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. Intestinal M cells contribute to maintenance of gut immune homeostasis

Nakamura Yutaka, Yarimizu Chiaki, Murayama Syun, Kaisho Tsuneyasu, Jun Kunisawa, Kiyono Hiroshi, and Hase Koji

The specialized epithelial microfold (M) cells actively transport luminal antigens to organized lymphoid follicle to induce antigen-specific IgA response in the gut mucosa. However, it still remains unknown whether the M cell-dependent antigen uptake contributes to the establishment and maintenance of gut immune homeostasis. Here we investigated the biological significance of M cells by taking advantage of Spi-B knock-out (KO) mice that lack mature M cells. We first analyzed the development of gut-associated lymphoid tissues (GALTs), and found that Spi-B KO mice possess smaller colonic patches with fewer number of germinal center B cells compared to wild type (WT) mice. Correspondingly, commensal bacteria-specific IgA and helper T cell responses were attenuated in Spi-KO mice. Thus, M cell-dependent antigen transport is essential for the maturation of GALT as well as mucosal immune response to commensals. We further analyzed the role of M cells in the protection against mucosal infection with *Citrobacter rodentium*. Spi-B KO mice were highly susceptible to *C. rodentium* infection compared to WT mice, as evidenced by body weight loss, severe fecal clinical score, and enhanced bacterial translocation into the body. Chronic inflammatory symptoms characterized by edema and inflammatory cell infiltration were evident in the colonic lamina propria of Spi-B KO mice at the late stage of infection. Based on these observations, M cell-dependent antigen uptake shapes mucosal barrier functions by inducing mucosal antigen-specific immune response, which minimizes bacterial invasion and prevents development of chronic inflammation.

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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)

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$CD103^+$ dendritic cells (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 Ag-specific Th17 and Th1 cells in response to flagellin. Intraperitoneal injection of activated antigen(Ag)-loaded $CD11c^{hi}CD11b^{hi}$ DCs induced Ag-specific IgG in serum and IgA in stool samples, Th1 and Th17 responses and strong 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 and found that β1,3-glucan is a strong inducer of Raldh 2. We will check immune responses by using β1,3-glucan as a mucosal adjubant.

2. Analysis of resident macrophages in small intestinal LP

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$CD11c^{int}CD11b^{int}$ 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 of TLR ligand and compared signaling pathways among them. We found several candidate genes which specifically express in $CD11c^{int}CD11b^{int}$ cells. We will generate gene-targeting mice and will examine the in vivo function of them in $CD11c^{int}$ $CD11b^{int}$ cells.

3. Role of intestinal eosinophils in radiation injury

Naoki Takemura[1], Kouta Matsunaga[1], Yasuo Ouchi[2], Shintaro Sato[3], Yosuke Kurashima[3], Shizuo Akira[4], Hiroshi Kiyono[3], Satoshi Uematsu[1,2]: [1]Division of Innate Immune Regulation, International Research and Development Center for Mucosal Vaccines, The Institute of Medical Science, The University of Tokyo. [2]Department of Mucosal Immunology, School of Medicine, Chiba University. [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.

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 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. We are further generating an antibody which can deplete eosinophils and analyze its therapeutic effects on RIF.

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 Rgulation, 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 syn-

drome (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 further analyzed 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

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Gastrointestinal tract is a unique organ which is constitutively exposed by various antigens including commensal microbiota. In order to create symbiotic environment to non-pathogenic luminal 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 α1, 2-fucosylation:

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α1, 2-fucosyl linkages located to 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*. Therefore, disruption of Fut2 leads to dysbiosis both in mice and human and predisposed to the development of inflammatory diseases such as Crohn's disease. Despite of the importance for intestinal and systemic homeostasis, the molecular and cellular mechanisms of the induction of epithelial Fut2 and subsequent α1, 2-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 α1, 2-fucosylation.

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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