International Research and Development Center for Mucosal Vaccines

Division of Mucosal Barriology
粘膜バリア学分野

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Humans harbor over 100 trillion bacteria in the distal intestine. These intestinal bacteria have long been appreciated for the benefits they provide to the host, the most obvious being their capacity to metabolize indigestible food components to small metabolites that are utilized as nutrients by host cells. Moreover, it is now clear that the presence of commensal bacteria contributes to shape epithelial barrier functions and the gut immune system. We explored the mechanism of the immune regulation by intestinal bacteria.

1. The epigenetic regulator Uhrf1 facilitates functional expansion of colonic regulatory T cells

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Mammals and gut microbiota have co-evolved over 100 million years to establish a unique ecosystem based on symbiotic interactions. During this time, the host has developed sophisticated defense functions as well as regulatory mechanisms to avoid excessive immune responses to commensal microbiota. Colonisation by gut microbiota facilitates expansion of colonic Foxp3^{+} regulatory T (Treg) cells, which play a pivotal role in suppression of excessive inflammation such as inflammatory bowel diseases (IBD). The molecular machinery controlling Treg homeostasis in the gut, however, remains largely unknown. We found that a DNA methylation adaptor Uhrf1/NP95 regulates homeostasis and functionality of colonic Treg cells. Colonisation of germ-free mice with gut microbiota upregulated the expression of Uhrf1 in Treg cells. Mice with T-cell-specific deletion of Uhrf1 (*CD4*^{cre}*Uhrf1*$^{fl/fl}$) displayed normal development of thymic Treg (tTreg) cells, but were defective in proliferation and functional maturation of colonic Treg cells in response to colonisation by gut microbiota. We also found that ablation of Uhrf1 de-repressed a cell cycle-dependent kinase inhibitor *Cdkn1a* due to hypomethylation of its promoter region, resulting in cell cycle arrest of Treg cells. *CD4*^{cre}*Uhrf1*$^{fl/fl}$ mice housed under specific pathogen-free (SPF) conditions spontaneously developed an IBD-like disease as a consequence of impaired Treg expansion. These observations suggest that Uhrf1-dependent epigenetic silencing of *Cdkn1a* is required for the maintenance of immune homeostasis in the intestine. This mechanism secures symbiotic host-microbe interactions without an inflammatory response.

2. Epithelial-stromal interaction via Notch signaling is essential for the full maturation of gut-associated lymphoid tissues

Yuuki Obata, Shunsuke Kimura, Gaku Nakato, Keito Iizuka, Yurika Miyagawa, Yutaka Nakamura, Yukihiro Furusawa, Machiko Sugiyama, Keiichiro Suzuki, Masashi Ebisawa, Yumiko Fujimura, Hisahiro Yoshida, Toshihiko Iwanaga,

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Intrinsic Notch signaling in intestinal epithelial cells restricts secretory cell differentiation. In gut-associated lymphoid tissue (GALT), stromal cells located beneath the follicle-associated epithelium (FAE) abundantly express the Notch ligand delta-like 1 (Dll1). Here, we show that mice lacking *Rbpj* -a gene encoding a transcription factor implicated in Notch signaling- in intestinal epithelial cells, have defective GALT maturation. This defect can be attributed to the expansion of goblet cells, which leads to the down-regulation of CCL20 in FAE. These data demonstrate that epithelial Notch signaling maintained by stromal cells contributes to the full maturation of GALT by restricting secretory cell differentiation in FAE.

Publications

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International Research and Development Center for Mucosal Vaccines

Division of Innate Immune Recognition
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 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 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. Now we are analyzing adjuvant effect of GM-CSF for the induction of mucosal immunity.

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. Unlike inflammatory macrophages, they do not produce IL-6 and IL-12p40 by TLR ligand stimulation. We are analyzing on signaling pathways of TLR4, 7 and 9 in these macrophages.

3. Analysis of eosinophils in intestinal LP

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In contrast to other organs, eosinophils are abundantly found in small intestinal LP. However, their function in homeostasis and roles in infection have not been fully elucidated. They highly express eosinophil markers such as CCR3 and siglec-F. They are activated by Th2 cytokines such as IL-4 and IL-13. They express only TLR4 and produce IL-6 in response to lipopolysaccharide (LPS). We are analyzing the roles of eosinophils in intestinal parasite infection by using Δdbl-Gata mice, in which eosinophils diminished. We also analyzing the function of eosinophils in intestinal tissue remodeling.

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.

Publications

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