While the intestinal immune system coexists with commensal bacterial flora through immunological tolerance, invading microorganisms are recognized and properly eliminated. However, it remains unknown what kinds of cells in the intestine initiate immune responses and how they activate host immunity. Recently, we identified a subset of CD11chICD11bhi lamina propria (LP) dendritic cells (DCs) as TLR5-expressing cells, which have the ability to activate adaptive immune responses. The LPDCs induced antigen-specific Th17 cells as well as Th1 cells in a TLR5-dependent manner. In addition, they acted on naïve B cells to induce their development to immunoglobulin A (IgA)+ plasma cells in response to flagellin, and such IgA+ plasma cell generation took place in a gut-associated lymphoid tissue (GALT)-independent fashion. Our findings demonstrate unique properties of LPDCs and the importance of TLR5 for adaptive immunity in the intestine. We also generated and examined mutant mice of ATG16L1. ATG16L1 is a component of autophagy machinery and has been reported to be a candidate gene responsible for susceptibility to Crohn’s disease. We discuss a novel role for autophagy in the regulation of the inflammatory immune responses in the intestine.

Key words: lamina propria; TLR5; IgA; Th17; autophagy

INTRODUCTION

The intestine is constantly exposed to food antigens and commensal bacteria. Although the intestinal immune system has developed mechanisms for maintaining immunological tolerance to food and commensal organisms, it recognizes invasive pathogens and properly induces protective immune responses for their elimination (24). Intestinal immunity is regulated by various kinds of cells. Epithelial cells exist as a monolayer in the intestine. They physically prevent any foreign substances from getting inside and recognize the invasion of pathogens. In the small intestine, antigen recognition and mucosal immune responses are mainly initiated in gut-associated lymphoid tissues (GALT), which consist of Peyer’s patches (PP), isolated lymphoid follicles and mesenteric lymph nodes (MLNs) (12). PPs are major inductive sites of intestinal immune responses. M cells, specialized epithelial cells, are frequently found in the follicle-associated epithelium of PPs and they transport luminal antigens into PPs by transcytosis. Subepithelial dendritic cells (DCs) in PPs uptake antigens and present them to T lymphocytes after processing (12). On the other hand, lamina propria (LP), which is parenchyma of the intestine located between the epithelial cells and muscle layers, is considered to be an effector site but not an inductive site of intestinal immunity. However, accumulating evidence has shown that various immune responses can be induced in LP. Intraepithelial DCs in LP send protrusions into the lumen of the small intestine and also uptake luminal antigens (17). If they engulf pathogens, they are activated, undergo maturation and migrate to T cell zones of MLNs for antigen presentation. These DCs identify pathogenic organisms via innate immune receptors. In this review, we will focus on the recently identified DC subset of LP and discuss their function in intestinal immunity (25). We also highlight a novel role for autophagy in the regulation of the inflammatory immune responses in the intestine.

ANTIGEN-PRESENTING CELL (APC) SUBSETS IN SMALL INTESTINAL LP

APCs in murine small intestinal LP consist of four subsets distinguished by differential expression patterns of CD11c and CD11b: two subsets of DCs (CD11chICD11bhi and CD11chICD11bhi), macrophages (CD11chICD11bint) and eosinophils (CD11chICD11bint) (Fig. 1a). CD11chICD11bhi and CD11chICD11bhi subsets have a DEC-205+ major
histocompatibility complex (MHC) class II-high CD80+CD86+CD103+ surface phenotype. Interestingly, the CD11chiCD11bhi subset has moderate expression of F4/80, suggesting that these DCs have a macrophage-like character. The CD11cintCD11bint subset consists of F4/80+DEC-205– MHC class II+ phagocytic macrophages. The CD11cintCD11bmid subset comprises eosinophils with uniquely shaped nuclei and eosinophilic granules, which express CD80 but not MHC class II (Figs. 1b and 1c).

TOLL-LIKE RECEPTOR (TLR) 5 AND INNATE IMMUNE RESPONSE

The TLR family, which is a key family for innate immunity, consists of 13 mammalian members (1). As the cytoplasmic portion of TLRs is similar to that of the interleukin (IL)-1 receptor family, it is called the Toll/IL-1 receptor (TIR) domain. However, the extracellular region of TLRs and IL-1R are markedly different. Whereas IL-1R possesses an Ig-like domain, TLRs contain leucine-rich repeats (LRR) in the extracellular domain (1). Recent genetic studies have revealed that TLRs play an essential role in the recognition of specific components of pathogens. TLRs are capable of sensing organisms ranging from bacteria to fungi. Recognition of microbial components by TLRs triggers activation of signal transduction pathways, which then induces dendritic cell maturation and cytokine production, resulting in development of adaptive immunity (1).

Although the role of TLRs has been closely examined, TLR responses of APCs in intestinal LP are not yet elucidated. TLR5 recognizes bacterial flagellin, which is
a major portion of flagella. Mouse intestinal LPCs have high expression of TLR5 (6). We isolated various kinds of cells from the intestine and found that CD11c\textsuperscript{hi}CD11b\textsuperscript{hi} LPDCs specifically express TLR5 (Fig. 1e) (1, 25). CD11c\textsuperscript{hi}CD11b\textsuperscript{hi} LPDCs produce IL-6 and IL-12p40 in response to flagellin in a TLR5-dependent manner. However, they do not produce IL-10, TNF-\alpha or IL-23 (Fig. 2). Unlike splenic DCs, CD11c\textsuperscript{hi}CD11b\textsuperscript{hi} LPDCs do not express TLR4 and show hyporesponsiveness to lipopolysaccharide (LPS). In contrast, other APC subsets in LP do not produce such cytokines in response to flagellin. Thus, CD11c\textsuperscript{hi}CD11b\textsuperscript{hi} LPDCs are responsible for TLR5-mediated innate immune responses in intestinal LP (25).

**CD11c\textsuperscript{hi}CD11b\textsuperscript{hi} LPDCs INDUCE IgA PRODUCTION**

The intestine is known as an organ that produces large amounts of secretory IgA. Intestinal IgA\textsuperscript{+} plasma cells are mainly generated in GALT such as PPs, ILFs and MLNs, by a mechanism dependent on antigen, T cells and the formation of germinal centers (23). CD103\textsuperscript{+} GALT DCs produce retinoic acid (RA), which induces the selective expression of gut-homing receptors such as integrin \alpha4\beta7 and CCR9 on differentiated IgA\textsuperscript{+} plasma cells for gut-homing (7, 15). However, differentiation of an IgA\textsuperscript{+} cell does not necessarily require T cell help and the formation of a germinal center (23). GALT DC-derived RA and cytokines synergistically act on naive B cells, leading to the generation of T cell-independent IgA\textsuperscript{+} cells (3, 15). Furthermore, it seems that some IgM\textsuperscript{+} B cells, especially peritoneal B1 cells, migrate directly to the gut LP in a sphingosine 1-phosphate-dependent manner, and differentiate into IgA\textsuperscript{+} plasma cells in the LP with the help of stroma cells (9, 23). Commensal bacteria induce natural secretory IgA, and this process is mediated by DCs loaded with commensal bacteria (11). Furthermore, DCs in small intestinal LP actively sample luminal bacteria through the formation of transmural dendrites by a mechanism dependent on the chemokine receptor CX3CR1 (17). We thus examined whether CD11c\textsuperscript{hi}CD11b\textsuperscript{hi} LPDCs are involved in the generation of IgA\textsuperscript{+} plasma cells, and showed that flagellinstimulated CD11c\textsuperscript{hi}CD11b\textsuperscript{hi} LPDCs efficiently induce the differentiation of B220\textsuperscript{−} IgA\textsuperscript{+} plasma cells in the absence of T cells in a TLR5-dependent way (25).

We also examined the in vivo function of TLR5 in IgA synthesis. Mice lacking the transcription factor Id2 do not develop GALT, yet they retain intestinal IgA production. In wild-type mice, around 20% of IgA\textsuperscript{+} plasma cells exist in small intestinal LP. Interestingly, we detected about 10% of IgA\textsuperscript{+} cells in the LP of Id2\textsuperscript{−/−} mice, which confirms that gut IgA can be generated without GALT. Although Tlr5\textsuperscript{−/−} mice did not have fewer IgA\textsuperscript{+} B cells, Id2\textsuperscript{−/−} Tlr5\textsuperscript{−/−} mice had far fewer IgA\textsuperscript{+} cells in the LP (Fig. 3). Thus, TLR5 signaling in CD11c\textsuperscript{hi}CD11b\textsuperscript{hi} LPDCs is essential for GALT-independent IgA synthesis in vivo (25).

Similar to GALT DCs, CD11c\textsuperscript{hi}CD11b\textsuperscript{hi} LPDCs specifically express Aldh1a2 mRNA, which encodes retinal dehydrogenase 2 (RALDH2) and induces IgA\textsuperscript{+} plasma cells via their derived RA. Thus, CD11c\textsuperscript{hi}CD11b\textsuperscript{hi} LPDCs can generate T cell-
independent IgA$^+$ cells with the characteristic ability to synthesize RA (25).

**CD11$^c$$^h$CD11$^b$$^h$ LPDC-MEDIATED CD4$^+$ HELPER T CELL (TH) RESPONSE**

CD11$^c$$^h$CD11$^b$$^h$ LPDCs are involved in the induction of Th responses as well as IgA production in the intestine (25). Splenic DCs produce IL-12 in response to TLR ligands and induce antigen-specific Th1 cells. Similarly, CD11$^c$$^h$CD11$^b$$^h$ LPDCs induce Th1 cells in response to flagellin. In addition, these DCs induced Th17 cells, which have recently emerged as a third T cell subset that produces IL-17. Th17 cells play an essential role in protection against certain extracellular pathogens and induce inflammation and severe autoimmunity. Th17 cell differentiation is initiated by TGF-$\beta$ and IL-6. IL-6 signaling activates Stat3 and the lineage-determining transcription factor ROR$\gamma$T. IL-21 is induced by the developing Th17 cells in response to IL-6 and acts on Th17 cells to amplify this population in an autocrine fashion. IL-23 serves to expand the previously differentiated Th17 cell population (8). CD11$^c$$^h$CD11$^b$$^h$ LPDCs produce IL-6 in response to flagellin, which is essential for the induction of Th17 cell differentiation (25).

A series of recent studies have shown that RA negatively regulates Th17 cell differentiation (2, 16, 22). However, the effect of RA on Th17 cell differentiation is different according to its concentration. Supplementation of in vitro cocultures of T cells and LPDCs with 10 $\mu$M RA effectively inhibited not only Th17 cell differentiation but also Th1 cell differentiation, suggesting that high concentrations of RA inhibit response to both Th17 and Th1 responses by CD11$^c$$^h$CD11$^b$$^h$ LPDCs. Notably, the RA inhibitor LE540 inhibited the differentiation of Th17 cells but not Th1 cells, which suggests that RA derived from CD11$^c$$^h$CD11$^b$$^h$ LPDCs is actually necessary for Th17 cell differentiation. Interestingly, LPS-stimulated SPDCs could induce IL-17-producing cells to the same extent as flagellin-stimulated CD11$^c$$^h$CD11$^b$$^h$ LPDCs following the addition of 1 nM RA. Furthermore, 10 $\mu$M RA abolishes Th1 cell differentiation induced by LPS-stimulated SPDCs (25). Thus, CD11$^c$$^h$CD11$^b$$^h$ LPDCs-derived RA may act as a positive regulator of Th17 cell differentiation. We have to consider the effect of RA on Th1 responses more cautiously.

**AUTOPHAGY**

Autophagy is an intracellular process in which cytoplasmic constituents are delivered by autophagosomes to lysosomes for degradation (10, 14, 19). Autophagy contributes to nutrient supply during starvation, cytoplasmic renewal, elimination of intracellular aggregate-prone proteins and pathogens, innate and acquired immunity, inflammation, and regulation of cell death (10, 14). Whereas transport vesicles are generated by budding from donor organelle membranes in other membrane trafficking processes such as endocytosis and secretory pathways, autophagosomes are formed de novo in the cytosol. A small flattened membrane sac called the isolation membrane, elongates and curves until its ends merge to enclose the cytoplasmic portions and form the double-membrane-bound autophagosome (18). Autophagy related proteins (ATG) have been identified by yeast genetic screening studies, which are well conserved from yeast to humans. Ubiquitin-like molecules, Atg12 and LC3 (homologue of yeast Atg8), are involved in autophagosome biogenesis. Atg12 is conjugated to Atg5 and forms an ~800-kDa high
molecular weight protein complex with Atg16L (referred to as Atg16L complex) (18). LC3 is conjugated to phosphatidylethanolamine and is associated with autophagosome formation, perhaps by enabling membrane elongation (4, 13). Previous studies have shown that Atg16L1 is a candidate gene for susceptibility to Crohn’s disease (5, 20). Recently, we generated Atg16L1 mutant mice and examined in vivo function of Atg16L1. Both autophagosome formation and degradation of long-lived proteins were severely impaired in Atg16L1-deficient cells. Atg16L1-deficient macrophages produce higher amounts of IL-1beta and IL-18 in response to LPS. In addition, Atg16L1-deficiency causes Toll/IL-1 receptor domain-containing adaptor inducing IFN-beta (TRIF)-mediated caspase-1 activation, leading to increased production of IL-1beta. Also chimeric mice with Atg16L1-deficient haematopoietic cells showed high susceptibility to dextran sulphate sodium-induced acute colitis due to the elevated production of IL-1beta and IL-18 (21). Thus, Atg16L1 is an essential for autophagosome formation as well as regulation of the endotoxin-induced inflammatory immune response. Since Atg16L1 deficiency causes severe symptoms in chemically induced colitis, it will be necessary to examine the involvement of autophagy in the pathogenesis of Crohn’s disease in the future.

CONCLUSION

In this review, we have summarized the unique characteristics of CD11c<sup>hi</sup>CD11b<sup>hi</sup> TLR5-expressing LPDCs. These DCs recognize invasive flagellated bacteria by TLR5 and induce innate immune responses. They also work against bacterial infection by inducing ‘local’ IgA secretion and ‘systemic’ T helper responses through TLR5 stimulation (Fig. 4). Since IL-17 can influence on various kinds of cells to produce proinflammatory cytokines and can induce the activation and migration of neutrophils, CD11c<sup>hi</sup>CD11b<sup>hi</sup> LPDCs and Th17 cells may be involved in the pathogenesis of intestinal bowel diseases such as Crohn’s disease. In addition, the ability of CD11c<sup>hi</sup>CD11b<sup>hi</sup> LPDCs to induce Th1 responses and IgA synthesis suggests that CD11c<sup>hi</sup>CD11b<sup>hi</sup> LPDCs might be useful targets of mucosal vaccination.

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