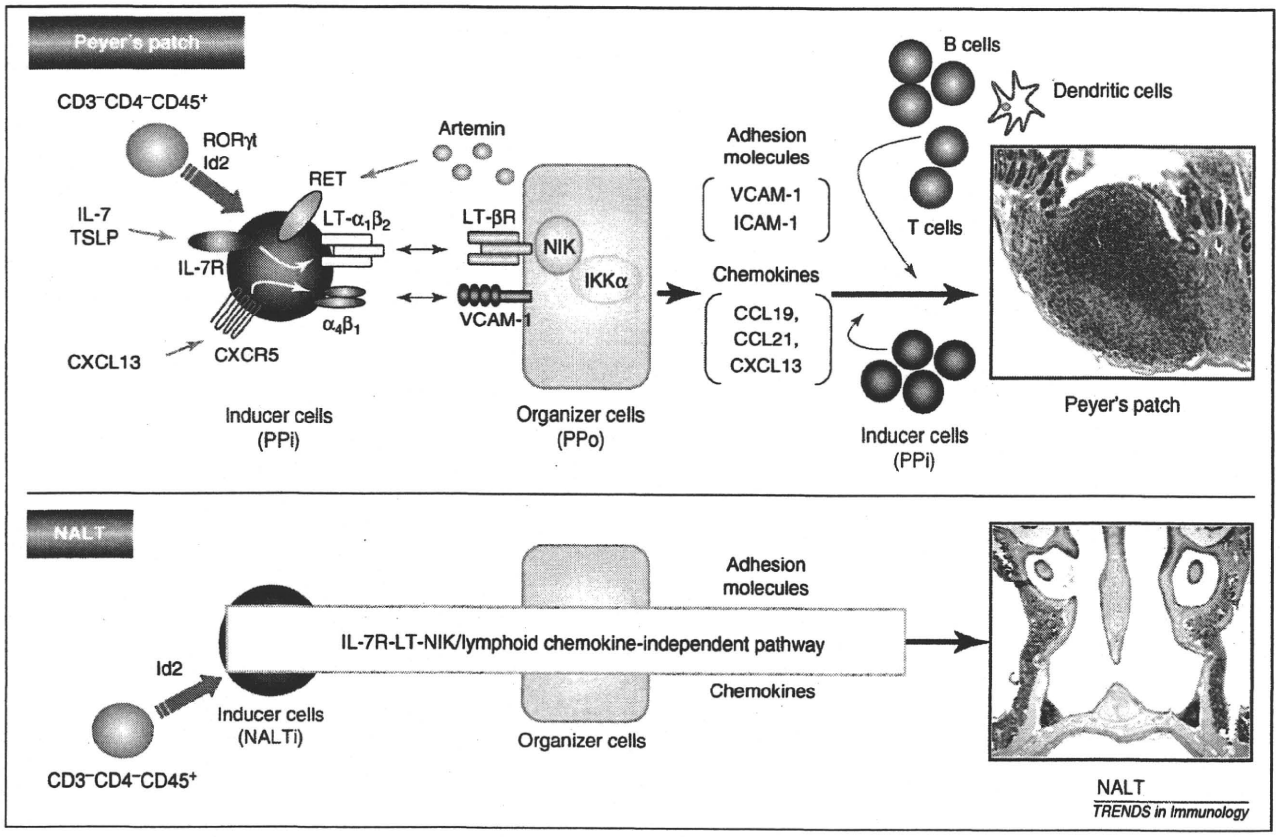

Figure 2. Distinct pathways for mucosa-associated lymphoid tissue development in the intestinal and respiratory tracts. The initial phase of the tissue genesis program of Peyer's patches (PP) operates during embryogenesis. RET⁺IL-7R⁺CD3⁻CD4⁺CD45⁺ PP-inducer cells (PPi) require Id2 and RORγt genes for their development and accumulation at the tissue anlagen for interaction with artemin⁺ VCAM-1⁺ PP organizer cells (PPo). This interaction induces subsequent activation of LTßR-associated molecules, such as NIK and IKKα, and induces the expression of adhesion molecules and chemokines by PP organizer cells; this leads to the further recruitment of T cells, B cells, and DCs. In comparison to the PP tissue genesis program, NALT organogenesis is initiated postnatally and is totally independent of the IL-7R-LT-LTßR-NIK-mediated pathway. The development of NALT-inducer cells (NALTi), like PPs, requires Id2 for the differentiation of CD3⁻CD4⁺CD45⁺ NALT-inducer cells but does not require RORγt.

formation, and thus the loss of any part of this signaling program results in the disruption or impairment of PP development (Table 1) [1–3]. Further evidence supports this model by showing that mice lacking CD3⁻CD4⁺CD45⁺IL-7R⁺ inducer cells due to genetic deletion of the transcription regulators Id2 or RORγt also completely lack PPs (Table 1) [35–37].

In contrast to the embryonically initiated program of PP development, the NALT formation program occurs postnatally (Figure 2 and Table 1) [38]. In addition to these chronological differences, the molecular requirements for PP and NALT tissue genesis are also different [1–3]. Indeed, normal NALT structure is observed in mice otherwise lacking PPs due to a deficiency in the LTßR-, IL-7Rα-, or chemokine (e.g. CCL13, CCL19, and CCL21)-mediated cascade (Table 1) [15,38,39]. Intriguingly, both PP and NALT structures are impaired in mice lacking Id2, whereas deletion of RORγt, results in the suppression of PP but not NALT development [38,40]. These findings suggest that although NALT and PP development depends on the phenotype of CD3⁻CD4⁺CD45⁺ inducer cells, these inducer cells can be categorized into at least into two subsets, NALT inducer (NALTi) and PPi cells, on the basis of the transcriptional regulators, which are either dependent on Id2 alone or dependent on both Id2 and RORγt, respectively (Figure 2 and Table 1).

The mucosal decision for inflammatory versus quiescent immune responses

In the diffuse LP regions of the aero-digestive tract, a wide variety of T cell subsets in the effector tissues are prepared for induction of active or silent immune responses against continuously encountered inhaled or ingested antigens. Therefore, in addition to the active form of immune responses mediated by effector T cells, various regulatory T cell (Treg) subsets are present in the gut for the creation of an immunologically quiescent environment for food-derived antigens and commensal microbiota [1].

Recent studies have identified unique molecular mechanisms mediated by various cytokines in the determination of whether T cells differentiate into pathogenic Th17 or regulatory-type T cells (e.g. Tr1 and Treg) (Figure 3). For example, transforming growth factor-β (TGF-β), which is abundantly produced in intestinal tissues, is an essential molecule for induction of both Foxp3⁺ Treg cells and Th17 cells; however, Th17 cells additionally require IL-6 for their development (Figure 3) [41,42]. A recent study showed that IL-23 plus IL-6 and TGF-β is an additional nonredundant factor in the full differentiation of Th17 cells (Figure 3) [43], whereas IL-27 plus IL-6 and TGF-β induced the differentiation of IL-10-producing T cells that resemble Tr1 cells (Figure 3) [44,45]. Several independent studies have identified retinoic acid (RA)

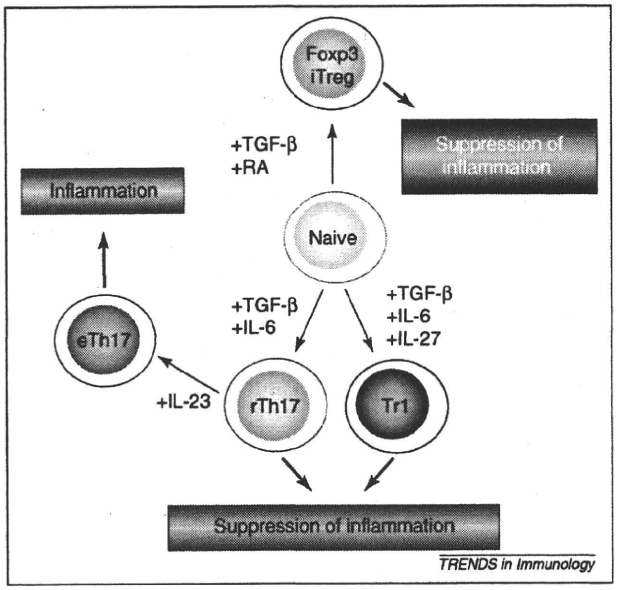

Figure 3. The generation of mucosal inflammatory and regulatory T cells is controlled by a complex network of signals. Naïve T cells are primed and stimulated via a molecular network with the recognition of peptide antigen presented by professional antigen-presenting cells (APC) and their associated cytokines and bioactive molecules. TGF-β and retinoic acid (RA) produced by dendritic cells lead to the default induction of Foxp3$^+$ regulatory T (Treg) cells. By contrast, IL-6 inhibits this pathway and alternatively enhances the induction of cells that differentiate into regulatory Th17 (rTh17) cells. IL-23 and IL-27 determine whether the rTh17 cells differentiate into effector Th17 (eTh17) or IL-10-producing type 1 regulatory (Tr1) cells, respectively.

produced by intestinal DCs as a key molecule to enhance the conversion of TGF-β-treated T cells to Treg cells and simultaneously suppresses their differentiation to Th17 cells (Figure 3) [46–49]. Because RA production is specifically observed in gut DCs, the respiratory tract likely employs other mechanisms for maintaining quiescence. In this regard, respiratory DCs alter their ability to induce inflammatory and/or noninflammatory responses [20,50–53] although the details of this process await elucidation. In addition, intestinal macrophages have been shown to behave as a member of mucosal regulatory cells by preferential production of IL-10 [54,55]. In a similar manner, the lung contains unique macrophages, known as alveolar macrophages, in the alveolar space. The alveolar macrophages by default inhibit immune responses through interaction with TGF-β activated by $\alpha_v\beta_6$ integrin$^+$ ECs [56], providing an additional pathway for inhibitory events in the lung. These findings collectively suggest the presence of a multivalent mucosa-associated regulatory system of unique mononuclear cells (e.g. DCs, macrophages, Th17, Tr1, and Treg cells), cytokines (e.g. TGF-β, IL-6, IL-10, IL-23, and IL-27), and other biological molecules (e.g. RA) that determine and control the qualitative and qualitative aspects of antigen-specific mucosal immune responses (Figure 3).

Regulation of secretory IgA (S-IgA) production in the aero-digestive tract

In addition to Th17, Tr1, and Treg cells, classical helper T cells producing IL-5, -6, and -10 allow IgA$^+$ plasmablasts to differentiate into PCs that produce polymeric forms of IgA (pIgA) joined by J-chains [57]. The polymeric IgA (pIgA) produced by PCs binds to polyimmunoglobulin receptors (pIgR) expressed on the mucosal ECs and are transported to the apical surface. Extracellular proteolytic fragments of the pIgR (secretory component, SC) and pIgA are secreted as secretory IgA (S-IgA) [58].

Mucosal IgA can be discriminated into two groups according to their affinities [1,59]. The high-affinity form of IgA plays an important role in neutralization of microbial proteins including toxins, and it is considered to originate from B2 (i.e. conventional) B cells, which are induced in MALTs [22]. The low-affinity type of IgA is thought to originate from T-independent B1 B cells in the peritoneal and pleural cavities, leading to the inhibition of adhesion of commensal microbiota [60,61]. Unlike the CD40-dependent differentiation of B2 B cells, several lines of evidence have revealed that B1 B cells use other molecular interactions for their development, such as B cell-activating factor of the TNF family (BAFF) and A-proliferation-inducing ligand (APRIL) [62]. Indeed, T-independent IgA responses are decreased in mice lacking the transmembrane activator and CAML interactor (TACI), a receptor for both BAFF and APRIL [63].

In addition to affinity, structural differences of human IgA separate it into two subclasses, known as IgA1 and IgA2 [64]. This difference is attributable to the deletion of 13 amino acids in the hinge region and is associated with the resistance of IgA2 to proteases. Distinct distributions of IgA1 or IgA2 are noted in different parts of the immunological tissues [64]. Systemic lymphoid tissues (e.g. spleen, lymph nodes, and bone marrow) are dominated by IgA1-producing cells. In most of the mucosal tissues (nasal, gastric, and small intestinal mucosa), IgA1 producers are still the major subclass, but these tissues have greater numbers of IgA2-producing cells than are found in the lymphoid tissues of the systemic compartments. Additionally, IgA2-producing cells are more frequent than IgA1 cells in the large intestine; this might account for the fact that naturally occurring S-IgA antibodies to bacterial endotoxin are associated with the IgA2 isotype [64]. To this end, a recent study revealed that intestinal ECs trigger sequential class switching from IgA1 to IgA2-expressing B cells in a T cell-independent but toll-like receptor (TLRs)- and APRIL-dependent manner [65]. Thus, it is likely that different circumstances (e.g. the nature of commensal microbiota) between respiratory and intestinal tracts might determine the different IgA1:IgA2 ratio.

Aero-digestive epithelium as a critical player in the mucosal immune system

In addition to their involvement in the transport of IgA, ECs are also involved in the regulation of mucosal immunity. Intraepithelial lymphocytes (IELs) are representative cells located among ECs, and consist mainly of T cells expressing either αβ TCRs or γδ TCRs, which allow the bridging innate and acquired immune responses at the surfaces of aero-digestive tracts through their interaction with classical and nonclassical MHC molecules expressed on ECs [66].

In addition to IELs, antigen-sampling M cells, termed villous M cells, are found in villous epithelium as an

alternative antigen-sampling pathway [67]. Additionally, DCs in the LP extend their dendrites into the lumen and sample antigen [68–70]. A recent study has suggested that these DCs are capable of initiating systemic IgG responses, whereas antigen transport by M cells into the PPs is required for the induction of intestinal IgA production [71], a finding that is consistent with another report that DCs in the PPs are responsible for intestinal IgA production [12]. Similar villous M cells and intraepithelial DCs have been reported in the respiratory tracts [72,73]. In addition, our recent studies have also suggested the presence of respiratory M cells in the nasal cavity (Kim *et al.*, submitted for publication). Taken together, these results suggest that the aero-digestive tissue is equipped with a diversified antigen-uptake and presenting system consisting of ECs, MALT M cells, villous M cells, and intraepithelial DCs.

Mucosal lymphocyte trafficking

Following T cell priming and CSR to IgA-committed B cells in the MALTs, lymphocytes move to distant mucosal effector compartments (especially the LP regions of the respiratory and gastrointestinal tracts). Accumulating evidence has revealed the existence of a highly sophisticated system regulating lymphocyte trafficking from MALTs into the LP regions of the aero-digestive tract [1] (also see the article by Agace in this issue).

A lipid mediator, sphingosine 1-phosphate (S1P) is now recognized as a general molecule in the regulation of lymphocyte trafficking [74]. We have recently shown that S1P contributes to the mucosal immune responses including intestinal S-IgA production from peritoneal and PP B cells as well as trafficking of IEL precursors from the thymus [24,75–77]. Other studies have demonstrated that S1P is also involved in the immune regulation of respiratory tissues [78]. Because the S1P-mediated system is also utilized in the trafficking of pathogenic cells, it is a potential target for the inhibition of inflammation in both respiratory and intestinal tracts [79–82].

In contrast to the shared function of S1P, aero-digestive tissue possesses a distinct pathway to achieve selective trafficking to its mucosa. Mucosal addressin cell adhesion molecule-1 (MAdCAM-1) expressed by high endothelial venules (HEVs) in the gastrointestinal tracts and $\alpha_4\beta_7$ integrin expressed by lymphocytes is the most important adhesion molecule in the cell trafficking to the intestine (Figure 4) [83]. Chemokines, especially CCR9, is selectively expressed on IgA-committed B cells and T cells activated in the PPs, and CCL25 (the ligand of CCR9) is produced dominantly by the intestinal epithelium, allowing PP-primed B and T cells to selectively migrate to the intestinal LP (Figure 4) [84]. Several lines of evidence suggest that PP DCs play a pivotal role in the induction of $\alpha_4\beta_7$ integrin and CCR9 on activated B and T cells through the production of RA from vitamin A [85,86]. Thus, significantly decreased numbers of intestinal T and B cells have been noted in the intestine of vitamin A-deficient mice and rats, which might in turn explain the increased level of child mortality arising from vitamin A deficiency induced diarrhea [85–87].

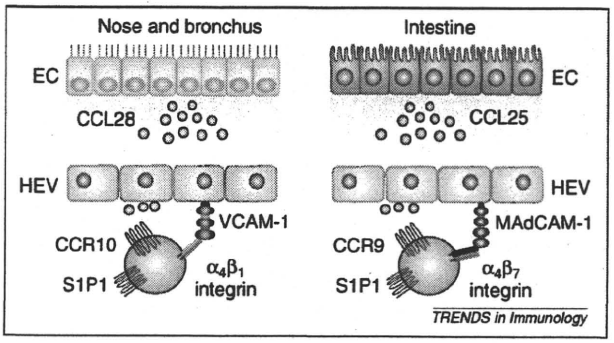

Figure 4. Mucosal T cell migration pathways for aero-digestive tracts. Mucosal lymphocyte migration is determined and controlled by the combination of adhesion molecules and chemokines. CCL28-CCR10 and VCAM-1-$\alpha_4\beta_1$ integrin interactions are involved in the trafficking of cells to the respiratory mucosa, whereas CCL25-CCR9 and MadCAM-1-$\alpha_4\beta_7$ integrin participate in the cell trafficking to intestinal tissues through high endothelial venules (HEV). S1P1 is a mucosal migration-associated molecule common to both respiratory and intestinal mucosa.

During lymphocyte trafficking to respiratory tissues, neither $\alpha_4\beta_7$ integrin nor MAdCAM-1 was expressed by human tonsil mononuclear cells or the tonsils and adenoids of rodent NALT HEVs, respectively. However, nasal immunization induces upregulation of $\alpha_4\beta_1$ integrin and CCR10, allowing selective trafficking of lymphocytes to LP regions of the nose, trachea and bronchus, where their ligands, VCAM-1 and CCL28, are strongly expressed (Figure 4) [88]. Several lines of evidence show that lymphocyte trafficking into the lung is regulated by adhesion molecules (VLA-1 and LFA1) and chemokines (e.g. CCL5) [89,90].

Leading bench mucosal immunology toward a new generation of self-administered vaccines

One of the major missions of basic research is of course the translation of bench discoveries to the bedside and public health. Understanding the molecular and cellular functions of the mucosal immune system associated with the aero-digestive tract has allowed us to create a general consensus that nasal or oral administration is an effective regimen for the induction of localized antigen-specific mucosal immune responses mediated by both S-IgA and CTL as well as systemic immune responses (e.g. IgG) [91] (also see the article by Belyakov and colleagues in this issue). Because mucosal immunization can effectively induce both layers of antigen-specific immunity, it has been considered that mucosal vaccination is an ideal strategy for the global control of mucosal infectious diseases [91]. In general, thanks to the above-described elegance of the imprinting system for tissue tropism of mucosally activated lymphocytes, the antigen-specific humoral and cellular immune responses induced by mucosal immunization most effectively occur at the site of antigen-deposited mucosal tissues with some mobility to the other mucosa-associated tissues. Therefore, a general view is that antigen administration via oral and nasal routes will be a suitable method for vaccination against gastrointestinal and respiratory infections, respectively [92].

Currently several oral vaccines, such as polio (OPV) and rotavirus vaccine (Rotarix® and RotaTeq®), are approved for human use (Table 2) [93,94]. These vaccines are forms of live-attenuated viruses that stimulate the gastrointes-

Table 2. Currently approved oral/nasal vaccine against mucosal infectious diseases

Pathogen	Administration route	
	Oral	Nasal
Polio virus	Live attenuated polio virus (strain: Sabin)	–
Vibrio cholera	Heat or formalin inactivated *V. cholerae* O1 (strain: Inaba and Ogawa) and cholera toxin B subunit (CT-B) (Dukoral®)	–
Rotavirus	Live attenuated rotavirus Strain: human rotavirus 89-12 (RotaRix®) Bovine rotavirus WC3 (RotaTeq®)	–
Influenza virus	–	Live attenuated influenza virus (strain: Fashion species) (FluMist®)

tinal immune system upon oral immunization, resulting in the effective induction of high levels of virus-specific protective immunity. In addition to oral vaccines, a nasal spray vaccine against influenza (FluMist®), composed of live-attenuated, cold-adapted influenza virus, is now approved and used in the United States (Table 2) [95]. Nasally administered FluMist® effectively induces influenza virus-specific protective immune responses via the airway mucosal immune system. In particular, the hemagglutinin-specific S-IgA secreted into the lumen of the respiratory tract plays a pivotal role in the inhibition of the virus entry through the epithelial surface of the respiratory tract. Despite their effectiveness as mucosal vaccines, it should be noted that these live-attenuated mucosal vaccines occasionally cause side effects because of their live nature. For instance, attenuated polio virus can, albeit very rarely, acquire neurovirulence after oral immunization and cause poliomyelitis [93]. In addition, nasal vaccination with the virus attenuated FluMist® is more likely to cause wheezing than the nasally administered inactivated influenza virus, especially in young children with a history of asthma [96]. For this reason, FluMist® is not yet approved for the immunologically infirm populations of infants and the elderly [97]. Therefore improvements to the safety of live-attenuated mucosal vaccines are an important prerequisite for their practical use.

By contrast, mucosal vaccines composed of inactivated viruses or bacteria killed by heat or formalin treatment have been energetically developed because they rarely cause side effects. An oral cholera vaccine (Dukoral®) composed of the O1 and B subunits of cholera toxin (CT-B) from inactivated *Vibrio cholerae* induces protective immunity, especially in the gastrointestinal tract (Table 2) [98]. By contrast, an injectable type of inactivated cholera vaccine is available, but it fails to fully protect against *V. cholerae* infection because it induces only systemic, and not mucosal immune responses [98]. Therefore, the development of an oral rather than injectable cholera vaccine is still a prudent approach and currently the subject of a worldwide effort. In addition to oral vaccines, the development of inactivated nasal vaccines against respiratory infectious diseases has been extensively investigated. It was recently reported that nasal vaccination with formalin-inactivated influenza virus (strain H5N1) induces protective immunity in both the systemic and mucosal compartments of nonhuman primates [99]. In addition, co-administration of the TLR3 agonist polyI:polyC$_{12}$U (Ampligen®) in combination with the H5N1 vaccine enhanced virus-specific S-IgA responses in the respiratory tract [100]. H5N1 is the highly pathogenic avian influenza virus, and these findings should facilitate the development of a nasal influenza vaccine for the global control of potentially catastrophic zoonoses caused by this strain of influenza virus.

Injectable vaccines are currently and commonly used in both developing and industrialized countries, despite our scientific knowledge of the advantages of mucosal immunization. One of the major practical obstacles to vaccination in the field, especially in developing countries, is storage of the vaccine under refrigeration (known as the cold-chain). In addition, injection of a vaccine with a needle and syringe requires skilled medical professionals at the time of inoculation and disposal of the used needles and syringes from mass vaccinations, which is now a major concern because medical waste can contaminate the environment. To overcome these practical concerns, a new generation of 'self-administrable cold-chain and needle and syringe-free vaccines' will need to be developed. To accomplish this goal, a plant-based vaccine is considered to be one attractive strategy, because plants can be used as natural bioreactors and transporters of vaccine antigens. Progress in plant genetic technology has enabled the development of plant-based oral subunit vaccines [91]. Among the several plant-based vaccines developed so far, a rice-based oral vaccine (or MucoRice™) has recently attracted interest as a vaccine production and delivery system because of its practical advantages [101]. For instance, a unique protein-storage organelle in the rice seed, named the protein body, provides a suitable vehicle for expression of vaccine antigens that are not only stable at room temperature for several years without loss of immunogenicity, but are also protected from digestive enzymes in the gastrointestinal tract [101]. In this context, we have recently reported that oral immunization with MucoRice™-expressed CT-B subunit in mice effectively induced antigen specific intestinal and systemic immune responses with a protective function against an oral challenge with cholera toxin [101]. By contrast, it should be noted that MucoRice™-expressed CT-B did not induce any detectable level of immune response against rice storage proteins. This discrepant but advantageous result might be due to the high antigenicity of MucoRice™-expressed CT-B compared with that of the rice storage protein. The MucoRice™ system therefore opens up novel avenues for developing both human and environmentally friendly vaccines as cold-chain-, needle- and syringe-free self-administered vaccines that will benefit both developing and developed countries.

The other recent progress in the development of new-generation mucosal vaccines is the creation of a delivery system that targets M cells. As discussed above, M cells in

FAE regions of PPs and NALT enable the selective transport of luminal antigens from the gastrointestinal or respiratory lumen to their respective MALTs for the initiation of antigen-specific immune responses [7]. However, because the number of M cells in mucosal epithelium is limited, the development of M cell-targeting systems for antigen delivery might facilitate the efficacy of mucosal vaccines for the induction of antigen-specific systemic and mucosal immune responses. Several approaches incorporating an M cell-specific lectin or peptide [e.g. *Ulex europaeus* agglutinin-1 (UEA-1) [102,103] and YQCSYTMPHPPV [104]] or microbial invasion molecules known to target M cells (e.g. reovirus σ1 protein and *Yersinia*-derived invasin) into the vaccine [91,105] have been examined as the cell-targeted delivery vehicles for nasal or oral vaccines, but their binding to other neighboring cells (e.g. UEA-1 binding to goblet cells [106]) has hampered their effective delivery of antigens to M cells. To overcome such obstacles, we have generated a monoclonal antibody (NKM 16-2-4), which specifically binds M cells but not other cells of the intestinal and respiratory tract [107]. NKM 16-2-4 also reacts with villous M cells, which are known to be alternative antigen-sampling cells located in the intestinal villous epithelium [67,107]. Thus, the use of NKM 16-2-4 as a delivery molecule for mucosal vaccines makes it possible to effectively target a vaccine antigen to both PP-associated and villous M cells, and the subsequent induction of antigen-specific immune responses in both mucosal and systemic compartments is more efficient than that of other M cell-targeted vaccines (e.g. UEA-1-conjugated vaccine). Indeed, we have shown that potent levels of antigen-specific protective immunity were induced when mice were orally immunized with small amounts of tetanus-toxoid- or botulinum-toxoid-conjugated NKM 16-2-4 [107]. Thus far we have not tested the application of NKM 16-2-4 to a nasal vaccine and several other issues, e.g. the requirement of a mucosal adjuvant and reactivity to human M cells, await future study. Development of an M cell-targeted mucosal vaccine using an M cell-specific antibody is a hopeful strategy for the development of a safe and effective mucosal vaccine.

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Chapter 31

The Mucosal Immune System

Hiroshi Kiyono, Jun Kunisawa, Jerry R. McGhee, and Jiri Mestecky

Add H1 "Introduction"?

The most important source of stimulation of the entire immune system is the external environment comprising the indigenous mucosal microbiota, potential pathogenic microorganisms, abundant food antigens (Ags), and allergens, all of which are encountered mainly at the vast surface areas of mucosal membranes. This enormous and highly variable antigenic load has resulted in a strategic distribution of cells involved in the uptake, processing and presentation of Ags, production of antibodies (Abs), secretion of cytokines, and cell-mediated immune (CMI) defenses at the front line of defense—mucosal tissues and associated secretory glands. Quantitative data concerning the distribution of phagocytic cells, T and B lymphocytes, and Ab-producing cells illustrate the point: mucosal tissues, particularly those of the intestinal tracts, contain more macrophages (MΦ), plasma cells (PCs), and T cells than any other lymphoid tissue in the entire immune system.

Notwithstanding the global importance of systemically acquired infections such as malaria and neonatal tetanus, the majority of infectious diseases worldwide either directly afflicts or is acquired through mucosal surfaces of the gastrointestinal (GI), respiratory, and genital tracts. Consequently, innate and adoptive immune mechanisms operational at mucosal surfaces are of great importance to the protection and survival in a hostile environment. The induction of preventive and protective immune responses to mucosal infectious agents, and to ingested food Ags and environmental allergens that would limit their absorption, is usually the most emphasized functional aspect of the mucosal immune system. Yet recently revived interest in the induction of systemic unresponsiveness to Ags applied first by the mucosal route, so called *oral* or *nasal* (mucosal) *tolerance*, has directed the attention of immunologists working in the field of autoimmunity, transplantation, and hypersensitivity to the exploitation of this fundamental

principle. Although there are limited numbers of clinical successes, the phenomenon of mucosal tolerance is an essential feature and critical functional component that efficiently prevents and suppresses otherwise unavoidable overstimulation of the entire immune system by the most common environmental Ags primarily of food and indigenous bacterial origins. The enhancement of protective mucosal immune responses to infectious agents sought by vaccinologists, and the desired suppression of systemic immune responses to autoAgs and transplantation Ags, may seem paradoxical. Yet such outcomes are not mutually exclusive due to the hierarchy in the quality of immune responses induced by mucosal Ag delivery: Mucosal immunity manifested by the appearance of secretory Abs and systemic tolerance evaluated by diminished CMI-responses may be concomitantly induced. Thus, the fundamental objectives of the mucosal immune system—containment of the vast onslaught of environmental Ags without compromised integrity of mucosal barriers and prevention of overstimulation of the systemic compartment—are achieved by concerted interactions of lymphoid and nonlymphoid cells, epithelial cells (ECs) in particular, and their respective products as a mucosal internet of communication. Thus, an orchestrated mucosal immune system consisting of innate immunity as well as acquired immunity including secretory IgA (S-IgA) Abs and mucosal cytotoxic T lymphocytes (CTLs), adds additional layers of host defense.

INNATE MUCOSAL IMMUNE SYSTEM

Epithelial Cells

Physical Barrier Function of ECs

The epithelium of the mucosa-associated lymphoid tissues (MALTs) of the lung, gut and genitourinary tracts, and, likely, others have been clearly shown to play an active role in both innate and adaptive types of mucosal immunity. Given the physical proximity of the ECs to the external milieu and, therefore, the primary site of initial Ag exposure, ECs may be a central cell type in both defining the Ags with which the mucosal immune system is confronted and regulating the ultimate responses to these antigenic exposures. Initially, prevention of luminal Ag transport is through a thick layer of mucus. Mucin 2 (MUC2) is a dominant intestinal mucus-formation molecule that is abundantly expressed by goblet cells located at the intestinal villous epithelium (1). Mucus not only provides a physical and biological protective barrier, but also ensures maintenance of an appropriate concentration of Abs at the mucosal surface by preventing Ag-specific S-IgA Abs from being physically carried away. Additionally, paracellular transport of luminal Ag is prevented by the juncture between adjacent ECs that is mediated by physical structures associated with the epithelium including the tight junctions (TJs) and the subjacent desmosomes and adherence junctions (2). The TJs are composed of a number of interacting cellular proteins, which include claudin, occuludin, ZO-1, ZO-2, and cingulin, among others. Under normal circumstances, the TJs exclude Ags greater than 6–12 Å (> 500–900 Daltons) in molecular diameter.

In addition to these physical barrier functions of ECs, the epithelium of the MALTs of the lung, gut and genitourinary tracts, and, likely, others have been clearly shown to play an active role in both innate and adaptive types of mucosal immunity by collaboration with adjunct neighboring ECs as well as subjacent parenchymal cells (fibroblasts and mesenchymal cells and their connective tissue substances) and hematopoietic cells (MΦ, dendritic cells (DCs), polymorphonuclear (PMN) lymphocytes and lymphocytes) and likely microbial components in the lumen (3).

Antimicrobial Peptides

The epithelium also secretes a variety of antimicrobial peptides (defensins, cathelicidins, crytdin-related sequence [CRS] peptides) and bacteriolysis enzymes (lysozyme, secretory phospholipase-A2 [PLA2], peroxidase, and lactoferrin), and others (Figure 31.1). In the intestinal epithe- **Fig. 31.1**
lium, ECs, Paneth cells, and PMNs mainly produce these molecules (4,5). Paneth cells reside at the base of the crypt regions of the small intestine, but not the stomach or colon. They produce α-defensins constitutively. In contrast, β-defensins are produced by ECs of the whole intestine, which requires microbial stimulation (4,5). Both defensins are cationic small peptides with a characteristic β-sheet–rich fold and a framework of six disulphide-linked cysteines and exhibit antimicrobial activity by damaging and permeabilizing the bacterial cell membrane by pore formation. Defensins also inhibit viral infection (e.g., human immunodeficiency virus [HIV], herpes simplex virus [HSV], vesicular stomatitis virus, and influenza virus) by interupting their invasion at an early step, such as receptor binding (4,5). In addition to the antimicrobial properties, defensins have chemotactic activities for monocytes, T cells, and B cells, implying that defensins may bridge between mucosal innate and acquired immunity via the augmentation of T and B cell interactions (6). The cathelicidin is also a cationic small peptide containing a cathelin-like domain produced by ECs, PMNs, and keratinocytes (4,5). The expression of cathelicidin by ECs is regulated by butyrate and other short-chain fatty acids produced by fermenting bacteria. The CRS peptide is produced by Paneth cells and shows antimicrobial activity through its cationic feature (4,5).

Antimicrobial enzymes are other molecules showing antimicrobial activities (Figure 31.1). PLA2 is a small enzyme produced by Paneth cells and PMNs, which

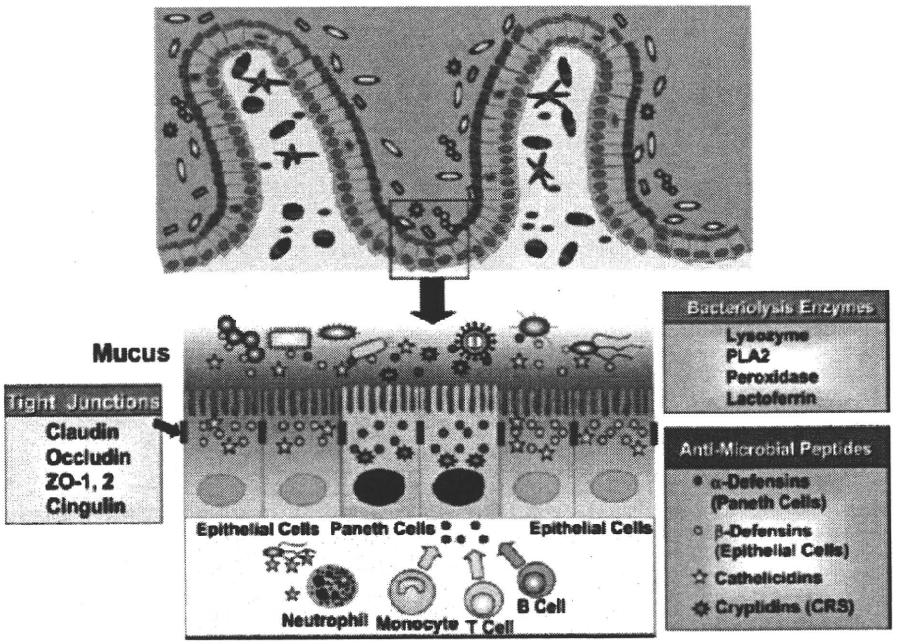

FIGURE 31.1 Various types of antimicrobial molecules protect mucosal surfaces against invading microbes. ECs and Paneth cells secrete bacteriolytic enzymes (lysozyme, PLA2, peroxidase, and lactoferrin) and antimicrobial peptides (defensins, cathelicidins and crytdin-related sequence [CRS]) in mucosal sites. Neutrophils also produce antimicrobial molecules. Defensins have been shown to possess the capability to recruit immunocompetent cells for the initiation of innate and adaptive immune responses.

degrades bacterial phospholipids and subsequently disrupts bacterial integrity (4,5). Lysozyme is another bactericidal component produced by Paneth cells, PMNs, and ECs. Lysozyme is a muramidase cleaving the glycosidic linkage between N-acetylglucosamine and N-acetyl muramic acid of peptidoglycan, and thus it is preferentially effective against gram-positive bacteria (4,5). Surfactant proteins A-D (SPs) are highly hydrophobic proteins in the lung produced by alveolar type II cells. Several lines of evidence revealed that SPs are actively involved in lung innate immunity following bacterial penetration into the lower airways (7). SPs bind to LPS and the interaction between SPs and CD14 may explain their ability to affect some LPS responses.

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Antimicrobial molecules are also produced by PMNs induced following infection by pathogens. It is well established that PMNs take up invading microorganisms through a complement lysis-dependent phagocytosis and kill them by antimicrobial tools such as toxic oxygen radicals, cationic peptides, and lytic enzymes in the phagocytic vacuoles. In addition, PMNs produce extracellular fibers containing DNA, histones, and granule proteins after stimulation by bacterial endotoxins (8). These fibers are known as *neutrophil extracellular traps* (NETs). NETs bind to both gram-negative and -positive bacteria and kill them by their esterase and antimicrobial peptides and enzymes.

Cytokines and Cytokine Receptors

ECs are able to secrete both constitutively and inducibly a large number of inflammatory and regulatory cytokines. Using EC lines, it has been shown that the epithelium can constitutively express proinflammatory cytokines such as IL-1-α, IL-1-β, IL-15, TNF-α, and IL-6 and anti-inflammatory and barrier-promoting cytokines such as TGF-β and IL-10, whose levels may be further increased

by interactions with pathogens and their toxic products (3). The production of these cytokines by the epithelium is likely to play an important role in both promoting intestinal inflammation (e.g., IL-1 and TNF-α), regulating the activation and expansion of mucosal T cells within the epithelium (e.g., stem cell factor [SCF], IL-5, IL-7, and IL-15), regulating local B cell production of immunoglobulins (Igs) (e.g., TGF-β, IL-6, and IL-10), and, finally, regulating barrier function, *per se* (IL-10, IL-15, and TGF-β). With regards to barrier function, ECs also express a large number of cytokine receptors (3). Intestinal EC (IEC) lines and freshly isolated IECs express mRNA for the common IL-2 receptor γ-chain and specific α-chains of the receptors for IL-2, IL-4, IL-7, IL-9, and IL-15. IECs also express receptors for TNF and IFN-γ, which not only regulate the expression of a wide variety of other immunologically important molecules such as the polymeric Ig receptor (pIgR), for example, but also tend to diminish epithelial barrier function. The expression of these cytokines and cytokine receptors thus further emphasize the integration of the epithelium into the network of cellular interactions associated with the MALTs. In this regard, bacterial infection can influence the interactions of the EC-mediated mucosal internet with mucosal T and B cells via IL-7/IL-7R and IL-15/IL-15R.

Transcellular Transport Functions of the Epithelium

Another aspect of epithelial barrier function that represents a link between the epithelium and the adaptive components of the MALT is the ability of the epithelium to transport macromolecules, especially Igs, transcellularly in a process termed *transcytosis*, which reflects the polarized nature of the epithelium. Two receptors for Ig have been shown to have such properties. The pIgR, whose itinerary is now well defined, transports polymeric forms of IgA (pIgA) and IgM (pIgM) in a basal to apical direction with unloading of its cargo in association with an extracellular proteolytic fragment of the pIgR receptor (secretory component, SC) (9). This pathway is not only able to deliver large quantities of secretory Ig onto the mucosal surfaces, but it is also able to exclude Ags that have entered the secretory pathway either apically or basally (discussed later). This type of defense, which takes advantage of a component of the adaptive immune response, is likely to be important in resistance against pathogenic viral infections.

In a related but distinct manner, the epithelium also expresses the neonatal Fc receptor for IgG (FcRn) (10). Recent evidence indicates that this molecule is expressed by adult human epithelium and MΦ of the intestine (and, likely, other surfaces) and thus is not strictly limited to neonatal life as predicted by earlier studies in rodents wherein the FcRn was responsible for the passive acquisition of IgG neonatally (10). In the context of expression postnatally in adult humans, FcRn may therefore be in a position to provide luminal immunosurveillance against pathogenic exposure. FcRn binds IgG, its cargo, in a pH-dependent process (pH 6 on, pH 7.4 off) due to critical histidine residues in the Fc-region of the IgG molecule. In contrast to the itinerary associated with pIgR-associated transport, the transport pathway associated with FcRn is bidirectional; both apical to basal and basal to apical (10). In addition, the FcRn is not associated with proteolytic cleavage allowing for reiterative rounds of transport. It is predicted, therefore, that the FcRn is at least in part responsible for the steady state distribution of IgG on either side of an epithelial barrier given the unlikely possibility that paracellular transport of this macromolecule occurs due to the molecular exclusion of the TJs.

As below

Pattern Recognition Receptors

Toll-Like Receptors

The extrinsic barrier functions of the epithelium associated with innate immunity are at least partially mediated by the interaction with luminal microflora. It is well established that pattern recognition receptors (PRRs) play an important role in the recognition of microbial products (Figure 31.2) (11,12). Most prominent among PRRs that regulate innate immune responses are an array of Toll-like receptors (TLRs). ECs in the respiratory, genital, and GI tracts were reported to express several types of TLRs binding to signature microbial products such as peptidoglycan of gram-positive bacteria (TLR2), viral double-stranded RNA (dsRNA) (TLR3), bacterial LPS of gram-negative bacteria (TLR4), bacterial flagellin (TLR5), and microbial CpG motifs of DNA (TLR9) (13,14). These molecules were initially characterized as the pathogen-associated molecular patterns (PAMPs), but these are produced by both pathogenic and commensal microorganisms. Although TLR signaling induces an inflammatory cascade in the sterile circumstance of the systemic immune compartment, mucosal TLRs should take a different strategy to distinguish pathogenic microorganisms from commensal microorganisms for the immunosurveillance in the mucosal tissues directly and continuously exposed to the large numbers of commensal as well as occasional pathogenic microorganisms.

Fig. 31.2

Evidence has been emerging that underlying molecular pathways exist to achieve the discrimination of pathogenic from commensal microorganisms in the innate mucosal system (Figure 31.2). First, it has been proposed that decreased surface expression of TLRs and coreceptors leads to the down-regulation of TLR signaling (13,14). Notably, peptidoglycan and LPS are abundantly produced by the commensal microflora, and thus their receptors (TLR2 and TLR4) should have an immune quiescent system against these commensal-derived PAMPs. Along these lines, TLR2

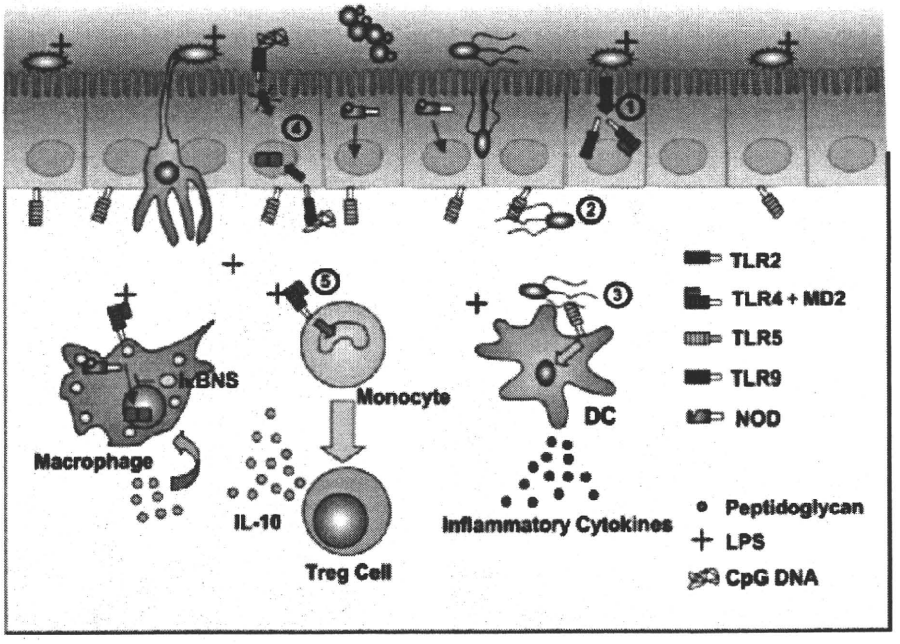

FIGURE 31.2 Uniqueness of recognition and discrimination of microbes. Toll-like receptors and NODs recognize bacterial products (peptidoglycan [PG], lipopolysaccharide [LPS] and CpG DNA). The host mucosal immune system employs various mechanisms to distinguish pathogenic from commensal bacteria: 1, continuous bacterial stimulation leads to down-regulation of TLR expression; 2, TLR5 is preferentially expressed on the basolateral side of ECs; 3, pathogenic bacteria are recognized by LP DCs which express TLR5; 4, TLR9 signals through the basolateral side induces the activation of the NF-κB pathway while apical TLR9 stimulation prevents NF-κB activation; 5, LPS from commensal bacteria induces regulatory T cell differentiation.

and TLR4 are expressed on the surface of ECs but prolonged stimulation with their ligands resulted in their down-regulation. Thus, TLR2 and TLR4 are expressed on fetal ECs and adult crypt ECs, but their expression was lost on mature ECs (15). In addition to ligand stimulation, down-regulation of TLR expression is induced by TGF-β, a regulatory cytokine predominantly produced in the intestine (12). In the case of TLR4, LPS recognition is coupled to CD14 binding of LPS, wherein LPS bound to CD14 interacts with a TLR4/MD-2 protein heterodimer. The absence or reduction of CD14 and MD-2 expression on ECs was reported, which presumably was associated with a lack of reactivity with LPS (14).

Secondly, the reduced reactivity of ECs to PAMPs is explained by the unique distribution of TLRs. For example, it has been shown that the LPS-induced reduction of TLR2 and TLR4 was mediated by the alteration of their distribution from the apical site to intracellular compartments (13). It is interesting to note that intracellular TLR4 maintains its activity to detect intracellular bacteria. This unique intracellular distribution of TLR4 but not outer cell membrane allows the EC to discriminate pathogenic cells invading the ECs and commensal cells generally attaching on their cell surface. Another example of this was shown by TLR5, which recognizes bacterial flagellin. TLR5 is exclusively expressed on the basolateral site of ECs, allowing them to sense bacteria when they invade into the lamina propria (LP) regions (14). A recent study has provided an additional example of polarity-mediated regulation of TLR signaling in the ECs by showing that TLR9 signals through

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the basolateral site and induces the activation of the NF-κB pathway, while apical TLR9 stimulation prevents NF-κB activation (16).

As the third mechanism, several negative regulatory pathways for TLR expression have been identified. It was reported that the loss of postnatal LPS responsiveness of ECs was associated with a posttranscriptional down-regulation of the IL-1 receptor-associated kinase 1 (IRAK1), an essential molecule for epithelial TLR4 signaling (15). ECs also express a negative regulator of TLR signaling (12–14). For instrance, tollip is induced in the EC by bacterial stimulation and plays an important role in the negative regulation of TLR signaling through its suppression of the IRAK activation pathway. Single Ig IL-1-related receptor (SIGIRR, also known as TIR8) is a negative regulator of IL-1 and TLR signaling expressed in the ECs, which attenuates the recruitment of receptor-proximal signaling components to the TLRs. Other negative regulators have been identified, such as intracellular antagonists of TLR signaling (MyD88s [splice variant of MyD88], IRAKM [homolog of IRAK1], and IRAK2c/d [splice variants of IRAK2]) and ubiquitin ligase of TLR-mediated signaling molecules (A20 and TRIAD3A), but their involvement in the down-regulation of TLR signaling in the mucosal tissues remain to be investigated (12–14).

Cells other than ECs also possess a unique system to achieve effective intestinal immunosurveillance without excess immune responses against commensal microflora. Like the ECs, LP MΦ lack reactivity to LPS due to failure to express CD14 (17). It is interesting to note that the unresponsiveness of intestinal MΦ to LPS was intrinsic but that of EC was acquired immediately after birth by exposure to exogenous LPS, as mentioned earlier (15). Additionally, LP MΦ selectively express IκBNS, an inhibitor of NFκB activation (18). In the case of mucosal DCs, a unique pathogen recognition system is achieved by the distinct expression of TLR4 and TLR5. Unlike conventional DCs in systemic immune compartments (e.g., spleen), intestinal LP DCs predominantly express TLR5, but not TLR4 (19). Thus, the LP DCs can detect pathogenic bacteria in a TLR5-dependent manner when luminal pathogens break the epithelial barrier and become exposed to LP DCs. However, these DCs do not secrete pro-inflammatory cytokines after exposure to commensal bacteria.

Cytoplasmic PAMPs Receptors

Besides the TLRs, other receptors for detecting cytoplasmic PAMPs have been identified (Figure 31.2). Nucleotide-binding oligomerization domain 1 (NOD1) and NOD2 are well-characterized cytoplasmic PRRs expressed by both ECs and MΦ, which recognize a peptidoglycan motif containing a diaminopimelate-containing N-acetylglucosamine-N-acetylmuramic acid tripeptide in gram-positive and gram-negative bacteria (11,12). Thus, the NOD family plays a crucial role in distinguishing invading pathogens and commensal bacteria. For the detection of invading viruses, retinoic acid inducible gene-I (RIG-I) and melanoma differentiation-associated gene 5 (MDA5) have been identified. Both recognize dsRNA generated during viral replication and trigger the activation of NF-κB and IRF3/7 with subsequent production of antiviral type I IFN (11,12). The family of intracellular PRRs provides another layer to the innate system for production of the mucosal epithelium and the immediate underlying region of the mucosal compartment enriched with T cells, B cells and Ag presenting cells (APCs).

AU: Is this acronym okay as used? In all previous chapters, APC was an acronym for antigen presenting cells, not Ag presenting cells.

Unique PRRs Function in Mucosal Immunity

Although these PRR-mediated signals activate mucosal immune responses by producing inflammatory cytokines and chemokines, the mucosal immune system is equipped with additional unique activation pathways for enhancing mucosal innate responses as well. For instance, TLR-mediated signals enhance antimicrobial peptide (e.g., β-defensin) production by ECs and Paneth cells (4,5). TLR ligand stimulation of ECs also leads to the tightening and sealing of the TJ protein ZO-1 (13,14). Simultaneously, mucosal TLR stimulation by commensal microflora enhances anti-inflammatory activities. Peroxisome proliferator-activated receptor γ (PPARγ) was induced by commensal microflora-mediated TLR signaling (14). PPARγ serves as an inhibitor of colonic inflammation through its ability to inhibit NF-κB activation. Additionally, LPS from the commensal microflora, but not from pathogenic bacteria, induces the development of regulatory T (Treg) cells producing an inhibitory cytokine, IL-10 (12). In the lungs, TLR stimulation induces the production of indoleamine 2,3-dioxygenase (IDO) in the parenchyma. The lung-specific production of IDO leads to the inhibition of T cell–mediated lung inflammation and airway hyperreactivity by inhibiting T cell migration into the lung and by killing T cells (20).

An Involvement of PRRs in the Development of Mucosal Inflammation and Allergy

As one may envision, a dysregulated mucosal innate system leads to inflammatory responses in mucosal tissues. For instance, TLR2 and TLR4 expression is upregulated during inflammatory bowel disease (IBD) development, and polymorphisms in TLR4 have been shown to be linked to IBD development (13). Similarly, mutation of NOD2 contributes to IBD pathogenesis (21). Additionally, mutation or down-regulation of negative regulators for TLR signaling (e.g., IκBNS) resulted in IBD development (18). It is interesting to note that MyD88-deficient mice showed a higher mortality than wild-type mice when exposed to dextran sulfate sodium (DSS)-induced colitis (22). The disease

susceptibility was at least partially attributable to an impaired epithelial proliferation caused by the TLR-mediated signaling. Commensal bacteria and TLRs are also involved in the development of food allergy, another serious mucosal disease. It has been shown that commensal flora-derived signaling through TLR provides a protective function against food allergy (14).

In addition to intestinal inflammation, PRRs are involved in airway inflammatory diseases such as asthma. It was previously reported that an insertion-deletion polymorphism of NOD was strongly associated with asthma (23). Additionally, a recent study suggests that TLR4-mediated signaling is involved in the development of allergic airway inflammation through a modification in mast cell function (24). These studies indicate that signaling through PRRs is also important for the creation and maintenance of a quiescent status in the immune environment of mucosal compartments. This includes intestinal homeostasis rather than induction of inflammatory responses since the destruction of the PRR system is associated with the development or acceleration of mucosa-associated diseases. Taken together, PRR-mediated signals play an important role in both immunosurveillance and immune homeostasis in mucosal tissues.

Intraepithelial T Lymphocytes

Close Communication between ECs and Intraepithelial Lymphocytes

The major interface between internal organs and the outside environment is the columnar IEC layer, which covers mucosal tissues. In addition to IECs, the columnar epithelium includes a population of lymphocytes commonly termed *intraepithelial lymphocytes* (IELs) (25). As their name implies, IELs reside between the basolateral surfaces of IECs. It has been estimated that 1 IEL occurs for every 4 to 10 IECs seen in the small intestine and for every 30 to 50 IECs found in the large intestine. This shows that large numbers of lymphocytes are situated in the surface regions of intestinal mucosal tissues. Thus, IELs have been shown to closely communicate with each other and with the IECs that surround them. Indeed, several interacting molecules were expressed between IECs and IELs. For example, CD103 (α_E integrin) expressed on IELs interacts with the E-cadherin expressed on IECs, playing an important role in the retention of IELs in the intestinal epithelium (26). Additionally, IEL retention may be mediated by expression of certain integrins (e.g., $\alpha_1\beta_1$, $\alpha_4\beta_1$, and β_2 integrins), the adhesion molecule, Ep-CAM and TJ molecules (e.g., ZO-1 and occludins) (25). These intimate biological interactions between IECs and IELs provide physiological barriers that act as a first line of innate defense in the intestine.

As mentioned earlier, IECs are in constant contact with the luminal microflora. In addition, IECs become the target of microbial pathogen attachment and replication leading to the establishment of infection. Given the presence of immune and inflammatory cells within the epithelium and their obvious changes during infection or inflammation, it is worthwhile to consider the role of IECs and IELs in orchestrating these responses. It is logical to assume that these IELs are important lymphoid cells that participate in the mucosal innate response. Indeed, the majority of human and murine IELs are classified as T cells because they express the CD3 molecule in association with either of the two forms of T cell receptor (TCR), $\gamma\delta$ or $\alpha\beta$. Concerning the expression of CD4 and CD8 by IELs, it has been shown that approximately 80% of small intestinal IELs belong to the CD8 subset; however, a substantial number of IELs can be grouped as CD4-bearing cells including $CD4^+CD8^-$ and $CD4^+CD8^+$ subsets. The CD8 molecules expressed on IELs consist of either $\alpha\beta$ heterodimeric or $\alpha\alpha$ homodimeric chains. $CD8\alpha\beta^+$ IELs express Thy-1 and express the $\alpha\beta$ TCR. In contrast, $CD8\alpha\alpha^+$ IELs and $CD4^-CD8^-$ double-negative (DN) IELs contain both TCR$\gamma\delta$ and TCR$\alpha\beta$ fractions (25).

Innate Homeostatic and Protective Immune Function of $\gamma\delta$ IELs

Among IELs, those expressing the TCR$\gamma\delta$ ($\gamma\delta$ IELs) have been considered to be involved in mucosal innate defense because cell-transfer studies have indicated that the $\gamma\delta$ IELs have only minimal pathogen-specific activity (25,27). The less Ag-specificity of $\gamma\delta$ IELs is supported by the finding that $\gamma\delta$ IELs are present in mice deficient in the transporter associated either with antigen processing (TAP) or with the classical class I molecules ($K^{b-/-}$ $D^{b-/-}$), while $\gamma\delta$ IELs were drastically reduced in numbers in β2-microglobulin–deficient mice (25,27), suggesting that $\gamma\delta$ IELs recognize a TAP-independent nonclassical MHC molecule (Figure 31.3). In humans, the TCR$\gamma\delta$ expressed by IELs predominantly use Vδ1. These human TCR$\gamma\delta$ recognize the MIC molecules MICA and MICB, members of the nonclassical MHC molecule family (27). MIC molecules on ECs are induced by stress such as heat shock and microbial infections and are not capable of presenting peptides, but instead act as ligands for $\gamma\delta$TCRs. In mice, $\gamma\delta$ IELs predominantly use the Vγ5 (also known as Vγ7) gene segment together with several Vδ genes. Although mice do not have a functional MIC gene ortholog, they express molecules that resemble MIC such as H60, members of the RAE class I–like family, and other nonclassical MHC class I molecules (e.g., T10/T22) (Figure 31.3) (27). **Fig. 31.3**

Upon TCR$\gamma\delta$-mediated stimulation by nonclassical MHC molecules, $\gamma\delta$ IELs synthesize an array of cytokines that includes IL-2, IL-3, IL-6, IFN-γ, TNF-α, and TGF-β (25,27). It was also shown that freshly isolated and activated $\gamma\delta$ IELs express high levels of mRNA specific for

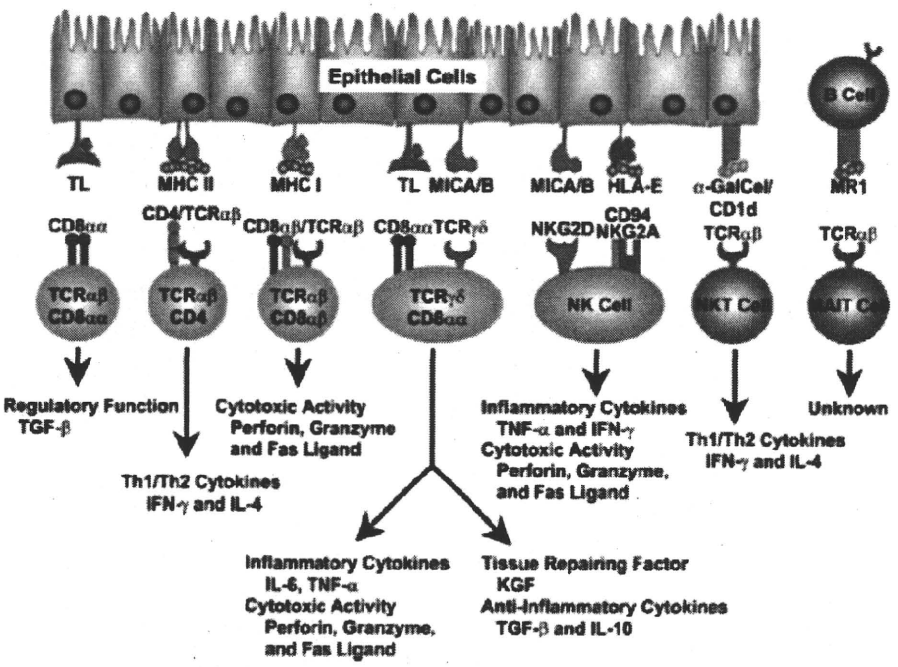

FIGURE 31.3 Molecular machinery for mucosal lymphocyte and EC interactions. Unique populations of mucosal lymphocytes are located in or underneath the intestinal epithelium. Immunological functions of these lymphocytes are regulated via molecular interactions between MHC family molecules and corresponding receptors. The ECs express MHC molecules including broad types of nonclassical MHC molecules interacting with specific receptors expressed on lymphocytes, which allow the establishment of induction of productive and quiescent immune responses in mucosal tissues.

lymphotactin, a chemokine important for $CD8^+$ T cell chemotaxis (27). These results suggest that IELs actively produce cytokines and chemokines to provide specific immunologic functions in the mucosal compartment. In addition to cytokine production, $\gamma\delta$ IELs produces cytotoxic molecules such as perforin, granzyme, and Fas ligand, and show cytotoxic activity against stressed or microbial infected IECs (28).

Alternatively, because activated $\gamma\delta$ IELs can produce keratinocyte growth factor (KGF), which is important for epithelial growth and repair of damaged tissues, some $\gamma\delta$ IELs could be involved in repair of tissue damage elicited during inflammatory responses (27). In addition to KGF, $\gamma\delta$ IELs synthesize anti-inflammatory and regulatory cytokines such as TGF-β and IL-10. In agreement with production of these cytokines, TCRδ chain–deficient mice show an increased susceptibility to epithelial damage caused by DSS-induced colitis (27). Thus, $\gamma\delta$ IELs also play a critical role in the maintenance of mucosal homeostasis in epithelial regions. However, not all immune responses mediated by $\gamma\delta$ IELs are beneficial. For example, it was shown that dysregulated production of IL-15 and overexpression of MICA/MICB on IECs led to the aberrant activation of IELs in the case of celiac disease (29).

To prevent the disruption of the epithelium by activated IELs, it is essential that IELs produce cytokines without self-proliferation. To achieve this opposite regulation (cytokine production without proliferation), the mucosal immune system has evolved a unique interaction between IECs and IELs. Thymus leukemia antigen (TL) is a nonclassical MHC molecule expressed almost exclusively by IECs of the small intestine (Figure 31.3) (27). Like

other nonclassical MHC molecules, TL does not present antigenic peptides but strongly interacts with CD8$\alpha\alpha$ on IELs. The interaction between CD8$\alpha\alpha$ and TL enhances cytokine production by IELs but inhibits self-proliferation and cytotoxic activity. By inhibiting proliferation, CD8$\alpha\alpha$-TL interactions prevent the disruption of a sheeted form of epithelium that results from attack by dividing IELs (27).

Recent studies show that a naïve population of IELs is made up of recent CD8$^+$ thymic emigrants (RTEs) (30). RTEs are distinguished by their ability to migrate into the small intestine without activation because of their expression of $\alpha_4\beta_7$ integrin, α_E integrin, and CCR9 in the thymus (30). After migrating directly into the intestinal epithelium from the thymus, these RTEs begin to proliferate in response to Ag exclusively present in the gut. These IELs show diverse TCR repertoires, which is important in their maintenance of TCR diversity in the intestine.

Unique Developmental Pathways for IELs

In addition to their immunological uniqueness, IELs also have a special development pathway, even though controversy remains as to what extent the IELs require thymic dependency (31,32). Like naïve lymphocytes circulating in systemic immune compartments, the major IEL populations in CD4 or CD8$\alpha\beta$ subsets expressing TCR$\alpha\beta$ originate from conventional single-positive (SP) thymocytes. In contrast, some populations of DN IELs and CD8$\alpha\alpha$ IELs expressing either TCR$\alpha\beta$ or TCR$\gamma\delta$ originate from unconventional thymocytes. Several lines of evidence have revealed that these IEL precursors in the DN thymocytes, including TCR$\alpha\beta^+$ DN thymocytes, TCR$\gamma\delta^+$ DN thymocytes, and TCR$\alpha\beta^-$ CD4$^-$ CD8$^-$ triple-negative (TN) thymocytes (31). TCR$\alpha\beta^+$ DN thymocytes are thought to be mature postselected DN thymocytes because they arose from the CD8$\alpha\alpha^+$ CD4$^+$ CD8$\alpha\beta^+$ triple-positive (TP) thymocytes after agonist selection and migration into the intestine where they further reinduce CD8$\alpha\alpha$ under the influence of IL-15 (31). In addition to the postselected DN thymocytes, some subsets among the TN thymocytes emigrated from the thymus during the CD44$^+$ CD25$^+$ TN2 or the CD44$^-$ CD25$^+$ TN3 stages and migrated into the intestinal epithelium where they characteristically expressed *c-kit* and IL-7 receptor (IL-7R) and subsequently expressed TCR$\alpha\beta$ or TCR$\gamma\delta$ (31). It was recently found that thymic IEL precursors could be divided into two groups based on the requirement of sphingosine 1-phosphate (S1P), a lipid mediator, in the regulation of trafficking of thymic IEL precursors into the intestine (33). CD4 or CD8$\alpha\beta$ naïve IELs originating from SP thymocytes express high levels of type 1 S1P receptor. In contrast, unconventional thymic IEL precursors, including RTEs and DN thymocytes expressing either TCR$\alpha\beta$ or TCR$\gamma\delta$, migrate into intestine in a S1P-independent manner (33).

AU: Is this acronym use okay here? Earlier, it was indicated that SP means surfactant protein[0].

In addition to the conventional and unconventional thymic IEL precursors, it has been proposed that certain populations of IEL subsets (e.g., TCR$\gamma\delta$ CD8$\alpha\alpha$ IELs) develop extrathymically (32,34). As a candidate lymphoid tissue for extrathymic IEL development, cryptopatches were identified (35). Cryptopatches (CryPs) were shown to be lymphocyte clusters in the crypt LP of the murine small and large intestine. Cells within the CryPs are composed mostly of lymphoid progenitors expressing SCF receptor (c-kit), and IL-7Rα, but lacking the lineage markers (CD3, B220, Mac-1, Gr-1, and TER-119). They possess transcripts for germline TCR genes, mRNA for CD3ε, as well as proteins (i.e., *RAG*-2 and preTα) involved in TCR gene rearrangement and are able to generate CD8$\alpha\alpha$ $\alpha\beta$ as well as $\gamma\delta$ IELs, albeit with a strong bias toward the generation of $\gamma\delta$ T cells, in irradiated severe combined immune-deficient (SCID) mice. These findings demonstrated that c-kit$^+$ CryP cells are committed to the T cell lineage and are competent for the generation of IELs.

Natural Killer and NKT Cells

In addition to the T cells, the epithelium includes natural killer (NK) and NKT cells. For instance, the human nonclassical MHC molecules MICA and MICB predominantly expressed on damaged or transformed IECs act as ligands for the NK receptor, NKG2D (Figure 31.3) (27). Interestingly, as mentioned earlier, TCR$\gamma\delta$ recognizes the same MICA and MICB molecules (Figure 31.3), implying that the mucosal immune system can use both TCR$\gamma\delta$ and NKG2D to recognize damaged or stressed IECs through the nonclassical MHC molecules. Additionally, the expression of HLA-E on the epithelium is associated with ligation of killer inhibitory-related receptors (CD94/NKG2) on activated mucosal NK cells in humans (Figure 31.3) (29). It was previously shown that the cytotoxic effects of NK IELs were enhanced by IL-15 through the up-regulation of IFN-γ production and Fas ligand-mediated killing activity and simultaneous enhancement of MICA expression (29).

NKT cells also play an important role in mucosal innate immunity (Figure 31.3). NKT cells express invariant TCR. The TCRα chain comprises a Vα14 in the murine system, and TCR Vα24 is a homologue expressed on human NKT cells (36). In contrast to the invariant expression of TCRα, NKT cells possess a wide variety of TCRβ chains (36), which allows them to contribute to various immune responses including mucosal homeostasis. These NKT cells recognize lipid-derived Ag presented by the CD1d, one of the nonclassical MHC molecules (Figure 31.3). IECs and DCs in the intestinal compartments express the CD1d (37). Although α-galactosylceramide (αGalCer) is a well-known Ag presented by CD1d, αGalCer is derived from a marine sponge, but not from microorganisms. However, several studies have identified lipid Ags presented by CD1d, such as α-glucuronosylceramide and α-galacturonosylceramide

from nonpathogenic sphingomonas bacteria and a diacylglycerol from pathogenic *Borrelia burgdorferi* (36). In addition, it has been proposed that infection with bacteria (e.g., *E. coli, Bacillus subtilis, S. aureus,* or *Mycobacterium bovis-Bacillus Calmette Guerin*) or their derived bacterial components such as LPS, lipoteichoic acid, or $Pam_3CysSerLys_4$ (P_3CSK_4) allows $CD1d^+$ cells to present endogenous glycosphingolipid, isoglobotrihexosylceramide, and stimulate NKT cells, which may contribute to initial sensing of pathogenic or infected cells (Figure 31.3) (36).

After stimulation via CD1d, NKT cells can secrete both Th1- and Th2-type cytokines (Figure 31.3). It is still unclear how the hierarchy between Th1- and Th2-biased NKT cells is determined, but this may account for the contribution of NKT cells to both protective and anti-inflammatory functions (36). Consistent with this, it was reported that CD1d-deficient mice were susceptible to infections (e.g., *Listeria* and *P. aeruginosa*) at mucosal sites. NKT cells are also involved in the amelioration of DSS-induced colitis through their ability to produce regulatory cytokines such as IL-4 and IL-10. NKT cells are also thought to be involved in the suppression of allergen-induced airway hyperreactivity by the induction of a Th1 shift from an allergy-associated Th2 environment or the creation of anergy. These results generally suggest a critical role for NKT cells in the down-regulation of inflammatory responses; however, other studies demonstrated that NKT cells induced asthma. Although the NKT cell subset is a minor population of mucosal immune compartments, the cells are involved in the recognition of self- and exogenic-glycolipid Ags as a part of the mucosal innate defense system (36).

Mucosa-Associated Invariant T Cells

A recent study has discovered MHC-related 1 (MR1)–restricted mucosal-associated invariant T cells (MAIT cells) as a novel subset of unconventional T cells abundantly present in the intestinal LP (Figure 31.3) (38). MAIT cells express invariant TCR α chain, TCR $V\alpha7.2\text{-}J\alpha33$ in humans and TCR $V\alpha19\text{-}J\alpha33$ in mice. Like conventional T and NKT cells, MAIT cells develop in the thymus, but their selection is independent of the TAP and invariant chain, suggesting that putative ligands presented by MR1 are different from those presented by conventional MHC class I and II molecules. In this context, several lines of evidence have revealed that MAIT cells can be activated by both peptide Ag and glycolipid Ags (e.g., α-GalCer and other α-mannosylceramides) (Figure 31.3). MAIT cells additionally require $MR1^+$ B cells for their development. It is interesting to note that MAIT cells are markedly decreased in germ-free mice, suggesting that some microbial stimulation is required for the selection, migration, and expansion of MAIT cells. MAIT cells have been suggested to be involved in the immunosurveillance and the establishment of immunological homeostasis in the intestine, because MAIT cells possess regulatory functions where the cells inhibit autoimmune responses (39).

ACQUIRED MUCOSAL IMMUNE SYSTEM

Common Mucosal Immune System for Acquired Immunity

In addition to the innate mucosal immune system, the mucosal immune system is equipped for well-organized and controlled acquired immunity. The mammalian host has evolved organized secondary lymphoid tissues in the upper respiratory and GI tract regions that facilitate Ag uptake, processing, and presentation for priming immunocompetent cells for subsequent induction of Ag-specific mucosal immune responses. Collectively, these tissues are termed mucosal inductive sites. The gut-associated lymphoid tissues (GALT) consist of several family members of inductive sites including the Peyer's patches (PPs), colonic patches (CPs), the appendix, and isolated lymphoid follicles (ILFs) (34). The major inductive tissues for nasal/inhaled Ags in humans, primates, mice, and rats appear to be the palatine tonsils and adenoids (nasopharyngeal tonsils), which together form a physical barrier of lymphoid tissues termed the *Waldeyer's ring*, now more frequently referred to as a nasopharynx-associated lymphoid tissue (NALT) (40). To summarize, then, NALT and GALT in humans and mice and possibly primates comprise a MALT network.

Through the interaction with APCs in MALTs, naïve B and T cells are primed by Ag and then emigrate from the inductive environment via lymphatic drainage, circulate through the bloodstream, and home to mucosal effector sites, especially the LP regions of the intestinal, respiratory, and reproductive tracts where they further differentiate into effector cells that protect mucosal surfaces (41,42). These mucosal networks are known as the common-mucosal immune system (CMIS) bridging between the inductive (e.g., MALTs) and effector sites (e.g., LP), a network that plays a key role in the induction of Ag-specific acquired immunity against mucosally encountered Ag.

Structure and Cellular Composition of Mucosal Inductive Sites

Among several MALTs, the most extensively studied mucosal inductive tissues are the PPs of the murine GI tract. PPs are large enough to be observed upon gross examination, and usually number 8 to 10 in murine small intestine. In humans, up to 200 PPs were detected. Like PPs in the small intestine, there are lymphoid organs in the large intestine. These tissues are known as CPs, or rectal-associated lymphoid tissue (RALT), which are smaller in size than PPs (34). An additional lymphoid structure

resembling PPs and CPs in composition and architecture has been identified as numbering at least 30,000 (in humans), and 100 to 200 (in mice) clusters on the antimesenteric wall of the murine small intestine, which are now known as solitary lymphoid follicles or ILFs (34). In the respiratory tract, NALT is found on both sides of the nasopharyngeal duct dorsal to the cartilaginous soft palate in rodents (40). In humans, there are unpaired nasopharyngeal tonsils (adenoids) and the paired palatine tonsils that play an important role for human airway immunity. The latter makes up most of Waldeyer's ring in humans (40). Further, a NALT-like structure of lymphocyte aggregation with follicle formation was identified in human nasal mucosa, especially that of the middle concha in children less than 2 years old (43). In the bronchial tract, bronchus-associated lymphoid tissues (BALT) was classically defined as an aggregated lymphoid structure separated from the bronchial lumen by a specialized lymphoepithelium in several species, including rats, rabbits, and sheep, although the presence of murine and human BALT as an aggregated lymphoid follicle under normal conditions remains a subject of debate (40).

Although each MALT exists in different regions of the mucosal tissues, MALTs share several interesting features associated with their role as the major mucosal inductive tissue (41,42). First, MALTs are unique in that they contain efferent lymphatics but no afferent lymphatics, reducing the possibility that an Ag will be encountered via the afferent lymphatics. Instead, MALTs are covered with the specialized EC termed a *follicle-associated epithelium* (FAE). FAE contains a specialized cell type called a *microfold/membraneous cell* (*M cell*) that is closely associated with lymphoid cells (Figure 31.4) (44). M cells are noted in the MALTs of both humans and rodents (e.g., PPs, ILFs, CPs, and NALT). The M cells, which have short microvilli, small cytoplasmic vesicles, and few lysosomes, are adept at uptake and transport of luminal Ags, including proteins and particulates such as viruses, bacteria, small parasites, and microspheres, allowing the selective transport of these Ag into the MALTs. The human palatine and nasopharyngeal tonsils (adenoids) are largely covered by a squamous epithelium and are often not appreciated as mucosal inductive tissues. However, the palatine tonsils usually contain 10 to 20 crypts that increase their surface area where M cells locate in the deeper regions (44). Although this issue remains a subject of debate, several studies demonstrated that M cells could be differentiated from the absorptive ECs through interactions with B cells (45).

Fig. 31.4

MALTs contain organized regions for the generation of IgA-committed B cells. Distinct B cell zones are located beneath the dome area of PPs and contain germinal centers (GCs) where significant B cell division is seen (Figure 31.5). These GCs contain the majority of surface IgA-positive ($sIgA^+$) B cells (46), but, unlike the spleen and secondary lymph nodes (LNs) in the systemic compartment, PC development does not effectively occur. In addition to the GCs, the underlying dome region of the PPs consist of sparse $sIgA^+$ cells that may provide a first line of IgA-mediated defense for the dome region of PPs. Since germ-free mice present PPs but lack GCs, it has been postulated that the continuous exposure of the PPs to the commensal bacteria or viruses from the outside environment induce the constant GC formation seen in PPs (47). In contrast to the PPs, GCs are absent in the NALT of normal mice (41,42). Thus, PPs contain a high frequency (10% to 15%) of $sIgA^+$ B cells, while NALT was found to contain fewer IgA-committed B cells, even though nasal immunization induces higher numbers of $sIgA^+$ B cells in

Fig. 31.5

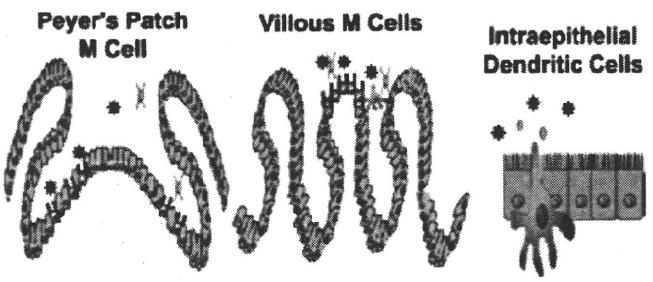

FIGURE 31.4 Multiple Ag uptake pathways for the induction of mucosal immune responses. At least three distinct Ag sampling sites have been reported. M cells were originally discovered in FAE of MALTs including PPs, ILFs, and NALT for sampling of orally administered Ags. In addition to MALT-associated M cells. M cells are also capable of developing at the tip region of villous epithelium from FAE of MALTs and have been termed villous M cells. Finally, DCs can extend their dendrites expressing the TJ molecule claudin between ECs and directly take up GI tract luminal Ags.

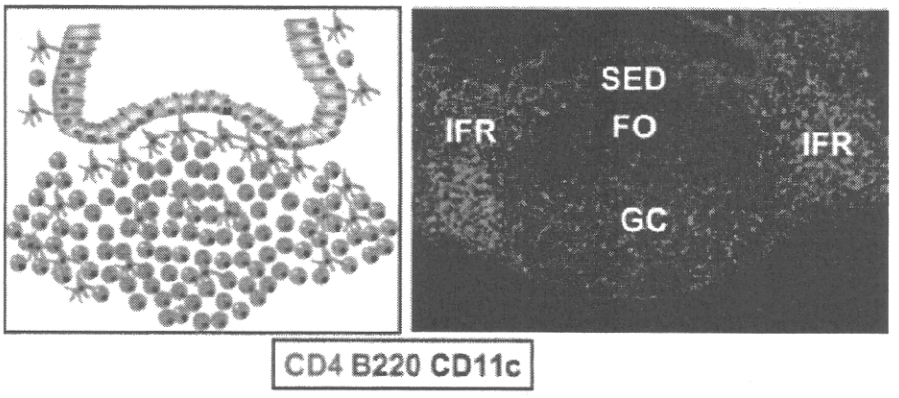

FIGURE 31.5 Segregated cell distribution in the PPs. $CD4^+$ T cells (green) are mainly present in the intrafollicular regions (IFRs) and B cells (red) are located in the subepithelial dome (SED) and follicle (FO) regions. DCs (blue) are distributed in the SED and IFRs. GCs are enriched in B cells with small numbers of T cells and DCs for the creation of a cellular environment for the efficient generation of IgA-committed B cells.

NALT (41,42). In human tonsils, approximately one half of tonsillar cells are B lymphocytes, and they mainly occur in follicle-containing GCs (46). Most human tonsillar B cells are actually surface IgG-positive ($sIgG^+$); however, significant numbers of $sIgM^+$ and $sIgA^+$ B cells are also present. The human palatine tonsil also contains a distinct subepithelial B cell population, as is seen in the FAE region of PPs. This B cell subset differs from both GCs and follicular mantle B cells (46). These subepithelial B cells located in NALT and GALT may play a crucial role in immediate Ab production toward Ag taken up through M cells.

All major T cell subsets are found in the T cell–dependent areas adjacent to follicles, the interfollicular regions (IFRs) (Figure 31.5). The parafollicular T cells are mature and >97% of these T cells use the $\alpha\beta$ heterodimer form of TCR. Approximately two-thirds of $TCR\alpha\beta^+$ T cells in the PPs are $CD4^+$ and exhibit properties of Th cells, including support for IgA Ab responses (48). Approximately one-third of the $TCR\alpha\beta^+$ T cells in the PPs are $CD8^+$; this cell subset contains precursors of CTLs (28). These IFRs contain high endothelial venules (HEVs), a main entry site for lymphocytes into PPs. The HEVs express mucosal vascular addressin cell adhesion molecule 1 (MAdCAM-1), a ligand for the $\alpha_4\beta_7$ integrin, which determines a selective gut-tropism migration for lymphocytes (49,50). The T cell-rich IFRs overlap with the B cell follicles in some areas, providing an important place for initial T–B cell interactions.

Immunohistologic studies have revealed the unique distribution of DCs. In the PPs, DCs are divided into at least three distinct populations (51,52). First, myeloid-type DCs expressing CD11b are located in the subepitheliual dome (SED) region. The second DC population in the PPs is $CD8\alpha^+$ lymphoid DCs residing in the T cell–rich IFRs. Additionally, PPs contain DCs expressing neither CD11b nor CD8α, which are called DN DCs. The DN DCs are exclusively found in both SED and IFRs. The distribution of different populations of DCs in the PPs is at least partially determined by chemokines and their receptors (51,52). $CD11b^+$ myeloid DCs express CCR6, which allow their migration toward CCL20 selectively expressed by FAE. Consistent with this, $CD11b^+$ DCs were markedly reduced in the SED of PPs in CCR6-deficient mice (53). In addition to the interaction between CCR6 and CCL20, the interaction between CCR1 and CCL9 plays a nonredundant role in the migration of $CD11b^+$ DCs into the FAE of PPs. Additionally, all DCs in the PPs expressed CCR7, allowing them to migrate into the IFRs toward cells producing CCL19 and CCL21. It has been demonstrated that DCs also play an important role in the respiratory immune system; however their distribution remains unclear.

Distinct Pathway for MALT Organogenesis

GALT Organogenesis

Although similar in terms of anatomy and histology, the MALTs of the respiratory and intestinal immune system differ in their organogenesis (34,41,42). The models describing the development of PPs have been studied in some detail in mice. A cluster of mesenchymal-lineage $VCAM\text{-}1^+/ICAM\text{-}1^+$ cells occur in the upper small intestine

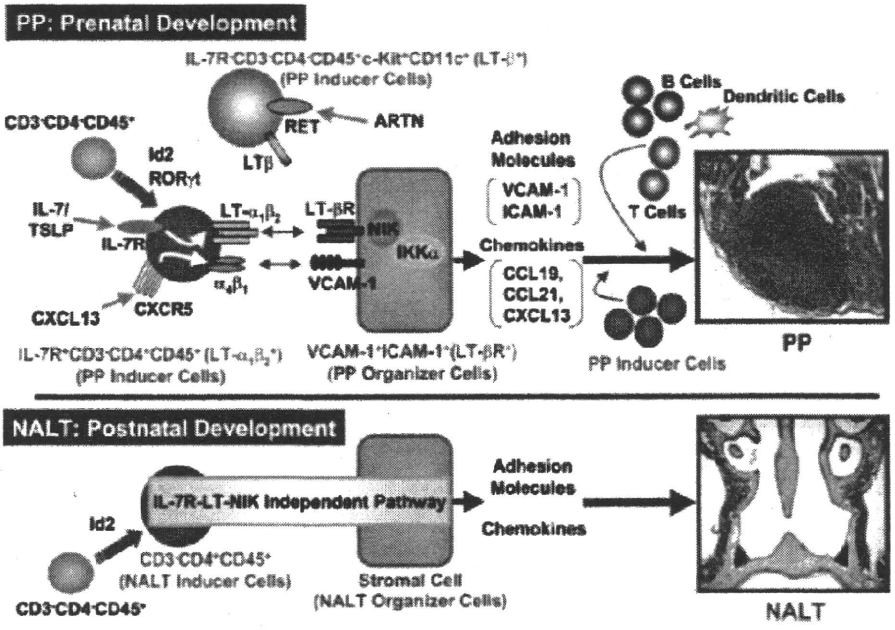

FIGURE 31.6 Distinctively orchestrated organogenesis of MALTs of PPs and NALT. Initial phase of the tissue genesis program of PPs operates during embryogenesis. The development of IL-7R$^+$CD3$^-$CD4$^+$CD45$^+$ PP inducer cells requires Id2 and RORγt genes. IL-7R$^+$CD3$^-$CD4$^+$CD45$^+$ PP inducer cells accumulate initially at the LN anlagen and specifically interact with VCAM-1$^+$ PP organizer cells. This cell-to-cell interaction induces subsequent activation of LT-βR–associated molecules such as NIK and IKKα for the induction of adhesion molecules and chemokines by PP organizer cells, which leads to the further recruitment of T cells, B cells, and DCs. Recently, RET$^+$CD11c$^+$ cells expressing LT-β have been identified as a new member of inducer cells that contribute to the initiation phase prior to the appearance of IL-7R$^+$CD3$^-$CD4$^+$CD45$^+$ PP inducer cells. In comparison to the PP organogenesis program, NALT organogenesis is initiated in a postnatal manner and is totally independent of IL-7R-LT/LT-βR-NIK–mediated tissue genesis signaling pathway. For the development of NALT inducer cells, like PPs, Id2 is necessary for the differentiation of CD3$^-$CD4$^+$CD45$^+$ NALT inducer cells but does not require RORγt.

Fig. 31.6

beginning at embryonic day 15 to 16 (Figure 31.6). These cells are termed *PP organizers* and express lymphotoxin β receptor (LT-βR). Subsequently, lymphoid-lineage IL-7R$^+$CD3$^-$CD4$^+$CD45$^+$ PP inducer cells appear to be the anlagen of the PPs at embryonic day 17.5. Following stimulation signals provided through IL-7R, PP inducer cells express LT-$\alpha1\beta2$ to activate PP organizer cells through LT-βR, and then PP organizer cells produce chemokines such as CXCL13 and CCL19 to stimulate PP inducer cells through CXCR5 and CCR7 (Figure 31.6). The reciprocal interaction between inducer and organizer cells through the chemokine and cytokine receptors is essential for the initiation of PP formation, and the loss of any part of the signaling program is sufficient to disrupt PP development, as evidenced by the loss of PPs in LTβR$^{-/-}$ and IL-7R$\alpha^{-/-}$ mice and the partial reduction in the formation and number of PPs in CXCR5$^{-/-}$ mice (34,41,42). In addition, alymphoplasia (*aly/aly*) mice, with a mutation in the NF-κB-inducing kinase, which appears to act downstream of LT-$\alpha1\beta2$/LT-βR signaling, also fail to develop PPs (54). Further evidence in support of this model comes from studies showing that mice lacking the CD3$^-$CD4$^+$CD45$^+$ IL-7R$^+$ inducer cells due to genetic deletion of the transcription regulators Id2 or RORγt also completely lack the formation of PPs and LNs (34,41,42). Another study revealed that defects in the Foxl1 gene, which encodes a