3CR1 in intestinal LP. Their phagocytotic activity is very strong. Although they express MHC class II, they cannot move from LP to draining lymph nodes effectively, suggesting that they may be involved in local immune responses in intestine. They express TLR4, TLR7 and TLR9 and produce TNF-α and IL-10 by TLR stimulation. We performed microarray analysis in the $CD11c^{int}$ $CD11b^{int}$ cells, $CD11c^{hi}CD11b^{hi}$ cells, splenic $CD11c^{+}$ DCs and peritoneal macrophages with or without stimulation by TLR ligand and compared signaling pathways among them.

3. Role of intestinal eosinophils in radiation injury

Naoki Takemura[1], Kouta Matsunaga[1], Yasuo Ouchi[1], Shintaro Sato[2], Yosuke Kurashima[2], Eun Jeong Park[2], Lamichhane Aayam[2], Kazuyo Moro[4], Shigeo Koyasu[4], Shizuo Akira[3], Hiroshi Kiyono[2], Satoshi Uematsu[1]: [1]Division of Innate Immune regulation, International Research and Development Center for Mucosal Vaccines, The Institute of Medical Science, The University of Tokyo. [2]Division of Mucosal Immunology, Department of Microbiology and Immunology, The Institute of Medical Science, The University of Tokyo. [3]Laboratory of Host Defense, WPI Immunology Frontier Research Center, Osaka University. [4]Department of Microbiology and Immunology, Keio University School of Medicine.

Radiation-induced intestinal fibrosis (RIF) is a serious complication after abdominal radiotherapy. We show RIF is mediated by eosinophil interactions with α-smooth muscle actin $(\alpha\text{-SMA})^{+}$ stromal cells. Abdominal irradiation induced fibrosis of the submucosa (SM) associated with the excessive accumulation and degranulation of eosinophils in the absence of lymphocytes. Eosinophil-deficiency markedly ameliorated RIF, suggesting their importance. Chronic crypt necrosis post-irradiation elevated extracellular adenosine triphosphate levels, which induced C-C motif chemokine 11 (CCL11) and granulocyte-macrophage colony-stimulating factor (GM-CSF) expression by pericryptal $\alpha\text{-SMA}^{+}$ cells that attracted and activated eosinophils, respectively. Transforming growth factor-β1 from GM-CSF-stimulated eosinophils promoted collagen expression by $\alpha\text{-SMA}^{+}$ cells. Upon co-stimulation with GM-CSF and CCL11, eosinophils released granule protein, which up-regulated CCL11 and profibrotic matrix metalloproteinase expression by $\alpha\text{-SMA}^{+}$ cells, facilitating eosinophil-mediated fibrogenesis. Thus, the mutual activation of eosinophils and $\alpha\text{-SMA}^{+}$ cells creates a positive feedback loop that mediates RIF progression. These findings aid the development of effective therapeutic strategies.

4. Blockade of TLR3 protects mice from radiation injury.

Naoki Takemura[1], Kouta Matsunaga[1], Takumi Kawasaki[2], Jun Kunisawa[3], Shintaro Sato[4], Kouji Kobiyama[5], Taiki Aoshi[5], Junichi Ito[6], Kenji Mizuguchi[6], Thangaraj Karuppuchamy[2], Shoichiro Miyatake[7], Nobuko Mori[8], Tohru Tsujimura[9], Yutaro Kumagai[2], Taro Kawai[2], Ken J Ishii[5], Hiroshi Kiyono[4], Shizuo Akira[2], Satoshi Uematsu[1]: [1]Division of Innate Immune regulation, International Research and Development Center for Mucosal Vaccines, The Institute of Medical Science, The University of Tokyo. [2]Laboratory of Host Defense, WPI Immunology Frontier Research Center, Osaka University. [3]Laboratory of Vaccine Materials, National Institute of Biomedical Innovation. [4]Division of Mucosal Immunology, Department of Microbiology and Immunology, The Institute of Medical Science, The University of Tokyo. [5]Laboratory of Adjuvant Innovation, National Institute of Biomedical Innovation. [6]Laboratory of Bioinformatics, National Institute of Biomedical Innovation. [7]Laboratory of Self Defense Gene Regulation, Tokyo Metropolitan Institute of Medical, Science. [8]Department of Biological Science, Graduate School of Science, Osaka Prefecture University. [9]Department of Pathology, Hyogo College of Medicine.

High-dose ionizing radiation induces severe DNA damage in the epithelial stem cells in small intestinal crypts and causes gastrointestinal syndrome (GIS). Although the tumor suppressor p53 is a primary factor inducing death of crypt cells with DNA damage, its essential role in maintaining genome stability means inhibiting p53 to prevent GIS is not a viable strategy. Here, we show that the innate immune receptor Toll-like receptor 3 (TLR3) is critical

for the pathogenesis of GIS. *Tlr3*$^{-/-}$ mice show substantial resistance to GIS owing to significantly reduced radiation-induced crypt cell death. Despite showing reduced crypt cell death, p53-dependent crypt cell death is not impaired in *Tlr3*$^{-/-}$ mice. p53-dependent crypt cell death causes leakage of cellular RNA, which induces extensive cell death via TLR3. An inhibitor of TLR3-RNA binding ameliorates GIS by reducing crypt cell death. Thus, we propose blocking TLR3 activation as a novel and preferable approach to treat GIS. We are further analyzing the role of TLR3 in radiation-induced oral mucositis.

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International Research and Development Center for Mucosal Vaccine

Group I
グループ I

Project Associate Professor　Yoshiyuki Goto, Ph.D.　特任准教授　医学博士　後　藤　義　幸

Gastrointestinal tract is a unique organ which is constitutively exposed by various antigens including commensal bacteria. In order to create symbiotic environment to non-pathogenic microorganisms, epithelial cells (ECs) and immune cells cooperatively establish homeostasis of intestinal microenvironment. We aim to identify the mechanisms of epithelial α1, 2-fucosylation, one of symbiotic factors between host and microbiota and uncover the role of ECs-immune cell network in the establishment of intestinal homeostasis.

1. Innate lymphoid cells govern intestinal epithelial fucosylation

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α1, 2-fucose added to the terminal carbohydrate moiety expressed on intestinal epithelial cells is catalyzed by fucosyltransferase 2 (Fut2). Epithelial α1, 2-fucose is one of symbiotic factors which mediate host-microbiota interaction. For example, epithelial α1, 2-fucose is utilized as a dietary carbohydrate by various symbiotic bacteria such as *Bacteroides*. However, the molecular and cellular mechanisms of the induction of epithelial fucosylation remain unknown. We found that group 3 innate lymphoid cells (ILC3) are critical inducers of intestinal epithelial Fut2 expression and fucosylation that is mediated by the production of interleukin 22 and lymphotoxin from ILC3 in a commensal bacteria-dependent and -independent manner, respectively. Fut2-deificient mice are susceptible to the infection by pathogenic microorganisms. These data unveil a novel function of ILC3 in creating the appropriate symbiotic environment and protective platform against pathogenic microorganisms through regulating the epithelial glycosylation.

2. IL10-producing CD4 T cells negatively regulate epithelial fucosylation

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Fucosylated glycans expressed on the epithelial surfaces contribute to regulate intestinal homeostasis by serving as a nutrient source for symbionts. However, the detail mechanism of the regulation of

epithelial α1, 2-fucose is still unknown. We found that epithelial α1, 2-fucosylation is negatively regulated by IL-10-producing $CD4^+$ T cells. The number of fucosylated ECs was increased in mice lacking T cells, especially those expressing αβ T cell receptor (TCR), CD4, and IL-10. No such effect was observed in mice lacking B cells and other subsets to T cells. Adoptive transfer of TCRαβ $chain^+$ $CD4^+$ T cells from normal mice, but not IL-10-deficient mice, normalized fucosylation of ECs. These findings suggest that IL-10 produced by $CD4^+$ T cells contribute to the maintenance of the α1, 2-fucosyation of ECs.

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*equally contribution

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