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A marvel of mucosal T cells and secretory antibodies for the creation of first lines of defense

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Abstract. The mucosal immune system acts as a first line of defense against bacterial and viral infections while also playing a crucial role in the establishment and maintenance of mucosal homeostasis between the host and the outside environment. In addition to epithelial cells and antigen-presenting cells (dendritic cells and macrophages), B and T lymphocytes form a dynamic mucosal network for the induction and regulation of secretory IgA (S-IgA) and cytotoxic T lymphocyte (CTL) responses. This review seeks to shed light on the pathways of induction and regulation of these responses and to elucidate the role they simultaneously play in fending off pathogen invasion and maintaining mucosal homeostasis.

Key words. Secretory IgA; cytotoxic T lymphocyte; $\gamma\delta$IEL; M cell.

Introduction

Intact or injured sites of the respiratory and digestive tracts represent major entry sites for pathogens from the lumen via inhalation and digestion, respectively. Several physical and biological barriers associated with the innate immune system protect these sites from invasion and help to maintain their mucosal homeostasis. The first line of defense is offered by a barrier structure made up of epithelial cells (ECs) joined firmly by tight junction proteins such as occludin, claudins and zonula occludens [1, 2]. In addition, the attachment and penetration of pathogenic microorganisms to mucosal sites are impeded physically by brush-border microvilli and a dense layer of mucin at the apical site of the EC, and biologically by the production of antimicrobial peptides such as a β-defensin [3]. Additionally, Paneth cells secrete biological defensive molecules, including lysozyme, type II phospholipase A2, and α-defensins, in response to bacterial infection [4, 5].

In addition to these physical and innate defense systems, mucosal tissues contain immunocompetent cells for adaptive immunity. As drawn in figure 1, numerous populations of T and B lymphocytes, dendritic cells (DCs), macrophages and granulocytes form a mucosal network known as the common-mucosal immune system (CMIS) [6]. The CMIS links inductive and effector tissues and also plays a key role in the induction of antigen-specific immune responses. The primary CMIS inductive site for orally administered antigen is the Peyer's patch (PP) of the gastrointestinal tract, and for nasally administered antigen, the nasopharynx-associated lymphoid tissue (NALT). Isolated lymphoid follicles (ILFs), which are located throughout the intestine, were recently identified and characterized by Dr Ishikawa's and our groups as an additional inductive site for the digestive tract [7]. These different organized lymphoid structures are generally known as mucosa-associated lymphoid tissues (MALTs). Despite variations in organogenesis [7–9], the MALTs share several interesting features associated with their role as inductive tissues. First, MALTs are overlaid by a follicle-associated epithelium (FAE) containing antigen-sampling M (microfold) cells, allowing selective transport of antigens to underlying antigen-presenting cells (APCs) in the inductive tissues. Second, they consist of an assembly of naïve B cells, often including a germinal center, supported by a network of follicular DCs and $CD4^+$ T cells. Upon activation by antigens, B and T cells emi-

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grate from the inductive tissue, circulate through the bloodstream and home to distant mucosal compartments, especially the lamina propria regions of the intestinal, respiratory and reproductive tracts. The diffused lamina propria region and the epithelium have been considered effector sites, where the MALT-originated, immunoglobulin A(IgA)-committed B cells differentiate into IgA plasma cells for the secretion of dimeric or polymeric forms of IgA. Effector tissues contain a variety of T cell subsets, which exhibit helper, regulatory and cytolytic activities and so help to regulate protective immunity at the mucosal surface. Additionally, a unique T cell population, known as intraepithelial lymphocytes (IELs), is located between ECs. IELs have been shown to possess a cytotoxic function against pathogen-infected cells.

Accumulating evidence suggests that the mucosal immune system not only protects from bacterial or viral infection, but also aids in the maintenance of mucosal homeostasis between the host and outside environmental antigens. This review focuses on the cellular and molecular mucosal network for the induction and regulation of mucosal antibody and T cell responses.

Antigen uptake, processing and presentation at mucosa

Following oral or nasal administration, foreign antigens follow the sequence of uptake, transport, processing and presentation at the inductive tissues, such as PPs and ILFs, or NALT, respectively. For selective uptake of antigens, the epithelium covering the inductive tissues develops FAE consisting of professional antigen-sampling epithelial cells, known as M cells (fig. 1) [10]. M cells are distinguished from the surrounding ECs by some unique histological and biochemical features, including the lack of brush borders, a limited mucus production and a lower level of degradation activity [11, 12]. Conversely, M cells exhibit a high transcytosis activity and are characterized by a unique pocket structure, where numerous kinds of immunocompetent cells, including DCs, macrophages, T cells and B cells, are located [12, 13]. These unique biological characteristics allow M cells to take up antigens from the lumen into their pocket structures and so selectively transport them to APCs. Hence, mice who lacked PPs because the tissue genesis cytokine cascade of interleukin 7 receptor (IL-7R) and lymphotoxin β receptor (LTβR) had been disrupted showed alternative and/or less ability to take up bacteria and particulate antigens from the intestinal lumen [14, 15]. Once antigens have been taken up from the lumen by M cells and transferred to the M cell pocket, APCs, including DCs, can process the antigens and migrate into the interfollicular areas of the PP, where they present epitopes to T cells [13, 16] (see Iwasaki's review, this issue).

Although FAE-associated M cells at inductive tissues (e.g., PP) are thought to be a major gateway for antigen uptake from the lumen for the initiation of antigen-specific immune responses, an alternative induction pathway may exist for the mucosal immune system, since antigen-specific immune responses have been induced in PP-null mice following oral immunization [15, 17]. At least three different scenarios have been offered regarding this alternative pathway. First, our group has recently identified M cells on intestinal villous epithelium (villous M cells) that is not in the vicinity of PP [14]. Intestinal villous M cells are developed in various PP/ILF-null mice and are capable of taking up bacterial antigens. Thus, villous M cells represent one novel gateway for antigen uptake in the intestine, as well as a possible new site for invasion of pathogenic microorganisms. Second, an M cell-independent pathway is operated by mucosal DCs, which express tight junction-associated proteins (e.g., occludin, claudin 1 and zonula occludens 1) and thus are capable of extending their dendrites between ECs [18]. On a similar note, CD18-expressing phagocytes have been reported to be involved in an M cell-independent pathway for bacterial invasion [19]. By protruding dendrites into the lumen, mucosal DCs located between ECs are able to sample gut antigens and then present them to T and/or B cells [18]. The third pathway for antigen uptake are ECs themselves. Some evidence has shown that ECs could process and then present antigens to T cells via major histocompatibility complex (MHC) class I as well as class II molecules [20]. In addition to sampling a wide variety of foreign antigens, the mucosal immune system must contend with the high number of apoptotic ECs, which result from the rapid turnover of epithelium. Although most of these apoptotic ECs are ceded by the epithelium to the lumen, some of them are potentially immunogenic and can be transported to T cell areas of mesenteric lyrrph nodes (MLNs) by mucosal DCs [21].

Like the intestinal tract, NALT and bronchus-associated lymphoid tissue (BALT) of the respiratory tract have been shown to contain M cells along their epithelium for antigen sampling [22, 23]. Thus, nasal immunization has been shown to be effective for the induction of Ag-specific immune responses. Our previous study showed that nasally administered fusogenic liposome-containing vaccine antigens were effectively taken up by M cells located on the NALT epithelium [24]. The efficacy of NALT-mediated immunity was further demonstrated by the use of σ-1 protein-coupled DNA vaccine [25]. These NALT- and BALT-associated M cells were of course also entry sites for pathogens [26]. Currently, far less is known about the antigen uptake pathways for the respiratory tract than for the intestinal tract, and indeed, it is not yet known whether alternative gateways even exist in the respiratory tract.

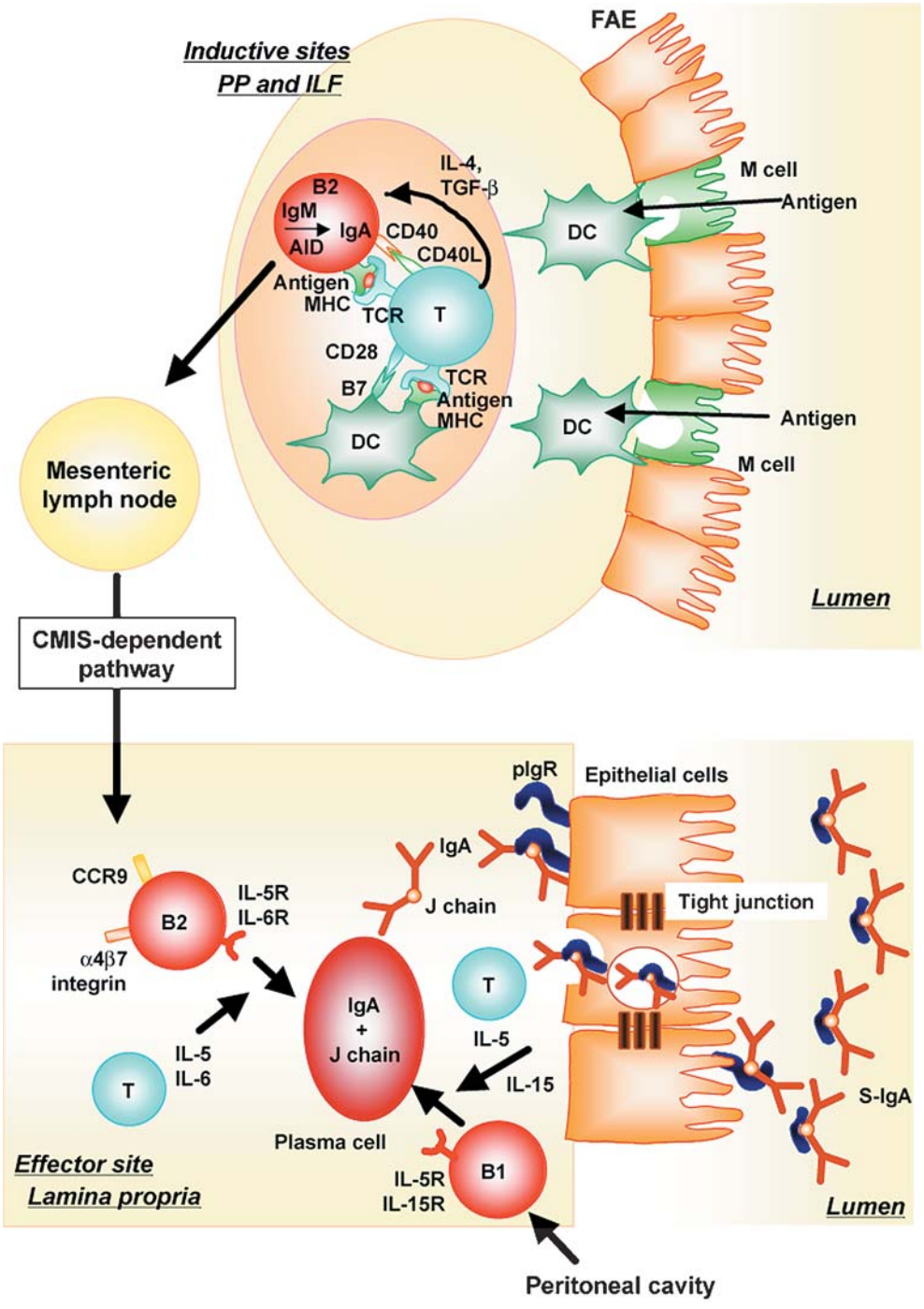

Figure 1. Multistep model to generate secretory IgA (S-IgA) responses in the intestine. In the common-mucosal immune system (CMIS)-dependent pathway, naïve B cells, also known as B2 lymphocytes, are stimulated in a T cell-dependent manner within Peyer's patches (PPs) and isolated lymphoid follicles (ILFs), where several factors (e.g., CD40 and cytokines) induce class-switch recombination from IgM to IgA. The IgA-committed B cells exit through the lymph and home through the thoracic duct and peripheral blood to mucosal effector sites such as the lamina propria of the gut. Intestinal homing is mediated by adhesion molecules and chemokine-mediated interaction. At the effector site, IgA-committed B2 cells receive several signals, resulting in the generation of plasma cells. The plasma cells produce IgA as a dimer joined by a J-chain, and the dimeric form of IgA binds to poly Ig receptor (pIgR) on epithelial cells, is transported across the epithelium and is released in the intestinal lumen as S-IgA, which acts as a first line of defense against pathogens and maintains mucosal homeostasis. Another lineage of B cells, B1 cells, are derived from the peritoneal cavity and act as the other source of intestinal secretory IgA. AID, activation-induced cytidine deaminase; APC, antigen-presenting cell; CCR9, CC-type chemokine receptor; FAE, follicle-associated epithelium; IL-4, interleukin 4; MHC, major histocompatibility complex; TCR, T cell receptor; TGF-β, transforming growth factor β.

Unique B cell network for mucosal IgA production

To provide a first line of defense at the mucosal surfaces of the aerodigestive and reproductive tracts, the mucosal immune system selectively uses IgA as a major isotype of antibody for the formation of secretory IgA (S-IgA). In order to induce the secretory form of IgA, mucosal B cells have to undergo two major molecular and cellular events in the organized inductive and diffused effector tissues interconnected by the CMIS. In PP, for example, a μ to α class switch recombination (CSR) occurs under the influence of transforming growth factor β (TGF-β) and antigen stimulation (fig. 1) [27]. After IgA isotype switching, IgA-committed B cells leave PP, migrate to distant effector tissues such as the intestinal lamina propria, and then, under the influence of IgA-enhancing cytokines such as IL-5 and IL-6, enter the terminal differentiation process to become IgA plasma cells (fig. 1) [28, 29]. Dimeric or polymeric forms of IgA produced by these plasma cells then interact with the poly Ig receptor (pIgR) expressed on the basal membrane of ECs and are transported to the apical membrane, where they form S-IgA [30].

As shown in figure 1, at least three different types of cells have to harmoniously form a mucosal internet for the induction of S-IgA at the diffused effector site: (i) IgA-committed B cells originated in PP, (ii) T helper 2 (Th2)-type cells producing IgA-enhancing cytokines (IL-5 and IL-6) and (iii) ECs expressing pIgR. Once in place, S-IgA antibodies also play a key role in establishing a cohabitant environment with commensal microorganisms in the intestinal tract [31].

Contribution of conventional B cells (B2 cells) to IgA responses

In the mucosal immune system, IgA is produced by two subsets of B cells, namely B1 and B2 cells [32]. For example, the murine intestinal lamina propria region contains equal numbers of B1 and B2 cells committed for IgA [33]. When MALTs such as PPs, ILFs and NALT were examined, the inductive tissues were found to contain numerous B2 cells originating from bone marrow-derived precursor cells. IgA-committed B cell development in these inductive organs seems to depend on antigenic stimulation of germinal centers, where B cells interact with both antigens trapped on follicular DCs and local $CD4^+$ T cells to induce the μ to α isotype CSR and somatic hyper mutation [34]. Similarly, NALT revealed the presence of germinal centers and μ to α isotype switching after antigen stimulation [35, 36]. The CSR in PPs is mediated by the CD40/CD40 ligand and by TGF-β [27, 37]. Also essential to CSR is the interaction between the inducible co-stimulator (ICOS), which is expressed on activated Th cells, and its ligand, ICOS-L, which is constitutively expressed on B cells [38]. Following stimulation by these molecules, multiple transcription factors induce the CSR. For example, an element for binding to Smad, which is a TGF-β-induced transcriptional factor, is located in the $C_{H\alpha}$ promoter region, and this pathway co-operates with acute myeloid leukemia (AML) transcription factors [39]. The discovery of activation-induced cytidine deaminase (AID) has led to a dramatic breakthrough in our understanding of the CSR and somatic hyper mutation in germinal centers [40]. AID is specifically expressed in germinal center B cells and may also exhibit an RNA- or DNA-editing cytidine deaminase activity. Surprisingly, the expression of AID alone induced CSR on artificial substrates in fibroblasts, indicating that AID per se can induce CSR [41, 42]. However, the molecular mechanism by which AID initiates this reaction in B cells and recognizes the specific immunoglobulin loci has yet to be clarified. By AID-mediated CSR together with TGF-β and antigen signaling, IgM^+B220^+B cells undergo μ-to-α gene rearrangement via the formation of an Iα-Cμ circular transcript. The expression of an Iμ-Cα transcript indicates the completion of the isotype switching for the generation of $IgM^-IgA^+B220^+$ B cells [40].

The post-switched IgA^+ B cells exit PP and NALT and migrate to MLNs and cervical lymph nodes, respectively, where they proliferate further and differentiate into B blasts (fig. 1). The B blasts migrate preferentially into the mucosal effector tissues (e.g., the gut lamina propria and the nasal passage) through the thoracic duct and blood circulation. Accumulating evidence suggests that the IgA^+ B cell trafficking to the gut lamina propria is facilitated by changes in the expression of adhesion molecules and chemokine receptors. IgA^+ B cells produce $\alpha4\beta7$ integrin that interacts specifically with mucosal vascular addressin cell adhesion molecule 1 (MAdCAM-1) expressed by blood vessels in the lamina propria [43]. Later, CCR9 is selectively expressed on IgA-, but not IgM- or IgG-, committed B cells [44]. The ligand of CCR9 is CCL25, also known as thymus-expressed chemokine (TECK), which is produced dominantly by the intestinal epithelium, determining the selective homing of IgA^+ B cells into the intestinal lamina propria [44]. Although the detailed mechanism remains to be investigated, it has been reported that the migration of IgA^+ cells from NALT to the nasal passage might be due to the expression of mucosae-associated epithelial chemokine (MEC)/CCL28 [45].

Role of B1 cells in mucosal IgA responses

The peritoneal cavity may be another source of intestinal B cells (fig. 1) [46]. Early research demonstrated that peritoneal cavity-derived B1 cells differ from conventional B2 cells in origin, surface marker expression (e.g.,

B220, IgM, IgD, CD5 and Mac-1) and growth properties [32, 47]. Of note, B1 cells exhibit different V_H repertoires and Ig specificities, and they are thought to be specialized in responding to T cell-independent antigens conserved on common pathogens, such as DNA and phosphatidylcholine. In contrast, the response of B2 cells to most protein antigens requires activation by DCs and Th cells [48–51]. Consistent with this notion, IgA production from B1 cells was noted in MHC class II-deficient mice as well as in T cell receptor (TCR) β and δ chain-deficient mice [52, 53].

B1 and B2 responses have distinct cytokine requirements. Our previous studies demonstrated that, like IL-5, a well-known IgA-enhancing cytokine, IL-15, promotes proliferation and differentiation into IgA-producing cells of B1 but not of B2 (fig. 1) [33, 54]. Indeed, a disruption in the IL-5 receptor gene or treatment with anti-IL-15 antibody resulted in the severe paucity of B1 cells at effector sites such as the intestinal lamina propria and nasal passage but did not affect B2 cell number [33, 54]. A previous report proposed that the homing pathway of B1 cells to the peritoneal cavity depended on the CXCL13 (also known as B lymphocyte chemoattractant, BCL) produced by peritoneal macrophages [55]. Another study using alymphoplasia (*aly*) mice that carried a point mutation in the nuclear factor κB-inducing kinase (NIK) demonstrated a complete absence of B cell population in the intestinal lamina propria of *aly* mice, and a defective migration of peritoneal cells to intestinal effector compartments [56, 57]. These data imply that the NIK-mediated pathway is involved in the B1 cell mucosal migration, which might be dependent on specific but not yet identified chemokine receptors. We previously reported that B1 cells existed in the nasal passages [33], but the actual molecular machinery of B1 cell migration into the nasal passages remains an open question.

Recent results obtained from $AID^{-/-}$ mice suggest an alternative pathway for CSR induction at diffused effector sites (e.g., the intestinal lamina propria), one that does not involve the organized inductive tissues, such as PPs [58]. In this study, stromal cell-derived TGF-β in the intestinal lamina propria was shown to trigger IgM^+B220^+ B cells to undergo μ-to-α CSR and to become IgA-switched B cells. Thus, the intestinal lamina propria might be able to act as both inductive and effector sites. However, the recent discovery of ILFs that are equipped like mucosal inductive sites challenges this hypothesis [7]. Because $AID^{-/-}$ mice were shown to exhibit numerous hyperplasia of ILFs, it is possible that IgA-switching of B cells was triggered within ILFs [59]. In accordance with these observations, the expression of a series of IgA isotype CSR molecules, including AID, the Iα-Cμ circular transcript and the Iμ-Cα transcript, were detected only in the organized tissues (e.g., PPs, ILFs and NALT), and not in diffused effector tissues [34]. Although this finding directly demonstrates that organized lymphoid structures are key to CSR in B2 cells, it does not rule out the possibility that IgA-specific CSR for B1 cells may not occur in the organized lymphoid tissues. In this regard, a majority of B cells belonging to the organized MALT were found to be of B2 lineage, and the diffused lamina propria regions of the aerodigestive tract and peritoneal cavity were observed to be rich in B1 cells [32].

Formation and transport of S-IgA by epithelial cells via pIgR

Two essential steps for the production of IgA antibody in the lumen and secretions have already been outlined: (i) the switching of B cells to IgA at inductive sites (e.g., PPs, ILFs, and NALT) and (ii) the migration of those IgA-committed B cells to effector sites (the intestinal lamina propria and nasal passages). Additionally, IgA production requires the expression of the joining chain (J-chain) and pIgR (fig. 1). The J-chain gene expressed in B cells is a small polypeptide that regulates polymer formation of IgA and IgM, but not that of other types of Ig [60, 61]. J-chain synthesis is tightly regulated at the transcription level. Transcription is induced by antigen recognition, which is dependent on IL-2-induced chromatin remodeling of the J-chain locus and interaction of specific transcription factors with the J-chain promoter [62, 63]. It is interesting to note that the expression of the J-chain has been identified in invertebrates (Mollusca, Annelida, Arthropoda, Echinodermata and Holothuroidea) that lack B cell development in the phylogenic tree [64]. Since mucosa-oriented, IgA-committed B cells produce dimeric or polymeric forms of IgA in the effector tissues, while serum IgA is generally a monomeric form, the expression of the J-chain is essential for the formation of S-IgA.

Similarly, pIgR expressed by the basal membrane of ECs is a prerequisite for the formation and transport of S-IgA [30]. Dimeric or polymeric IgA containing the J-chain shows a high affinity for pIgR, thereby accelerating the internalization and transport of the complex to the apical site via transcytosis [65]. Thus, elevated serum IgA and decreased fecal IgA levels were observed in J-chain knockout mice due to the decreased affinity for pIgR [66, 67]. At the apical site, S-IgA antibodies are produced by endoproteolytic cleavage of the pIgR domain to become secretory components. As in J-chain knockout mice, disruption of the pIgR gene results in a defective transport of IgA into the intestinal lumen and, thus, in the reduction of IgA antibodies in the gut secretions, despite the presence of high numbers of IgA plasma cells in the intestinal lamina propria [68, 69]. The high levels of pIgR constitutively expressed by ECs are regulated at the transcription level by specific transcriptional factors (USF-1 and USF-2) [70, 71]. Additionally, the constitutive expression of pIgR is further upregulated by a group of Th1, Th2 and in-

flammatory cytokines [e.g., interferon, (IFN-γ), IL-4 and tumor recrosis factor (TNF)], indicating that pIgR expression is also involved in increased local IgA production during the course of mucosal injury, such as infection [72]. This evidence further emphasizes the unique mechanism of S-IgA production, whereby PP- or NALT-originated Th1 and Th2 cells as well as IgA-committed B cells form a mucosal intranet together with ECs.

IgA as a mucosal guarding and symbiosis molecule

Several studies have shown that S-IgA is capable of neutralizing viruses and bacteria in cultures and of protecting the host from pathogenic microorganisms in vivo. For example, IgA derived from the saliva of mice nasally immunized with fimbriae prevented the adhesion of *Porphyromonas gingivalis* to ECs, which resulted in the subsequent inhibition of inflammatory cytokine produc-

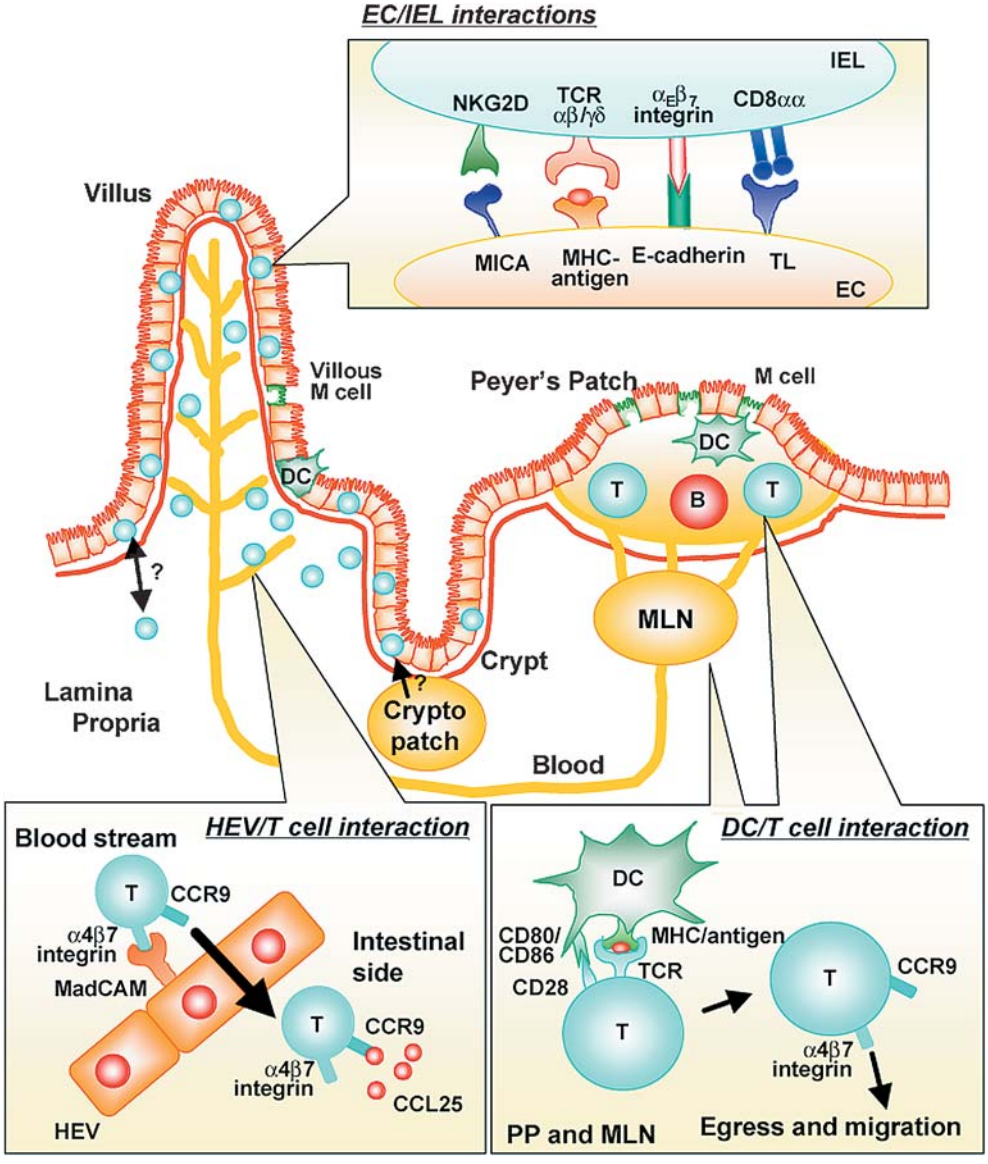

Figure 2. Induction pathway of intestinal T cells. Dendritic cells (DCs) in Peyer's patches (PPs) take up antigens transported through M cells and present them to T cells in an MHC-dependent manner. The activated T cells express $\alpha 4\beta 7$ integrin and CCR9, allowing them to migrate to mucosal effective sites, such as the lamina propria. $\alpha 4\beta 7$ interacts with intestinal high endothelial venules (HEV) expressing mucosal vascular addressin cell adhesion molecule 1 (MAdCAM-1), and CCR9 allows specific migration to the intestinal epithelial cell (EC)-chemokine, CCL25. These migrating lamina propria T cells exhibit cytotoxic activity primarily in an MHC-restricted manner. Another population of intestinal T cells is formed by intraepithelial lymphocytes (IELs), which are located between intestinal ECs. One population of IELs originates in the lamina propria, while the others are derived from cryptopatches. They express several molecules interacting with intestinal EC (e.g., TCR, NKG2D and CD8$\alpha\alpha$) and so act as a bridge between innate and acquired immunity. CCR9, CC- type chemokine receptor; MHC, major histocompatibility complex; TCR, T cell receptor; MLN, mesenteric lymph node.

tion [73]. S-IgA prevents mucosal infection with viruses and neutralized microbial toxins [74–76]. Additionally, the J-chain- and pIgR-mediated transport machinery through ECs is an effective physical system not only for the delivery of dimeric/polymeric IgA from the basolateral surface to the lumen, but also for the creation of a one-way transport pathway that blocks antigens from penetrating the body [77]. Furthermore, the mucosal IgA plays a pivotal role in impairing pathogen penetration by neutralizing pathogens during transcytosis by ECs, especially within the apical recycling endosome [78, 79].

The mucosal immune system acts as more than just the first line of defense against pathogenic microorganisms: the mucosal epithelium, especially the intestinal tract, serves as the means by which nonpathogenic commensal bacteria cross-talk with the immune system to foster the development and maintenance of the mucosal IgA production pathway [31]. For example, the unusually small and flattened PPs of germ-free mice, which also showed a paucity of IgA-producing B cells, matured normally once commensal bacteria were introduced, and an increase in the number of IgA plasma cells was seen as well [80]. It has also been reported that disruption of the AID gene resulted in ILF hyperplasia and a high degree of germinal-center formation, as discussed above [59]. These observations were associated with an increase in anaerobic flora and with the antibiotic treatment meant to destroy them, indicating that ILF development was also regulated by the interaction with commensal bacteria [59]. Further, most recent studies suggest that secretions of intestinal IgA are a key factor in the regulation of commensal microflora [81]. Thus, an altered bacterial flora characterized by an aberrant increase in segmented filamentous bacteria was observed in the intestinal tract of IgA-deficient mice.

Since both commensal and pathogenic bacteria express conserved molecular features of microbes (so-called pathogen-associated molecular patterns; PAMPs) necessary for stimulation of innate immunity and eventually, of acquired immunity, one obvious question would be why commensal bacteria do not induce inflammatory responses [82]. Several recent investigations offer plausible explanations for this intriguing interaction between intestinal commensal microflora and the host immune system. First, induction of S-IgA responses against commensal bacteria is derived from T cell-independent B1 cells, while the S-IgA response against pathogen-derived epitopes required antigen-specific T cell help presumably belonging to B2 cells [53]. As mentioned above, the T cell-independent IgA antibodies originating from B1 cells possessed reactivity to conserved bacterial products, which resulted in the undiscriminating blockade of commensal bacteria attachment to mucosal surfaces. It was further demonstrated that intestinal macrophages rapidly kill commensal bacteria, while intestinal DCs retain small numbers of live commensal organisms and migrate only into MLNs, and do not stray beyond them. This function ensures a commensal bacteria-specific IgA response that is specifically produced at gut mucosa, but not at systemic compartments [83]. In contrast, pathogenic *Salmonella enterica* serovar Typhimurium are detected in both DCs and macrophages from the MLNs as well as the spleen, which allows bacteria to persist longer and induce more pathogenic effects at both the local and the systemic compartments [83].

Second, it was reported that avirulent *Salmonella* were capable of disrupting inflammatory cytokine synthesis from intestinal ECs by inhibiting ubiquitin-mediated degradation of IκB, leading to the blocking of nuclear factor kappa B (NF-κB)-mediated transactivation of the inflammatory gene [84]. The third possible mechanism of inhibiting inflammatory response at mucosal sites is the generation of tolerance to subsequent stimulation from bacterial products. Otte et al. reported that repeated contact with bacterial components (e.g., lipopolysaccharide) induced downregulation of Toll-like receptors (TLRs) on the surface of ECs, and inhibition of intracellular signaling though TLRs by upregulation of Tollip [85]. These data suggest mechanisms by which inflammatory responses induced by commensal bacteria are inhibited to create and maintain an immunological silence at the intestinal mucosa. However, the exact means by which the mucosal immune system cleverly distinguishes commensal from pathogenic bacteria remains to be clarified.

Cytotoxic functions of mucosal T cells as a cellular barrier

The mucosal immune system does not rely solely on S-IgA-mediated humoral immunity to provide an effective first line of defense. Since the mucosal immune system is continuously facing harsh environmental stress, and because a rupture of this first defense line can lead to serious disease, the system must be equipped with multiple layers of protective immunity. The experiments using $IgA^{-/-}$ mice pointed out that compensatory mechanisms other than S-IgA might be responsible for protection from viral or bacterial infection [86, 87]. In this respect, there is substantial evidence that mucosal T cells harbor cytolytic activity and are thus capable of killing cells infected with virus or bacteria [88–90]. Like IgA-producing B cells, large numbers of mucosal T cells, including both $CD4^+$ and $CD8^+$ T cells, are situated in the intestinal lamina propria for the delivery of protective functions, including cytotoxicity (fig. 2). Moreover, a unique mucosal T cell population exists in the intestinal epithelium. Next we focus on the cytotoxic effects of intestinal T cells as a major provider of cell-mediated immunity at the mucosal surface.

Intestinal lamina propria T cells with cytotoxic function

Intestinal lamina propria T cells are largely composed of $\alpha\beta$TCR lymphocytes expressing either CD4$^+$ or CD8$\alpha\beta^+$. In addition, CD4$^+$ and CD8$^+$ T cells distribute in different areas of intestinal tissue sections, as observed by immunostaining. CD4$^+$ T cells are largely located in the lamina propria, while CD8$^+$ T cells reside along the epithelium [91]. Although we have no explanation for this histological segregation, the most obvious interpretation would be that mucosal CD8$^+$ T cells with cytotoxic activity are situated close to the entry sites for pathogenic invaders to ensure the immediate elimination of the pathogens and infected ECs. Most of these mucosal T cells are thought to derive from the CMIS-dependent induction pathway. Recent studies have demonstrated that PP- and MLN-derived DCs determine the gut tropism of lamina propria lymphocytes (LPLs) by the induction of high levels of $\alpha_4\beta_7$ integrin and CCR9 expression, resulting in selective migration to the small intestine (fig. 2) [92–94]. Thus, oral antigen-educated mucosal T cells originating from PP migrate to distant effector sites by obtaining the mucosal trafficking molecules (e.g., $\alpha_4\beta_7$ and CCR9) via the CMIS.

At the periphery, CD8$^+$ T cells recognize the antigens derived from the cytosolic antigen as a complex with MHC class I molecules [95, 96]. Heterodimeric CD8 (CD8$\alpha\beta$) T cells are involved in the subsequent killing of target or virus-infected cells [97, 98]. Thus, the α chain of the CD8 molecule associates with MHC class I molecules, and the β chain acts as a TCR co-receptor for the recognition of cytotoxic T cell epitope antigens. Consistent with the expression of CD8$\alpha\beta$ on LPL T cells, these CD8$^+$ LPLs present cytotoxic activities against MHC class I-restricted antigens originating from various kinds of intracellular antigens [99, 100]. Similar to peripheral CD8$^+$ T cells, CD8$\alpha\beta$ LPLs express the pore-forming protein perforin and cytolytic granules containing granzyme proteases to exhibit cytotoxic activity against pathogenic cells [101].

Intraepithelial T cells, an anonymous cell population, are important as a first line of defense

An additional unique feature of the mucosal immune system is the presence of T cells in the intestinal epithelium known as IELs (fig. 2). IELs are located at every four to nine ECs and are mainly composed of heterogeneous groups of T cells based on the usage of TCRs as well as CD4 and CD8 [102]. LPL CD8$^+$ T cells are exclusively $\alpha\beta$TCR-positive cells with heterodimeric CD8$\alpha\beta$ (70% of CD8$^+$ LPLs are $\alpha\beta$TCR-positive, and 15% are $\gamma\delta$TCR-positive). In contrast, few $\alpha\beta$TCR CD8$\alpha\beta$ *T* cells are found in IELs (about 10%), and most CD8$^+$ IELs are either $\gamma\delta$TCR- or $\alpha\beta$TCR-positive cells with homodimeric CD8$\alpha\alpha$ (about 50%) [103]. Similar to CD8$^+$ T cells at the periphery and lamina propria, CD8$\alpha\beta$ IELs develop in the thymus and migrate specifically into the mucosal compartments by the selective expression of CCR9 and $\alpha_4\beta_7$ integrin [104]. However, CCR9-deficient mice exhibited a modest decrease of IELs, indicating that other chemokines might be involved in IEL migration [105, 106]. Thus, several studies suggest the contribution of other chemokines and chemokine receptor pathways as mediators of gut tropism [107–109]. Intriguingly, TCR clonotypes the CD8$\alpha\beta$ $\alpha\beta$TCR IELs were almost identical to those of the CD8$\alpha\beta$ LPLs and thoracic duct CD8$^+$ T cells [110], implying that CD8$\alpha\beta$ IELs were primed to antigen in PPs and migrated into the intestinal region via the CMIS-dependent pathway. The finding that DCs in PPs and MLNs induce $\alpha_4\beta_7$ integrin and CCR9 expression on IELs as well as LPLs lends support to this theory (fig. 2) [93, 94]. Under the influence of TGF-β, a cytokine produced by ECs and numerous activated lymphocytes and macrophages, inhibition of α4 expression occurs simultaneously with the induction of αE, leading to the expression of αEβ7, a hallmark of IELs for the cell-to-cell interaction with E-cadherin [111]. The presence of a two-way communication of T cells between the epithelial region and the lamina propria was also predicted [112], but the exact governing of it remains to be elucidated. At the least, these findings suggested that IELs provide an additional layer of defense over and above IgA-committed B cell-mediated humoral immunity.

In contrast to so-called thymus-dependent CD8$\alpha\beta$ IELs, at least some populations of CD8$\alpha\alpha$ IELs, such as $\gamma\delta$TCR T cells, are thought to be thymus-independent and thus develope in gut-associated cryptopatches (CPs) [113]. CP lymphocytes do not originate from the thymus, because nude mice contain CPs of identical size, structure, number and cell phenotype with normal mice. In contrast, CPs are absent in mice that have a defective cytokine-receptor γ chain gene and that also lack CD8$\alpha\alpha$ $\alpha\beta$TCR and $\gamma\delta$TCR IEL fraction, but contain thymus-dependent CD4$^+$ and CD8$^+$ $\alpha\beta$TCR IELs [114, 115]. The main population of CP cells displayed a c-kit, IL-7R and CD44-positive, but lineage markers (CD3, B220, Mac-1, Gr-1 and TER-119)-negative lympho-homopoietic stem cell phenotype [116]. Consistent with the IL-7R expression on CP lymphocytes, gut epithelium-derived IL-7 has been shown to be important in the induction of CD8$\alpha\alpha$ IEL T cells and CP maturation, since IL-7$^{-/-}$ mice do not have $\gamma\delta$TCR IELs and CPs. The introduction of IL-7 into IL-7-deficient mice results in the recovery of $\gamma\delta$TCR IELs and CPs [117, 118]. In vivo studies demonstrated that CPs had an ability to generate both $\alpha\beta$TCR and $\gamma\delta$TCR IELs without the influence of the thymus [115, 116]. However, other studies questioned the thymus-independent nature of IELs and implied that CD8$\alpha\alpha$ $\alpha\beta$TCR and $\gamma\delta$TCR IELs developed at the thymus [119, 120]. The most recent study has demonstrated that all of

the intestinal T cells expressing $\alpha\beta$TCR, regardless of co-expression of heterodimeric or homodimeric CD8, are progeny of $CD4^+CD8^+$ thymocytes [121]. Although the issue of thymus-independent development of IELs remains controversial, CPs are still considered key members of the gut-associated lymphoid tissue network and at least serve as one immunological nest for the development of some populations of intestinal T cells. Some researchers have reported the expression of CCR6 by CP lymphocytes and have noted that the expression of E-cadherin on ECs could be a tethering molecule for IELs, helping them migrate to and reside in the intestinal epithelium [111, 122]. However, the molecular sequence mechanism for IEL egress from CP and migration into the EC compartment is still obscure.

Another key difference between CD8$\alpha\beta$ IEL T cells and CD8$\alpha\alpha$ IEL T cells was revealed using MHC class I-deficient mice. The experiments demonstrated that CD8$\alpha\beta$ $\alpha\beta$TCR IELs were dependent on MHC class I, while CD8$\alpha\alpha$ IELs were not [123, 124]. Thus, CD8$\alpha\beta$ $\alpha\beta$TCR IELs showed cytotoxic activity against nonself cytoplasmic antigens in an MHC class I-restricted manner, whereas CD8$\alpha\alpha$ IELs exerted extremely low cytotoxic activity against antigens associated with MHC class I [89]. These observations raise the obvious question about the nature of antigens and presentation molecules interacting with CD8$\alpha\alpha$ IELs. Mice lacking the MHC-regulating molecules β2-microglobulin (β2m) and transporter associated with antigen processing (TAP) shed new light on the process of antigen presentation and recognition. The number of CD8$\alpha\alpha$ IELs were markedly reduced in β2m-deficient mice compared with TAP-deficient mice, implying that nonclassical MHC molecules might contribute to antigen presentation to subpopulations of IELs [125, 126]. In support of this hypothesis, intestinal ECs express several nonclassical MHC molecules, including thymus leukemia antigen (TL), Qa-1, Qa-2, CD1 and MHC class I-related molecules (MICA and MICB) (fig. 2) [127]. Some populations of these nonclassical MHC molecules (TL and MICA) are capable of interacting with their ligand without antigen, but the other populations present lipid antigen (e.g., CD1). As expected, Qa-$2^{-/-}$ mice contained a few CD8$\alpha\alpha$ IELs, and the mice were susceptible to parasitic infections [128, 129]. The other molecules interacting with $\gamma\delta$TCR IELs are MICA, capable of activating Vγ1V$\delta1^+$ IELs (fig. 2) [130]. Additionally, MICA interacts with an 'activating type' of natural killer (NK) receptor, NKG2D [130, 131], and CD8$\alpha\alpha$ $\gamma\delta$TCR IELs display both T and NK cell markers and cytotoxic feasibility [132]. Since MICA is not expressed constitutively on normal ECs but is induced by bacterial or viral infection [133, 134], it has been thought that CD8$\alpha\alpha$ $\gamma\delta$TCR IELs recognize infected ECs via MICA, hampering systemic dissemination of virus or bacteria. These responses mediated by nonclassical MHC molecules were induced promptly after infection; hence, it has been proposed that $\gamma\delta$TCR IELs provided a bridge between rapid innate responses and slower acquired immune responses [135]. In support of this idea, a recent study using a *Listeria* infection model demonstrated that MHC class I-restricted memory T cells regulate H2-M3 (one of the nonclassical MHC molecules)-restricted memory T cells by limiting antigen presentation by DCs, thereby preventing the contribution of H2-M3-restricted protective mechanisms at late stages of infection [136].

In addition to the interaction between nonclassical MHC and TCR or NKG2D, CD8$\alpha\alpha$ itself interacts with TL, a β2m-dependent nonclassical MHC class I molecule [137]. TL is constitutively expressed by the ECs of the small intestine and, like the other nonclassical MHC molecules, does not present peptide antigens [138, 139]. Functional studies have demonstrated that the interaction of TL with CD8$\alpha\alpha$ on IELs promotes the production of cytokines but does not induce their proliferation and cytotoxic response [137]. These unique functions seem to lead to IEL-mediated protection without destruction of the EC layer. Regardless of the origin of IELs (thymic versus extra-thymic development), these gut-oriented T cells seem to be key players in establishing a surface barrier-associated immunological flow of innate and acquired immunity.

Concluding remarks

This review has been aimed at elucidating the functional aspects of the molecular and cellular regulation of mucosal B- and T-cell-mediated S-IgA and cell-mediated immunity as a first line of defense against invading pathogens. The mucosa-associated immunocompetent cells, including mucosal ECs, DCs, macrophages, Th1, Th2, CTL and IgA-committed B cells, harmoniously interact in both innate and acquired immunity at mucosal sites, thereby playing an important role in the early and late phases of pathogenic microorganism invasion, respectively. These facts have led to considerable efforts at developing a mucosal vaccine using mucosal adjuvant and/or mucosal delivery systems that could effectively upregulate the induction of protective immunity at the initial entry of pathogens via the aerodigestive and reproductive tracts [140, 141]. In addition to protecting against microorganism invasion at mucosa, the mucosal immune system is capable of inducing and regulating a mucosal homeostasis between host and outside environments. Thus, disruption of the system leads to the development of mucosal immune diseases such as inflammatory bowel disease, asthma and food allergies [142]. A comprehensive molecular and cellular understanding of the mucosal immune system will facilitate novel

approaches to mucosal immune therapy and mucosal vaccine design, eventually contributing to the improvement of public health.

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