International Research and Development Center for Mucosal Vaccines

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

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Innate immunity is the 'gateway' of an 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. Analysis of dendritic cells (DCs) in small intestinal lamina propira (LP)

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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 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. We found that small intestine CD103$^+$ DCs of the LP (LPDCs) could be divided into a small subset of CD8α$^+$ cells and a larger subset of CD8α$^-$ cells. Flow cytometry analysis revealed that CD103$^+$CD8α$^+$ and CD103$^+$CD8α$^-$ LPDCs were equivalent to CD11c^{hi}CD11b^{lo} and CD11c^{hi}CD11b^{hi} subsets, respectively. Whereas CD103$^+$CD8α$^+$ LPDCs expressed TLR3, TLR7 and TLR9, CD103$^+$CD8α$^-$ LPDCs expressed TLR5 and TLR9. Both LPDC types produced IL-6 and IL-12p40, but not TNF-α, IL-10 or IL-23, following TLR ligand stimulation. Only CD103$^+$CD8α$^-$ LPDCs expressed the retinoic acid-converting enzyme *Raldh2* and were involved in T cell-independent IgA synthesis and FoxP3$^+$ regulatory T cell induction. CD103$^+$CD8α$^+$ LPDCs induced Ag-specific IgG in serum, Th1 responses and cytotoxic T lymphocyte (CTL) activity. By contrast, CD103$^+$CD8α$^-$ LPDCs induced Ag-specific IgG in serum and IgA in stool samples, Th1 and Th17 responses and strong CTL activity. Thus, from both quantitative and qualitative viewpoints, CD103$^+$CD8α$^-$ LPDCs are suitable targets for oral vaccines in the intestine. We will generate a new mucosal vaccine, which targets on CD103$^+$CD8α$^-$ LPDCs as antigen-presenting cells for the induction of neutralizing IgA antibody.

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. Since they do not produce IL-6 and IL-12, they are regulatory macrophages rather than inflammatory macrophages. Interestingly, they strongly induce $FoxP3^+$ regulatory T cells in the presence of TGF-β. We are analyzing the function of $CD11c^{int}CD11b^{int}$ macrophages in oral tolerance induction. Although they produce IL-10 in response to TLR ligand stimulation, they do not express M2 macrophage markers. We will also analyze function and activation of these macrophages in intestinal parasite infection.

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. Innate Immune Functions in Radiation-Induced G I syndrome

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Recent studies have suggested that TLRs recognize microorganisms as well as endogenous factors released from damaged tissues as danger signal and trigger inflammation under aseptic condition. High-dose ionizing radiation induces fatal disorders in normal tissues, called radiation syndrome, which are dose-dependent and classically divided into three main manifestations; hematopoietic (>3 Gy), gastrointestinal (>8 Gy), and neurovascular syndromes (>15 Gy). In this study, we revealed that $Tlr3^{-/-}$ mice are markedly resistant to radiation. After 10 Gy of total body γ-irradiation, $Tlr3^{-/-}$ mice survived significantly longer than $Tlr3^{+/+}$ mice. $Tlr3^{-/-}$ mice exhibited greater alleviation of symptoms of gastrointestinal syndrome, including body weight loss and diarrhea, compared with $Tlr3^{+/+}$ mice. However, both groups equivalently showed a dramatic loss of bone marrow cells, a symptom of hematopoietic syndrome. Histological analysis demonstrated marked cell death in crypt epithelium and severe villus collapse in small intestine of $Tlr3^{+/+}$ mice after irradiation. In contrast, $Tlr3^{-/-}$ mice showed significant reduction in crypt cell

death and well-maintained villus structure accompanied with regeneration of crypt epithelium following irradiation. Using mice deficient in downstream mediators of TLR3, we showed that radiation-induced crypt cell death is mediated by TRIF, but not by IRF3 and type I interferon. Rather, treatment with a RIP1 inhibitor necrostatin-1 suppressed crypt cell death after irradiation, indicating the involvement of RIP1 as a downstream component of TRIF. Thus, the present study suggests that crypt cell death following irradiation is mediated by TLR 3-TRIF-RIP1-dependent pathway, which may critically contribute to breakdown of intestinal epithelium and eventually to gastrointestinal syndrome. We are now searching for a ligand responsible for TLR3-mediated crypt cell death after irradiation.

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International R&D Center for Muco Vac was established to develop new generation of "Mucosal Vaccine" which can contribute to the control of emerging/re-emerging infectious diseases as well as allergic diseases. 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. Spi-B plays a critical role in M-cell maturation

Kanaya T, Hase K, Takahashi D, Fukuda S, Hoshino K, Sasaki I, Hemmi H, Knoop KA, Kumar N, Sato M, Katsuno T, Yokosuka O, Toyooka K, Nakai K, Sakamoto A, Kitahara Y, Jinnohara T, McSorley SJ, Kaisho T, Williams IR, Ohno H

Intestinal microfold cells (M cells) are an enigmatic lineage of intestinal epithelial cells that initiate mucosal immune responses through the uptake and transcytosis of luminal antigens. The mechanisms of M-cell differentiation are poorly understood, as the rarity of these cells has hampered analysis. Exogenous administration of the cytokine RANKL can synchronously activate M-cell differentiation in mice. Here we show the Ets transcription factor Spi-B was induced early during M-cell differentiation. Absence of Spi-B silenced the expression of various M-cell markers and prevented the differentiation of M cells in mice. The activation of T cells via an oral route was substantially impaired in the intestine of Spi-B-deficient mice. Our study demonstrates that commitment to the intestinal M-cell lineage requires Spi-B as a candidate master regulator.

2. *Brucella abortus* exploits a cellular prion protein on intestinal M cells as an invasive receptor.

Nakato G, Hase K, Suzuki M, Kimura M, Ato M, Hanazato M, Tobiume M, Horiuchi M, Atarashi R, Nishida N, Watarai M, Imaoka K, Ohno H

Brucella abortus is a Gram-negative bacterium causing brucellosis. Although *B. abortus* is known to infect via the oral route, the entry site in the gastrointestinal tract has been unclear. We found that *B. abortus* was selectively internalized by microfold cells (M cells), a subset of epithelial cells specialized for mucosal Ag uptake. During this process, colocalization of cellular prion protein (PrP^C) and *B. abortus* was evident on the apical surface as well as in subapical vacuolar structures in M cells. Internalization of *B. abortus* by M cells of PrP^C-deficient ($Prnp^{-/-}$) mice was greatly reduced compared with that in wild-type mice. Furthermore, an oral infection study revealed that translocation of *B. abortus* into the Peyer's patch was significantly lower in $Prnp^{-/-}$ than in wild-type mice. These observations suggest that orally infected *B. abortus* invades the host through M cells by using PrP^C on the apical surface of M cells as an uptake receptor.

3. Epithelial Notch signaling secures lymphoid organogenesis and immune homeostasis in the gut

Obata Y, Takahashi D, Ebisawa M, Kakiguchi M, Yonemura S, Jinnohara T, Kanaya T, Fujimura Y, Ohmae M, Hase K, Ohno H

Intestinal epithelial cells (IECs) lining the mucosal surface comprise fist-line defense at the interface between the host's internal milieu from the external environment. IECs sense microbial attachment as well as inflammatory signals on the mucosa, and then serve as effector cells by releasing an array of chemokines and antimicrobial products. However, the molecular machinery that regulates epithelial immune functions remains to be clarified. Notch signaling regulates binary cell fate decision in secretory and absorptive cell lineages in the intestinal epithelium. We found that epithelial Notch signaling is a prerequisite for the development of gut-associated lymphoid tissues (GALTs). Mice harboring IEC-specific deletion of *Rbpj* (RBP-J^{IEC-KO}), a transcription factor responsible for Notch signaling, were defective in maturation of Peyer's patch (PP) and isolated lymphoid follicles (ILFs). This defect was attributed to malformation of follicle-associated epithelium that establishes the microenvironment essential for the PP compartmentalization. These findings uncovered a novel link between epithelial Notch signaling and the development of GALTs, raising the possibility that Notch signaling may contribute to the maintenance of gut immune homeostasis. Indeed, RBP-J^{IEC-KO} mice spontaneously develop colitis characterized by the accumulation of T_H17 cells in the lamina propria. Our observations suggest the unique role of epithelial Notch signaling in the establishment and maintenance of the gut immune system.

Publications

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