論文の内容の要旨

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平成 25 年度博士課程進学
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論文題目

Studies on Functions of Cells Involved in Immune Responses in the Intestinal Lymphoid Tissues

(腸管のリンパ組織における免疫応答に関わる細胞の機能についての研究)

§1 Introduction

The intestine is exposed to many foreign substances and organisms such as foods and pathogenic bacteria invading orally. Further, the intestine harbors a huge number of commensal bacteria, some of which have benefits for the host. Whereas the pathogens should be eliminated, immune responses to beneficial or unharmful foreign substances and organisms should be suppressed to maintain host homeostasis. To accomplish precise regulation of these two opposite responses, the intestinal immune system has developed characteristic antigen-specific responses, oral tolerance and immunoglobulin A (IgA) secretion. These responses are induced in the lymphoid tissues in the intestine.

Oral tolerance is defined as systemic immune-hyporesponsiveness toward orally-administrated antigens. This has implication for therapeutic strategy for immunological pathologies, such as allergy and autoimmune diseases. Oral tolerance is dependent on induction of the oral antigen-specific suppressive T cell subset, regulatory T cells (T_{reg} cells). The oral antigen-specific T_{reg} cells have been reported to be highly induced by dendritic cells (DCs) in mesenteric lymph nodes (MLNs).

IgA is secreted into the intestinal lumen and contributes to eliminating pathogens and, further, to regulating the commensal bacteria. Antigen-specificity of antibodies, including IgA, increases in the process of affinity maturation in germinal centers of lymphoid tissues. In the intestine, germinal centers are constantly observed in Peyer's patches (PPs) which are dispersed along the small intestine. In addition, PP is also a site for class-switch recombination which results in IgA^+ B cells. Thus, PPs are important for antigen-specific IgA induction.

The present studies investigated immune cells involved in above-mentioned antigen-specific

responses in the intestinal lymphoid tissues. First, the author investigated T_{reg} cell induction by MLN DC subsets. Then, analyses on a specific T cell subset involved in germinal center reaction, follicular helper T cells (T_{FH} cells), in the intestine were performed in subsequent chapters.

§2 CD11b^{-}CD103^{+}PD-L1^{High} Dendritic Cells Highly Induce Regulatory T Cells in Mesenteric Lymph Nodes.

DCs are classified into phenotypically and functionally distinct subsets. Previous studies have revealed that MLN CD103^{+} DCs highly induce T_{reg} cells through transforming growth factor (TGF)-β and retinoic acid. Another group has reported that MLN DCs from mice deficient for PD-L1 or PD-L2 cannot induce T_{reg} cells. The author's group previously showed that MLN DCs are classified into four subsets expressing CD103 or PD-L1, and, among the subsets, CD11b^{-}CD103^{+}PD-L1^{High} DCs highly expressed *Aldh1a2* gene, which encodes an enzyme metabolizing retinal to retinoic acid.

In the present study, the author observed that the MLN CD11b^{-}CD103^{+}PD-L1^{High} DC subset highly induced Foxp3^{+} T_{reg} cells compared with the other subsets. Such difference in capacity to induce T_{reg} cells among the DC subsets was not abrogated by blocking PD-L1 or PD-L2, and by exogenous supplementation with retinoic acid. Thus, these factors are not critical for T_{reg} cell induction by the DC subset. The author found that the CD11b^{-}CD103^{+}PD-L1^{High} subset highly expressed gene encoding integrin β_8, which is involved in TGF-β activation, and further, that exogenous supplementation with TGF-β abrogated the difference in T_{reg} cell induction. These results suggest that MLN CD11b^{-}CD103^{+}PD-L1^{High} DCs highly induce T_{reg} cells through TGF-β activation.

In MLNs, two types of DCs exist, i.e. migratory subsets from the intestine and MLN-resident subsets. The CD11b^{-}CD103^{+}PD-L1^{High} DCs showed the migratory phenotype, highly expressing CC-chemokine receptor 7 and MHC class II molecule. Further, this subset from mice orally-administrated ovalbumin (OVA) induced proliferation of OVA-specific T cells in co-culture system. Collectively, the results suggest that CD11b^{-}CD103^{+}PD-L1^{High} DCs capture orally-administrated antigens in the intestine, migrate to MLNs, and induce T_{reg} cells specific to the captured antigens through TGF-β activation.

§3 Peyer's Patch Dendritic Cells Respond to Toll-like Receptor Stimulation and Induce Interleukin-21 of T Cells.

PPs constantly contain germinal center structure depending on commensal microbiota whereas systemic lymphoid tissues, such as spleen, has almost no germinal center in the steady state. Germinal center reaction is regulated by T_{FH} cells. T_{FH} cells is a helper T cell subset which is localized in germinal centers and produces interleukin (IL)-21. IL-21 supports IgA class-switch recombination and affinity maturation. T_{FH} cell differentiation is primed by DCs. These raised a possibility that PP DCs prime T_{FH} cell differentiation depending on microbial stimulation. Previous studies have reported that DCs could induce T_{FH} cells through IL-6, IL-12, IL-23, and inducible T cell co-stimulator (ICOS) ligand.

In the present study, the author compared T cells primed by PP DCs and splenic DCs. The results showed that PP DCs induced higher IL-21 gene expression than splenic DCs whereas induction of other T cell-signature cytokines was not different between those DCs. PP DCs and splenic DCs showed some differences in expression of genes encoding IL-12 and IL-23 subunits among T_{FH} cell inducing factors. Especially, the IL-23p19 subunit gene was highly expressed by PP DCs. This expression was diminished in germ-free mice. These results suggest that PP DCs produce IL-23 depending on stimulation by commensal bacteria.

Next, the author examined responses of DCs to Toll-like receptor (TLR) stimulation. TLRs recognize microbe-associated molecular patterns, such as lipoproteins and non-methylated CpG DNA. Previous studies of the author's group suggested the possibility that TLR2 suppressed TLR9-induced IL-12 and IL-23 production in PP DCs. Thus, the author investigated IL-12 and IL-23 responses to TLR stimulation in splenic DCs in addition to PP DCs in detail. As a result, both PP DCs and splenic DCs responded strongly to TLR2 ligand and TLR9 ligand. The response to each ligand was specific, i.e. TLR2 stimulation induced IL-12p35 and IL-23p19 gene expression whereas TLR9 stimulation induced IL-12/23p40 gene expression in PP DCs. Splenic DCs exhibited similar responses except for only slightly expressing IL-23p19 gene. On the other hand, the author confirmed that TLR2 ligand suppressed TLR9 ligand-induced IL-12 (p40/p35 heterodimer) and IL-23 (p40/p19 heterodimer). The same suppression was observed in IL-12/23p40 production and gene expression. In *Tlr2*$^{-/-}$ DCs, such suppression was abrogated, which supports that TLR2 stimulation induces the suppressive cross-talk. TLR2 did not suppress IL-12/23p40 gene up-regulation by TLR7 ligand or anti-CD40 stimulation. Thus, TLR2-induced suppression is specific to TLR9 responses. Further, supernatant of TLR2-stimulated DCs could not suppress the TLR9-responses. This indicates that the TLR2-dependent suppression is not mediated by secretory factors.

The author investigated other suppressive cross-talk between TLRs. To find out TLRs which suppress other TLR-responses, PP DCs were stimulated with mixture of several TLR ligands including TLR2, 3, 4, 5, 7, 9 ligands. When TLR5 ligand was removed from all ligands, IL-12/23p40 gene expression was up-regulated. This implies that TLR5 suppresses other TLR-induced IL-12/23p40 gene expression. Indeed, TLR5 ligand stimulation suppressed TLR9-induced response in PP DCs.

Collectively, PP DCs respond to TLR9 stimulation by IL-12 and IL-23 production. This response is suppressed when simultaneously stimulated with TLR2 or TLR5 through IL-12/23p40 gene suppression. Such positive and negative responses to the intestinal microbiota probably regulate DCs and subsequent T cell priming, including T_{FH} cell differentiation.

§4 Follicular Helper T Cells in the Small and Large Intestines Have Different Characteristics.

To maintain homeostasis in the intestine, the intestinal microbiota is precisely regulated by somatically mutated IgA produced through germinal center reaction. The germinal center reaction is regulated by T_{FH} cells. Previous studies on the intestinal T_{FH} cells have focused on PP T_{FH} cells. However, a composition and

the number of microbiota is largely different among regions of the intestine. Further, the antigen-specificity of IgA has been reported to be distinct between the small and large intestines. Thus, the author analyzed T_{FH} cells in other lymphoid tissues in the intestinal immune system.

In the present study, T_{FH} cells were highly detected in caecal patches (CaePs) of the caecum and colonic patches (CoPs) of the large intestine, in addition to PPs. The author compared gene expression of T_{FH} cells among PPs, CaePs, and CoPs and found that IL-4 and IL-21 genes in T_{FH} cells were inversely expressed; from the upper to the lower intestine, *Il4* gene expression was increased whereas *Il21* gene expression was decreased. IL-4 and IL-21 induce class-switch recombination to IgG1 and IgA, respectively. Thus, the author examined mRNA expression associated to class-switch recombination, and the results indicated that PPs contained less IgG1-switched and more IgA-switched B cells whereas CaeP and CoP contained more IgG1-switched and less IgA-switched B cells. Thus, T_{FH} cells in the intestinal lymphoid tissues may induce somewhat different immune responses.

Next, the author investigated cells involved in the difference of T_{FH} cells among PPs, CaePs, and CoPs. T_{FH} cells have been reported to be regulated by DCs and B cells. However, DCs and B cells from PPs, CaePs, and CoPs almost equally induced IL-4 and IL-21 gene expression when co-cultured with T cells. Thus, the difference in T_{FH} cells may be due to other cells. Then, the author found that PPs contain more group 3 innate lymphoid cells (ILC3s) than CaePs and CoPs. ILC3s have been reported to be involved in T cell-dependent IgA response. Thus, ILC3s in PPs might direct T_{FH} cells to produce high IL-21 and low IL-4.

Collectively, the intestine contained T_{FH} cells in lymphoid tissues dispersed throughout the small and large intestines, i.e. PP, CaeP, and CoP in the steady state. Those tissues probably regulate IgA responses toward commensal bacteria of each region. Further, the T_{FH} cells in the lymphoid tissues exhibited different cytokine patterns. This difference cause different balance of IgG1- and IgA-switching among the tissues. PPs contained more ILC3s than the other lymphoid tissues in the intestine. It raises a possibility that ILC3s regulate T_{FH} cell responses in PPs.

§5 Concluding Remarks

In the present studies, the author revealed several inter-cellular regulation of antigen-specific immune responses in the intestine. MLN $CD11b^{-}CD103^{+}PD\text{-}L1^{High}$ DC subset induces T_{reg} cells, which could suppress excessive immune responses toward orally-administrated antigens. PP DCs are involved in T_{FH} cell priming probably through TLR response induced by commensal bacteria. Some TLRs have suppressive effects on other TLR responses. Further, T_{FH} cells in the small and large intestines have different characteristics. These studies, in part, revealed a complex cellular network in the intestinal immune system and can contribute to development of way to improve the health through immune regulation by foods and probiotics.