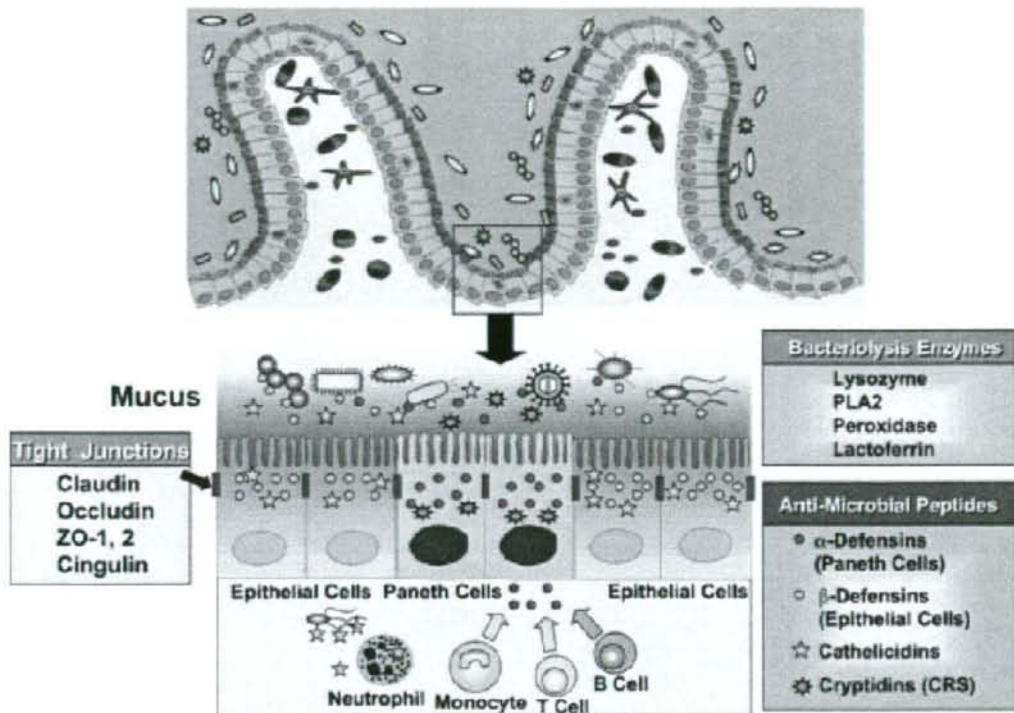

FIGURE 31.1 Various types of antimicrobial molecules protect mucosal surfaces against invading microbes. ECs and Paneth cells secrete bacteriolytic enzymes (lysozyme, PLA2, peroxidase, and lactoferrin) and antimicrobial peptides (defensins, cathelicidins and crytdin-related sequence [CRS]) in mucosal 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.

ED/PE: Necessary to spell out? First reference in chapter.

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

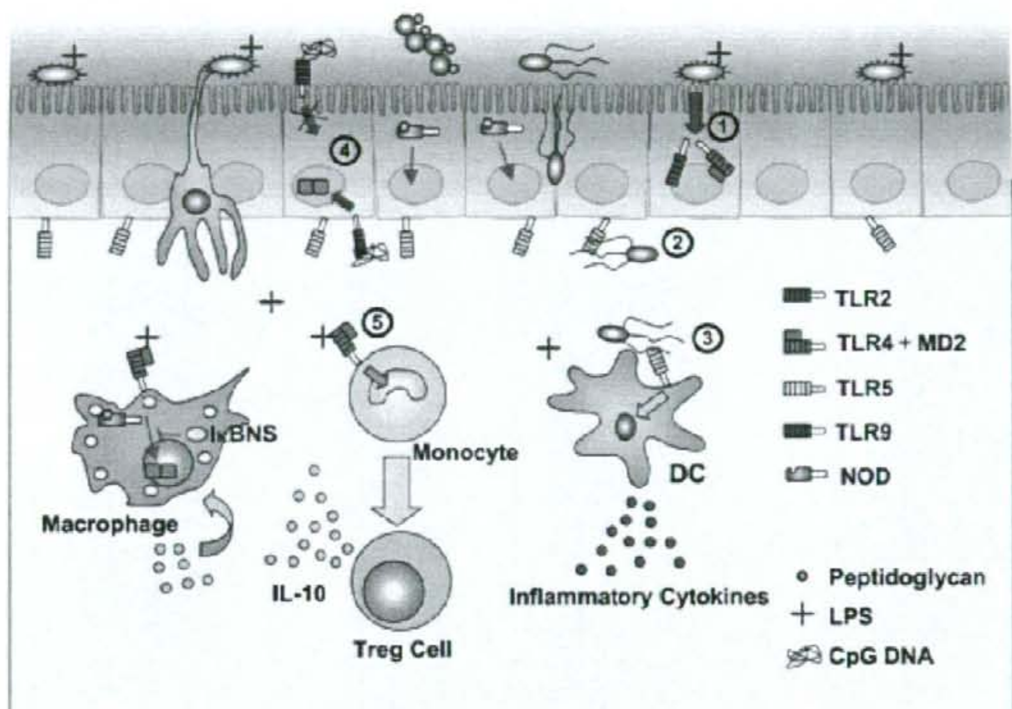

FIGURE 31.2 Uniqueness of recognition and discrimination of microbes. Toll-like receptors and NODs recognize bacterial products (peptidoglycan [PG], lipopolysaccharide [LPS] and CpG DNA). The host mucosal immune system employs various mechanisms to distinguish pathogenic from commensal bacteria: 1, continuous 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

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

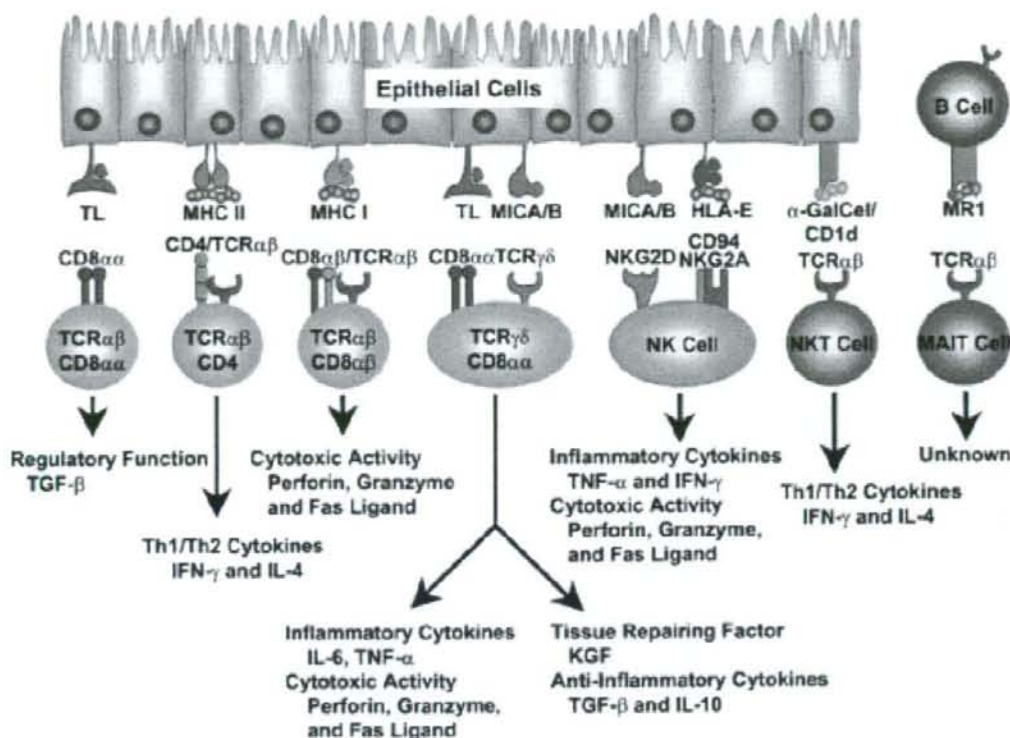

FIGURE 31.3 Molecular machinery for mucosal lymphocyte and EC interactions. Unique populations of mucosal lymphocytes are located in or underneath the intestinal epithelium. Immunological functions of these lymphocytes are regulated via molecular interactions between MHC family molecules and corresponding 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 CD8αα on IELs. The interaction between CD8αα and TL enhances cytokine production by IELs but inhibits self-proliferation and cytotoxic activity. By inhibiting proliferation, CD8αα-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 CD8αβ subsets expressing TCRαβ originate from conventional single-positive (SP) thymocytes. In contrast, some populations of DN IELs and CD8αα IELs expressing either TCRαβ or TCRγδ 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 CD8αα 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 TCRαβ or TCRγδ (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 CD8αβ 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 TCRαβ or TCRγδ, 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., TCRγδ CD8αα 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 CD8αα αβ as well as γδ IELs, albeit with a strong bias toward the generation of γδ T cells, in irradiated severe combined immune-deficient (SCID) mice. These findings demonstrated that $c\text{-kit}^+$ 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, TCRγδ recognizes the same MICA and MICB molecules (Figure 31.3), implying that the mucosal immune system can use both TCRγδ 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 Vα7.2-Jα33 in humans and TCR Vα19-Jα33 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

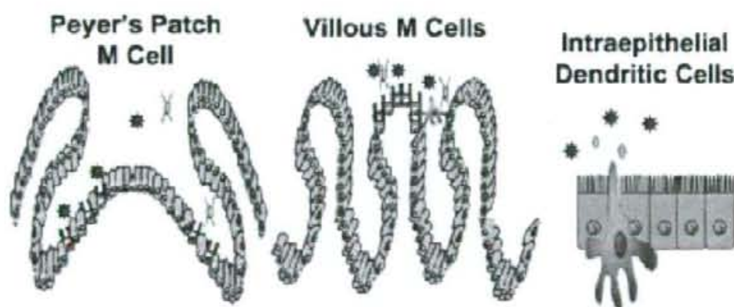

FIGURE 31.4 Multiple Ag uptake pathways for the induction of mucosal immune responses. At least three distinct Ag sampling sites have been reported. M cells were originally discovered in FAE of MALTs including PPs, ILFs, and NALT for sampling of orally administered Ags. In addition to MALT-associated 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.

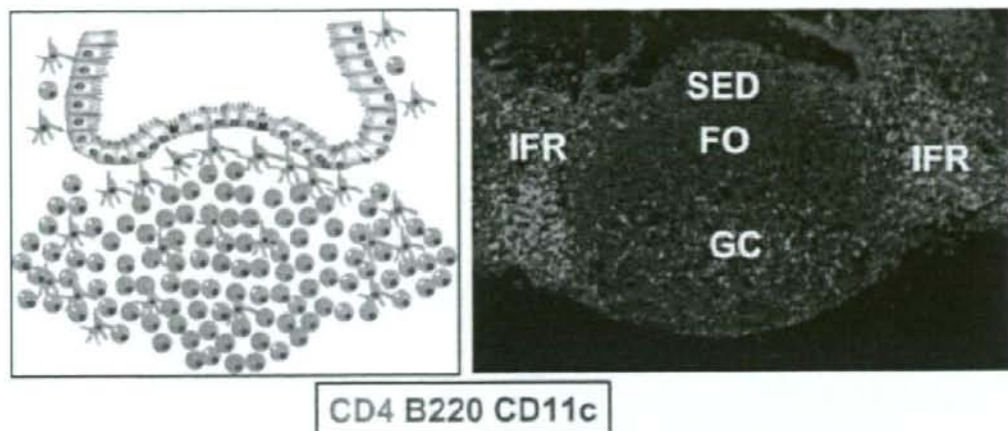

FIGURE 31.5 Segregated cell distribution in the PPs. CD4$^+$ T cells (green) are mainly present in the intrafollicular regions (IFRs) and B cells (red) are located in the subepithelial dome (SED) and follicle (FO) regions. DCs (blue) are distributed in the SED and IFRs. GCs are enriched in B cells with 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 CD8α^+ 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-1$^+$/ICAM-1$^+$ cells occur in the upper small intestine

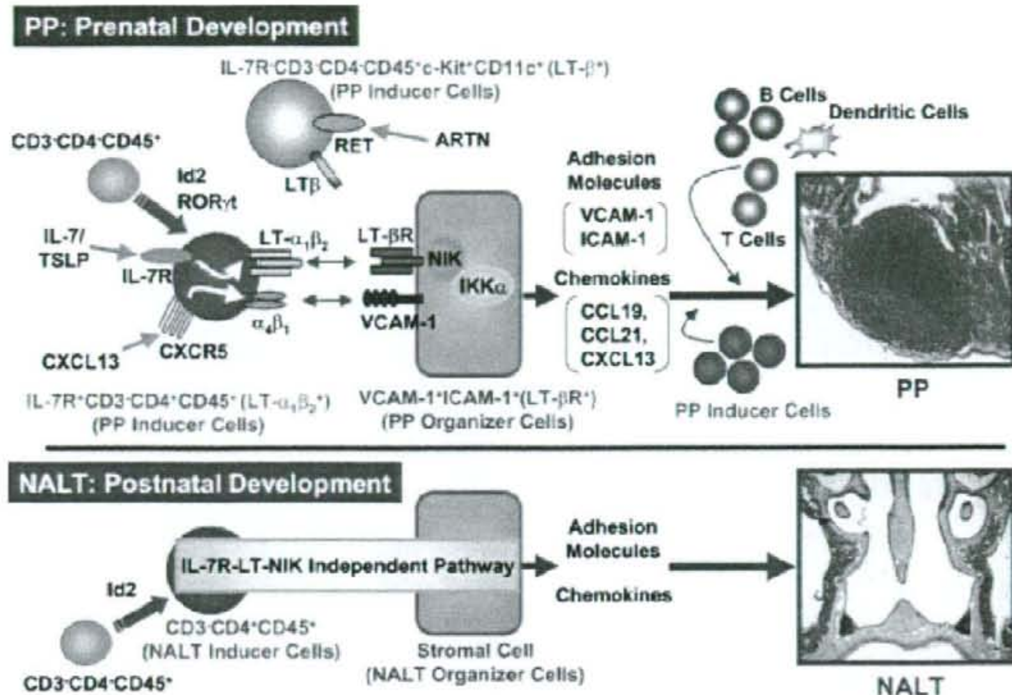

FIGURE 31.6 Distinctively orchestrated organogenesis of MALTs of PPs and NALT. Initial phase of the tissue genesis program of PPs operates during embryogenesis. The development of IL-$7R^+CD3^-CD4^+CD45^+$ PP inducer cells requires Id2 and RORγt genes. IL-$7R^+CD3^-CD4^+CD45^+$ PP inducer cells accumulate 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-$\alpha 1\beta 2$ 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 LT$\beta R^{-/-}$ 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-$\alpha 1\beta 2$/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

winged helix transcriptional regulator expressed in the mesenchymal layer of both the developing and mature GI tract resulted in the delayed formation of PP organizing centers as revealed by the expression of VCAM1 and IL-7R at 17.5 days postcoitus (55). In addition to IL-7R$^+$CD3$^-$CD4$^+$CD45$^+$ PP inducer cells and stromal organizer cells, a recent study has shown that the IL-7R-CD3$^-$CD4$^-$CD45$^+$c-kit$^+$CD11c$^+$ hematopoietic population expressing LT-β has an important role in the initiation stage of PP formation (56). These cells express the receptor tyrosine kinase (RET), which is essential for the mammalian enteric nervous system formation and is also crucial for PP formation. Thus, the RET ligand ARTN induces the formation of ectopic PP-like structures. In humans, PPs develop during prenatal life, a situation also seen in sheep, pigs, dogs, and horses, and it is thought that a similar tissue genesis program is involved.

The formation of ILFs also requires LT-β and LT-βR-dependent events (34,41,42). However, the LT-β and LT-βR–dependent events in ILF formation are chronologically different from PP development. ILF formation can occur postnatally and requires LT-β-expressing B lymphocytes (34). Consistent with this fact, treatment with LT-βR-Ig fusion protein during the postnatal period suppresses ILFs but not PPs (42). It was also demonstrated that immature ILFs with clusters of B220$^+$ cells are present in the intestine of germ-free mice, but exogenous stimuli including bacterial Ags/mitogens are required for the completion of the lymphoid organization of ILFs, including GC formation (34,41,42). In AID-deficient mice, ILFs developed hyperplasia. A subsequent study demonstrated that the lack of hypermutated IgA production into the intestinal lumen resulted in the expansion of segmented filamentous bacteria in the small intestine of AID-deficient mice (57). Since antibiotic treatment of AID-deficient mice abolished the hyperplasia, it was proposed that anaerobic bacterial growth induced ILF hyperplasia. Taken together, these findings suggest that postnatal and physiological inflammatory signals are essential for the formulation of ILFs in the small intestine.

AU: Necessary to spell out AID? First reference in chapter.

Like the PPs, inhibition of LT-β–mediated signaling during the embryonic stage leads to the inhibition of CP formation (34,41,42). In contrast, increased numbers and enlargement of CPs were noted in Foxl1-deficient mice, while the size and numbers of PPs were decreased (55). Additionally, ILFs in the large intestine showed a different requirement for LT-β signaling for their development. Normal ILFs were present in the small intestine of mice treated with LT-β-Ig fusion protein *in utero* (42). However, the same treatment resulted in the acceleration of ILF formation in the large intestine (58), suggesting that LT-mediated signaling behaves as a negative regulator for ILF formation in the large intestine during the gestational period. One study demonstrated a critical involvement of the GI tract flora for the development of ILFs in the small intestine. Thus, the development of ILFs in the small intestine did not occur in germ-free mice, but modest numbers of mature ILFs developed after the conventionalization of germ-free mice. In contrast, the development of ILFs in the large intestine is independent of the gut microflora (58). These data indicate that small and large intestines share some parts of a tissue genesis-associated molecular signaling program for their development, but they also possess unique development pathways to adjust to environmentally different circumstances that occur between the small and large intestines.

Distinct Features of NALT Organogenesis Compared with PPs

NALT formation has not been observed during embryogenesis or in newborn nasal tissue. The HEV structure is first detected in bilateral nasal tissue 1 week after birth, and the complete bell-shaped NALT formation with lymphoid cells is seen 8 weeks after birth (40–42). These findings indicate the presence of a distinct tissue genesis program in the PPs and NALT, although these tissues have a similar structure and immunological function as inductive tissues (Figure 31.6). As mentioned earlier, PP organogenesis requires a cytokine-signaling cascade involving IL-7R and LT-βR, and these deficiencies resulted in the lack of PP formation. However, mice lacking PPs or both PPs and LNs due to a deficiency in the LT-βR-mediated inflammatory cytokine cascade, including LT-β–deficient, IL-7R–deficient, and *aly/aly* mice, and mice treated *in utero* with LT-β-Ig fusion protein have a normal NALT structure (40–42). These findings further support the idea that NALT genesis does not follow the "programmed physiological inflammation" model typical of PPs. In contrast, deletion of Id2, which is responsible for the induction of CD3$^-$CD4$^+$CD45$^+$ inducer cells, impaired the genesis of all secondary lymphoid tissues including both NALT and PPs (Figure 31.6) (40–42). However, the deletion of the gene encoding RORγt, the additional transcriptional regulator for the development of CD3$^-$CD4$^+$CD45$^+$ inducer cells, resulted in the suppression of PP organogenesis, while NALT development was normal (40–42). These findings suggest that although NALT and PPs development depends on inducer cells of the same phenotype, CD3$^-$CD4$^+$CD45$^+$, those inducer cells can be categorized as those either dependent on Id2 alone (for NALT) or dependent on both Id2 and RORγt (for PPs).

Antigen Sampling and Presentation in MALTs

As mentioned earlier, MALTs have unique epithelial regions or FAE containing specialized ECs, termed *M cells*, to achieve preferential transport of luminal Ag (Figure 31.4) (44). M cells are characterized by a pocket structure (M cell pocket) at the basolateral side harboring a wide variety of

lymphoid cell subsets such as DCs, MΦ, and lymphocytes. Unique features of M cells include the fact that in spite of high uptake of luminal Ag via pinocytosis and endocytosis, they contain only a few lysosomes. In addition, IgA preferentially binds to the apical sides of M cells (44,59). Thus, it has been suggested that M cells contribute to the transport of luminal Ags to underlying APCs without any Ag digestion or processing.

In addition to serving as a means of transport for luminal Ags, the unique structural features of M cells (e.g., short microvilli and the thick glycocalx) and their predominant expression of receptors for some microorganisms also provide entry sites for pathogens (44,59). For instance, *Yersinia* adheres to M cells via the invasin and the $\beta1$ integrin expressed on the *Yersinia* and M cells, respectively. *Salmonella* initiate murine infection by invading the M cells of the PPs. Reoviruses also initiate infection of the mouse through the M cell, an ability that has been associated with the reovirus sigma protein. It has also been suggested that M cells act as the entry site for Prions. Lung M cells were reported to be the site for entry of *Mycobacterium tuberculosis* into the host, with subsequent uptake in draining LNs (60). Thus, M cells act as a gateway to the outside environment, delivering antigenic substrate to the underlying immune-competent cells for the subsequent induction of Ag-specific immune responses.

Several lines of evidence demonstrated that DCs are present in the MALTs. Among them, detailed studies have been performed mainly using PPs and showed that there are several distinct types of DCs in the PPs, which are distinguished by the surface expression of CD11b, CD8, and B220, as described earlier (51,52). In the FAE regions, $CD11b^+$ DCs and $CD11b^-CD8^-$ DN DCs are present, and some of them are associated with M cells, suggesting that they reside in the M cell pockets (44,52). Upon stimulation of $CD11b^+$ DCs and DN DCs through microbial infection or uptake of their products, $CD11b^+$ DCs and some populations of DN DCs begin to express CCR7, allowing them to migrate into the IFRs of PPs. Hence, it is likely that blood-derived $CCR6^+$ DCs migrate into FAE regions via interactions with CCL20, and their activation by microbial stimulation through M cells results in their migration into the IFRs. Here, they present Ag to T cells for priming and subsequent induction of the productive phase of the Ag-specific immune response. In the IFRs, additional types of DCs occur. These DC subsets express CD8, but not B220. The exclusive expression of CCR7 on $CD8^+$ DCs allows them to reside in the IFRs. A fourth population, the $B220^+$ plasmacytoid DCs (pDCs), are also present in the IFRs and FAE (51,52). The pDCs perhaps are involved in the generation of Treg cells for the establishment of a quiescent state of immune response (termed *tolerance*) in the harsh environment of mucosal tissues. These different types of DCs cooperatively regulate the activation or inhibition of immune responses induced via MALTs.

Priming of T Cells in Mucosal Inductive Sites

As $CD4^+$ Th cells mature in response to foreign Ags, they assume unique characteristics, such as production of distinct cytokine arrays. The naïve $CD4^+$ T cells have substantial plasticity for development of distinct effector or regulatory lineages (Figure 31.7). The environment and cytokine milieu greatly influences the differentiation of naïve T cells into Th1 (IFN-γ), Th2 (IL-4, IL-13), Th3 (TGF-β), Th17 (IL-17) T cells, or $CD25^+$ $Foxp3^+$ IL-10–producing Treg cells (48). For example, the differentiation of Th1 cells producing IFN-γ was induced by certain pathogens. These cells often develop following production of IL-12 by DCs. In this context, it was previously reported that both DN DCs and $CD8^+$ DCs secreted high levels of IL-12p70 following microbial stimulation, which led to the predominant Th1-type responses (51,52). However, $CD11b^+$ DCs predominantly exist in the FAE regions and produce high levels of IL-10 in response to microbial stimulation, but produce little IL-12, which leads to the induction of Ag-specific T cells secreting IL-4 and IL-10. Of note, IL-10–producing Treg cells are preferentially induced by $CD11b^+$ DCs, indicating that $CD11b^+$ DCs have a unique ability to induce Treg cells.

Fig. 31.7

Recently, an IL-17–producing T cell subset, termed *Th17 cells*, has also been identified as a new subset of the intestinal T cell repertoire and considered to be responsible for pathologic inflammatory reactions (61). It is interesting to note that stimulation of naïve T cells with both TGF-β and IL-6 resulted in the induction of Th17 cells, while IL-6 inhibited the development of Treg cells in the presence of TGF-β (62,63). Taken together, the mucosal immune system has unique T cell–inducing and balancing mechanisms for matching $CD4^+$ T cell effector and regulatory lineage specification, and clearly DCs play a central role in the education of naïve T cells to be an immunosuppressive and active under normal conditions.

Other T cell families involved in mucosal immunity are CTLs. Most CTLs are $CD8^+$ $TCR\alpha\beta^+$ and recognize antigenic peptides through MHC class I–restricted presentation by infected cells. An obvious question is how a CTL immune response is initiated given that mucosal inductive sites, which harbor CTL precursors (pCTLs), are separate from effector sites, such as infected ECs where activated $CD8^+$ CTLs function. A partial answer is that the M cell has specific receptors for mucosal viruses, best exemplified by reovirus. As described earlier, using the sigma one protein, the reovirus enters the M cell in both NALT and GALT (44,59). It is likely, though less well documented that other enteric viruses, such as rotavirus and respiratory pathogens, such as influenza and RSV, also enter the mucosal inductive pathway via M cells (44,59). Further, it was shown that administration of attenuated virus into the GI tract results in the induction of increased pCTL

AU: Spell out RSV? Respiratory syncytial virus?

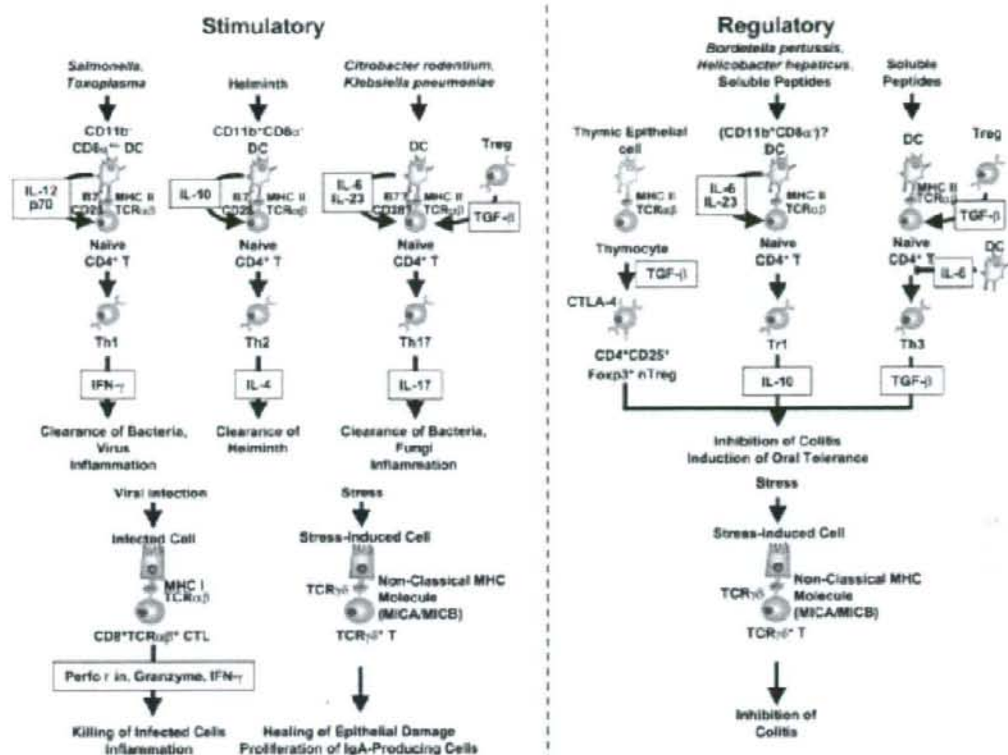

FIGURE 31.7 Various induction pathways for the generation of versatile mucosal T cells for the induction of productive and quiescent immune responses. Naïve T cells are primed and stimulated via a molecular network with the recognition of peptide Ag presented by MHC molecules, costimulatory molecules, and cytokines. After receiving stimulation, naïve T cells differentiate into immunologic (effector T cells) or tolerogenic (Treg cells) cell types for subsequent immunity.

frequencies in the PPs (64). Similarly, virus-specific CTLs were detected in NALT as well as in mediastinal, submandibular, and cervical LNs after nasal immunization with attenuated virus (65). These findings clearly demonstrate that PPs and NALT play a pivotal role in the induction of Ag-specific CTLs in addition to the generation of Ag-specific IgA-committed B cells; the molecular and cellular pathways underlying the CTL induction in the inductive tissues (e.g., involvement of DCs) remain obscure.

Immunoglobulin Isotype Switching in Mucosal Inductive Sites

T Cells for IgA Class Switching

The cross-talk among DCs, T cells, and B cells in mucosal inductive tissues promotes the IgA-commitment of B cells, which undergo μ to α isotype class swich recombination (CSR) (Figure 31.8) (66). This μ to α isotype CSR is likely dependent on Ag stimulation in GCs, where naïve B cells interact with local $CD4^+$ T cells and with follicular Ag-trapping DCs (FDCs) in the presence of specific cytokines. Hence, depletion of $CD4^+$ T cell subsets markedly affects mucosal immune responses, including diminished levels of $sIgA^+$ B cells (67). Consistent with this, clear evidence was presented that clones of T cells from murine GALT, when mixed with noncommitted $sIgM^+$ B cells, induced isotype switching to B cells expressing surface IgA (sIgA) (68). The initial studies with murine T switch (Tsw) cells used T cell clones derived by mitogen stimulation and IL-2 supported outgrowth, and when Tsw cells were added to $sIgM^+ sIgA^-$ B cell cultures resulted in marked increases in $sIgA^+$ cells (68). This result suggests that cognate interactions between Tsw and B cells are required for induction

Fig. 31.8

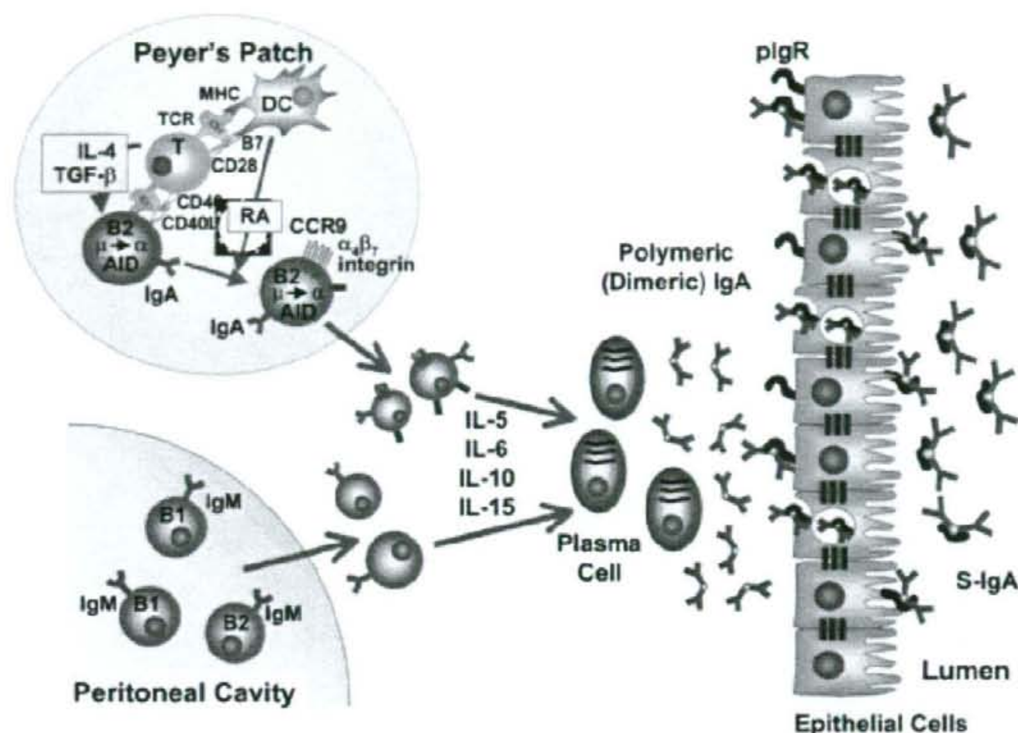

FIGURE 31.8 CMIS-dependent and alternative pathways for the production of intestinal S-IgA Abs. In the CMIS-dependent pathway, DCs take up luminal Ag via M cells, and then present the peptide to CD4$^+$ Th cells after intracellular processing of Ags in PPs. Ag-primed Th cells produce IL-4 and TGF-β, allowing B cells to undergo μ to α CSR. Simultaneously, RA produced by DCs increases gut homing receptors ($\alpha_4\beta_7$ integrin and CCR9) on Ag-primed IgA-committed B cells. In the LP, Th2 cell-derived IL-5 and IL-6 induce terminal differentiation of sIgA$^+$ B cells to become IgA-producing PCs secreting dimeric (or polymeric) forms of IgA. The dimeric IgA (dIgA) binds to the pIgR expressed on the basolateral surface of ECs and transports the dIgA into the lumen as S-IgA. In the alternative pathway, peritoneal B cells (mainly B1 cells) migrate into the LP, where they differentiate into IgA-producing PCs under the influence of IL-5 and IL-15.

of the IgA class switch. Other studies have revealed that T–B cell interactions support B cell switches and have postulated a major role for the CD40 receptor on GC B cells with CD40 L on activated T cells (66,69). Evidence for Tsw cells in human IgA responses has stemmed from an earlier study with T cell clones obtained from human appendix. These T cell clones and their derived culture supernatants exhibited preferential help for IgA synthesis (70).

Cytokines for IgA Class Switches

The most definitive studies to date suggest that several cytokines are involved in the B cell switching to IgA (66). The first studies showed that addition of TGF-β to LPS-triggered mouse splenic B cell cultures resulted in switching to IgA, and IgA synthesis was markedly enhanced by IL-2 (71) or IL-5 (72). It was also shown that TGF-β induced sterile Cα germline transcripts (72), an event that clearly precedes actual switching to IgA. Molecular pathways of TGF-β–induced Cα germline transcripts have been clarified (46). For example, transcriptional activation of the $C_H\alpha$ gene requires the TGF-β–mediated activation of Smad3/4 and the *de novo* synthesis of core-binding factor (CBF) α3 that binds to the Smad-binding elements (SBEs) and CBF sites on the intronic $C_H\alpha$ promoter region (46,66). Other studies showed that TGF-β induced human B cells to switch to either IgA1 or IgA2, an event clearly shown to be preceded by formation of Cα1 and Cα2 germline

transcripts (46). It can be presumed that TGF-β induces μ to α switches in normal physiologic circumstances, since sIgM$^+$, sIgD$^+$ B cells triggered through CD40 were induced to switch to IgA by TGF-β and to secrete IgA in the presence of IL-10 (73,74).

Although one would predict that deletion of the TGF-β gene would lead to a negative influence on the IgA immune system, the TGF-β gene knockout mice unfortunately die from a generalized lymphoproliferative disease 3 to 5 weeks after birth, making it difficult to use this model to investigate the role of TGF-β in IgA regulation *in vivo*. Nevertheless, conditional mutagenesis (Cre/loxP) was used to knock out the TGF-β receptor in B cells, showing that these mice exhibited expanded peritoneal B1 cells and B cell hyperplasia in PPs and a complete absence of serum IgA (75).

Molecular Mechanisms of IgA Class Switch Recombination

Isotype switching involves the recombination between tandem repetitive DNA sequences (switch or S regions) located 5′ of the respective C_H genes. Switching is an irreversible DNA deletional event in which recombination between upstream and downstream S regions forms a DNA circle containing the deleted intervening C_H genes. Isotype switching can also be induced by cytokines in combination with activation signals provided by mitogens such as LPS or through the more physiological T cell CD40 L and B cell CD40 interactions as discussed earlier (46,66). Several tangible events, including demethylation of 5′ flanking region DNA, DNAse hypersensitivity, and transcription of unrearranged H chain genes, precede cytokine-induced switching. Germline transcription initiates 5′ of the targeted C_H gene upstream of so-called *I region exons* that contain stop codons in all translational reading frames, thus the resulting transcripts are "sterile." I exons have been identified for all isotypes and subclasses, and in general their deletion, for example, in I exon-knockout mice, results in impaired switching to that isotype or subclass (76). An apparent exception has been observed for IgA switching, where replacement of the Iα exon with an irrelevant human gene construct in the gene transcriptional orientation did not impair B cell switching to IgA (77). These studies rule out a direct role for the I exon in controlling switch recombination. However, transcription of the Cα locus was found to be constitutive in the Iα-targeted mice, in contrast to other I region KO mice. It seems likely that cytokine-induced germline transcripts themselves direct cytokine-regulated isotype switching (46,66).

A dramatic breakthrough in our understanding of CSR came with the discovery of the AID gene, initially identified in GC B cells and subsequently cloned from B lymphoma cells stimulated with CD40 L, IL-4, and TGF-β, which were undergoing μ to α switches (78). AID exhibits a single-stranded DNA deaminase activity and associates with the CSR target chromatin in a germline transcription-coupled manner (78). As may be expected given the ability of PPs, ILFs, and NALT to induce the generation of IgA-committed B cells, AID expression and Iα-Cμ circle transcripts and their reaction products are always detected in these inductive tissues (79).

The dogma that μ to α switching only occurs in mucosal inductive sites is challenged by several recent findings. Overexpression of AID in μ^+ B lymphoma cells resulted in spontaneous class switching from IgM to IgA in the complete absence of TGF-β or other cytokines. Mice defective in the AID gene (AID$^{-/-}$) exhibit a hyper IgM syndrome with no evidence of downstream switching (78). However, studies revealed that AID$^{-/-}$ mice have a subset of B220$^+$ surface IgA$^+$ B cells in LP (an effector site) and the presence of circles of "looped out" DNA suggest that μ to α switching had just occurred in this site (80). Along these lines, it was also revealed that B cell–deficient μMT mice also exhibit LP IgA$^+$ PCs, suggesting that switches to IgA can occur even during preB cell development (81). Although this issue is still a subject of debate, μ to α B cell switches may occur throughout the mucosal immune system and in the complete absence of GCs.

In contrast to the dominant class switch to IgA in the PPs, B cell development in NALT leads to the production of both IgA and IgG (41,42). It had been previously established that the development of IgA-committed cells in the presence of TGF-β was characterized by sequential CSR from Cμ to Cα via a Cγ pathway-mediated by CD40 engagement (82). It is also interesting to note that human tonsils have been shown to contain a high frequency of IgG B cells in addition to IgA (83). These findings may explain the equal commitment of B cells to IgG and to IgA in NALT, but further analysis will be required to reveal the molecular mechanism involved in the generation of mucosal B cells with those two different isotypes.

Trafficking and Homing from Mucosal Inductive into Effector Sites Via the CMIS

Following T cell priming and CSR to IgA-committed B cells through their interaction with DCs, both B and T cells emigrate from the inductive tissue (e.g., PPs and NALT), circulate through the bloodstream, and home to distant mucosal effector compartments, especially the LP regions of the GI, respiratory and reproductive tracts (Figure 31.8).

Several early studies demonstrated that lymphocytes circulated from blood to LNs and that thoracic duct lymphocytes were retained primarily in the intestine (50). A direct route for B cell migration between PPs and distant LP was revealed by the finding that rabbit GALT B cells repopulated the gut with IgA PCs (84). Further, the mesenteric lymph nodes (MLNs) of orally immunized animals were found to contain Ag-specific precursors of IgA

PCs that repopulated the LP of gut and mammary, lacrymal, and salivary glands (50). Studies of the origin, migration, and homing of lymphoid cells from mucosal inductive to effector tissues were of basic importance for parallel attempts to induce Ag-specific immune responses. Consequently, specific Abs in glandular secretions could be induced in human and animal experiments by oral or bronchial immunization. These studies served as the basis for demonstrating the existence of a CMIS. This may explain the phenomena that immunization via one mucosal site often activates other, remote mucosal sites. However, immunization via certain mucosal inductive tissues can lead to the preferential induction of humoral immune responses in the same mucosal site, which is basically determined by site-specific combination of adhesion molecules and chemokines.

Recently, a lipid mediator, S1P has been identified as a key regulator of lymphocyte emigration from the organized lymphoid structures including the thymus and secondary lymphoid organs (85). Lymphocytes increase the S1P receptor, especially type 1 S1P receptor, when they emigrate from the secondary lymphoid organs, allowing them to emigrate from the lymphoid tissues to the blood where S1P is present at high concentration (100 nM–300 nM) (85). Several studies have now suggested that the S1P-mediated pathway is involved in the regulation of T cell emigration from mucosal inductive tissues (e.g., PPs) (85). In addition to T cells, it was recently found that B cell trafficking, especially IgA^+ plasmablasts, is selectively regulated by S1P in the emigration of B cells from the PPs and their entry into the CMIS pathway for the final destination of LP (Jun Kunisawa and Hiroshi Kiyono, unpublished data).

Lymphocyte Homing in the GI Tract

Lymphocytes enter mucosal or systemic lymphoid tissues from the blood through specialized HEVs, which consist of cuboidal endothelial cells. In GALT, HEVs are present in the interfollicular zones rich in T cells (41,42,50). The endothelial venules in effector sites such as the LP of the GI tract are less pronounced and tend to occur near villus crypt regions. Mucosal addressin cell adhesion molecule-1 (MAdCAM-1) is the most important addressin expressed by HEVs in the PPs and LP (Figure 31.9) (50). Likewise, peripheral lymph node addressin (PNAd) and VCAM-1 are the principal addressins expressed by peripheral lymph node (PLN) and skin HEVs, respectively. The major homing receptors expressed by lymphocytes are the integrins, a large class of molecules characterized by a heterodimeric structure of α and β chains. In general, the type of homing receptor is determined by the integrin expressed with the α_4 chain; the β_1 integrin characterizes the homing receptor for the skin and the β_7 integrin that for the GI tract. Thus, the pairing of α_4 with β_7 represents the major integrin molecule responsible for lymphocyte binding to MAdCAM-1 expressed on HEVs in the PPs and LP (Figure 31.9) (49). In addition to $\alpha_4\beta_7$ integrin, the C-type lectin family of selectins that includes L-, E-, and P-selectins, also serve as homing receptors. L-selectin has a high affinity for carbohydrate-containing PNAd, and this lectin addressin is of central importance in PLN homing of B and T cells (41,42,50). Despite this homing pair, L-selectin can also bind to carbohydrate-rich MAdCAM-1 and is an important initial receptor for homing into GALT HEVs. Interestingly, naïve B and T cells destined for GALT express L-selectin; moderate levels of $\alpha_4\beta_7$ ($\alpha_4\beta_7{}^+$) and memory lymphocytes destined for LP express higher levels of $\alpha_4\beta_7$ ($\alpha_4\beta_7^{high}$) and lack L-selectin (Figure 31.9). Similarly, human tissues revealed naïve T and B cells in HEVs, which expressed both L-selectin and $\alpha_4\beta_7$ integrin, while memory T and B cells in efferent lymphatics expressed $\alpha_4\beta_7$ integrin but not L-selectin (86). These data indicate that naïve and memory lymphocytes utilize a different regulation pathway for their migration into the GI tract.

Fig. 31.9

In addition to the integrin-mediated regulation, chemokines have been directly involved in lymphocyte homing, and different chemokine-receptor pairs control migration into different lymphoid tissues (Figure 31.9) (50,85). For example, loss of secondary lymphoid tissue chemokine (SLC/CCL21) results in lack of naïve T cell or DC migration into PPs or spleen. Further, the chemokine receptor CCR4, which responds to the thymus activation-regulated chemokine (TARC/CCL17) and MΦ-derived chemokine (MDC/CCL22), mediates arrest of skin-homing T cells but does not affect $\alpha_4\beta_7^{high}$ T cell migration in the GI tract. However, gut-tropism, especially into the small intestine, is determined by the CCR9, selectively expressed on IgA-, but not IgM- or IgG-committed B cells (Figure 31.9) (50,85). The ligand of CCR9 is CCL25, also known as thymus-expressed chemokine TECK, which is produced dominantly by the small intestinal epithelium, determining the selective homing of IgA^+ B cells into the small intestinal LP (50,85). Although the detailed mechanism remains to be investigated, it has been reported that the migration of IgA^+ B cells to the large intestine may be due to the expression of MEC/CCL28 (50). Similarly, human memory T cell migration into the LP of the GI tract is mediated by the TECK/CCL25 expressed on intestinal epithelium, and gut-homing $\alpha_4\beta_7^{high}$ T cells specifically express CCR9 (50). In addition to the LP T cells, $\alpha_E\beta_7^+$ and $\alpha_4\beta_7^{high}$ IELs also expressed CCR9 in human and mouse, suggesting that TECK-CCR9 is also involved in lymphocyte homing and arrest of IEL in the small intestinal epithelium (50).

Several lines of evidence have suggested that GALT DCs play a crucial role in determining the gut tropism of T and B cells (Figure 31.8) (87). $\alpha_4\beta_7$ integrin and CCR9 were induced on Ag-primed T cells by GALT DCs, but not other DCs, respectively interacting with the MAdCAM-1 expressed by HEVs in the intestinal LP and the TECK/CCL25

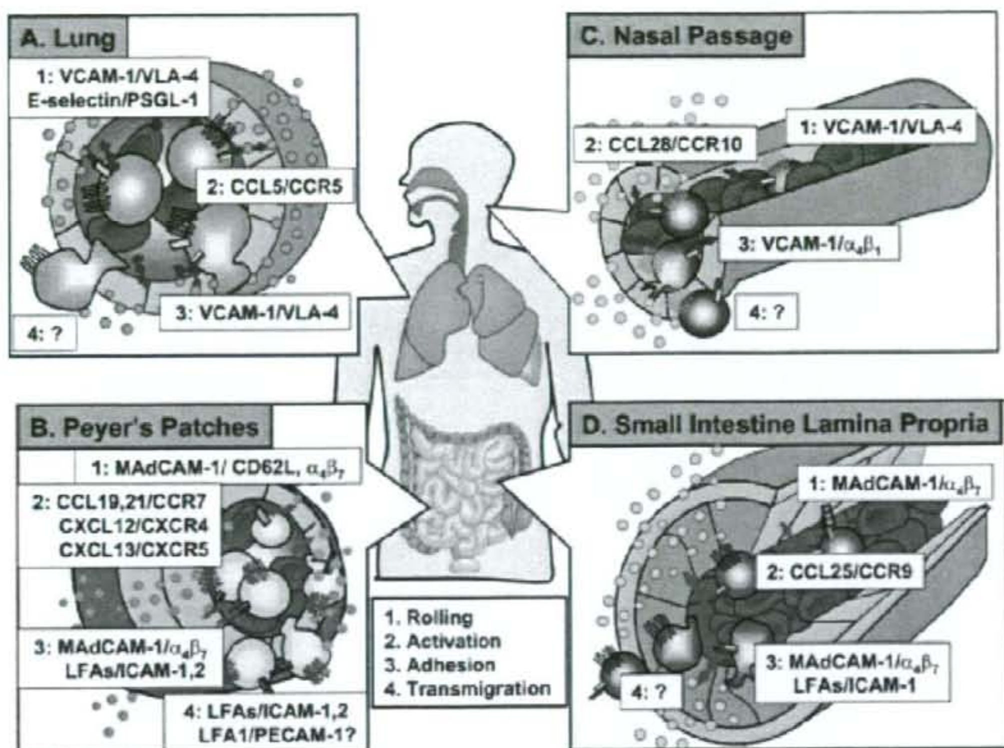

FIGURE 31.9 Mucosal lymphocyte migration-adhesion pathway. Lymphocyte migration is consistent with 1) rolling, 2) activation, 3) adhesion, and 4) transmigration or diapedesis. Effector/activated lymphocytes migrate to the mucosal effector sites, such as pulmonary tissue via the pulmonary vasculature (**A**), nasal passages (**C**), and intestinal LP (**D**) via endothelial venules. Naive lymphocytes migrate into the PPs with interaction of lymphocytes and HEVs (MadCAM-1/$\alpha_4\beta_7$ integrin) in a chemokine-dependent pathway (e.g., CCL19, 21/CCR7 (T cells), CXCL12/CXCR4, CXCL13/CXCR5 (B cells) (**B**).

produced by small IECs. Recent work has revealed that retinoic acid (RA) produced by GALT DCs is involved in the imprinting of T and B cells for gut homing (88,89). RA is a metabolite of vitamin A, and GALT DCs express RA-producing enzymes (alcohol dehydrogenases). Thus, significantly decreased numbers of gut T and B cells were noted in vitamin A–deficient mice (88,89).

Although $CD8^+$ CTLs utilize similar migration pathways into mucosal immune compartments, $CD8^+$ CTLs additionally migrate into the epithelium. This is reasonable because ECs are the primary cells infected with many viruses and bacteria and should be recognized by $CD8^+$ CTLs. Although mucosal effector tissues such as the intestinal epithelium contain high numbers of $\gamma\delta$ T cells as key players in innate immunity as mentioned earlier (25), virus-specific CTLs in IELs greatly contribute to the acquired phase of immunosurveillance in the epithelium itself. The homing of IELs into the epithelium is also determined by the adhesion molecules and chemokines (25). Like migration of other T cells into the intestinal compartment, CCR9s play an important role in the determination of IEL trafficking into the epithelium. In addition, the various chemokine receptors expressed on IELs, such as CCR3, CCR4, CCR5, and CXCR3, may play pivotal roles in the alternative pathway of IEL trafficking (Figure 31.9) (25). The $\alpha_4\beta_7$ integrin also contributes to the regulation of IEL trafficking. Thus, β_7 integrin-deficient mice showed critically reduced numbers of IELs in the intestine (49). Integrin-mediated interactions between IECs and IELs play a pivotal role not only in the migration of

the IELs into the intestine, but also in the retention of IELs at the epithelium. CD103 (α_E integrin) is exclusively expressed on IELs; it interacts with E-cadherin expressed on ECs (Figure 31.9) (26). It was previously reported that TGF-β induced down-regulation of $\alpha_4\beta_7$ integrin and simultaneously up-regulated CD103. Thus, $\alpha_4\beta_7$ integrin expression was reduced following IEL entry into the small intestinal epithelium, a reduction that coincided with an increase in CD103 expression (87). In addition to the TGF-β-mediated pathway, a recent study proposed that CCR9-mediated signaling promoted the induction of CD103, a retention molecule of IELs in the epithelium (87).

Lymphocyte Homing in NALT and Lung-Associated Tissues

Unlike PP HEVs, which are found in T cell zones, murine NALT HEVs are found in B cell zones and express PNAd either alone or associated with MAdCAM-1 (Figure 31.9) (41,42). Further, anti-L-selectin but not anti-MAdCAM-1 Abs blocked the binding of naïve lymphocytes to NALT HEVs, suggesting predominant roles for both L-selectin and PNAd. Consistent with this, the failure of human tonsillar cells to demonstrate selective $\alpha_4\beta_7$ expression and the lack of MAdCAM-1 expression on tonsil or adenoid HEVs make it likely that gut-homing does not extend to human NALT and associated LNs. However, nasal immunization induces up-regulation of $\alpha_4\beta_1$ integrin and CCR10, allowing selective trafficking of B cells to nasal passage epithelium expressing their ligands, VCAM-1 and CCL28, respectively (Figure 31.9) (41,42). It is interesting to note that the same molecules are involved in the trafficking to the genitourinary tract (41,42), which may explain why high levels of Ag-specific immune responses are induced in the genital tract after nasal immunization (Figure 31.9).

Early induction of VCAM-1, E-selectin, and P-selectin in the pulmonary vasculature was reported during pulmonary immune responses with an initially increased expression of P-selectin ligand by peripheral blood CD4$^+$ and CD8$^+$ T cells (50). The number of cells expressing P-selectin ligand then declined in the blood as they accumulated in the bronchoalveolar lavage fluid. The very late antigen (VLA-4) could be an important adhesion molecule involved in the migration of activated T cells into the lung since migration of VLA-4$^+$ cells into bronchoalveolar fluid is impaired following treatment with anti-α_4 Ab. Other investigators have shown that Ag-specific L-selectin^{low} CTL effectors rapidly accumulate in the lung following adoptive transfer to naïve mice with reduced pulmonary viral titers early during infection.

An interesting approach used to address the homing of human cells in the NALT was the analysis of tissue-specific adhesion molecules after systemic, enteric, or nasal immunization (90). This study showed that following systemic immunization, most effector B cells expressed L-selectin, with only few cells expressing $\alpha_4\beta_7$, while after enteric (oral or rectal) immunization the opposite held true. Interestingly, effector B cells induced by nasal immunization displayed a more promiscuous pattern of adhesion molecules with a large majority of these cells expressing both L-selectin and $\alpha_4\beta_7$ integrin.

S-IgA Formation and Transport

Distribution of Ig Isotypes

Measurement of combined synthesis of Ig of all isotypes indicates that in a 70 kg individual, around 8 g of Ig are produced every day (Table 31.1) (91). Divided by individual isotypes, humans produce ~5 g IgA, ~2.5 g IgG, ~0.6 g IgM, and trace amounts of IgD and IgE per day. Approximately one half of IgA is internally catabolized mainly in the liver, and the remainder is actively and passively transported into external secretions (91). It is estimated that ~50%–70% of total IgA is selectively transported into external secretions; daily, over 3 g of IgA are deposited on a large surface area of mucosal membranes. Studies that addressed the tissue origin of S-IgA convincingly demonstrated that ~99% is produced locally in mucosal tissues and glands. Extensive studies of the distribution of Ig-producing cells in various mucosal tissues and glands by the immunofluorescence technique and ELISPOT convincingly demonstrated a remarkable preponderance of

Tab. 31.1

TABLE 31.1 Levels of Immunoglobulins in Human External Secretions (μg/ml)[a]

	IgA	*IgG*	*IgM*
Tears	80–400	trace-16	0–18
Nasal fluid	70–846	8–304	0
Parotid saliva[b]	15–319	0.4–5	0.4
Whole saliva	194–206	42	64
Bronchoalveolar fluid	3	13	0.1
Colostrum and milk	470–12,340	40–168	50–610
Hepatic bile	58–77	88–140	6–18
Gallbladder bile	92	12	46
Duodenal fluid	313	104	207
Jejunal fluid	32–276	4–340	2
Colonic fluid	240–827	1	trace-860
Intestinal fluid[c]	166	4	8
Urine	0.1–1.0	0.06–0.56	
Ejaculate	11–23	16–33	0–8
Cervical fluid	3–133	1–285	5–118
Vaginal fluid	35	52	

[a]High variability in Ig levels is due to the method of collection, dilution of specimens by lavage fluids, methods of measurements, including the use of appropriate standards (S-IgA versus monomeric IgA) flow rates and stimulation of secretions, hormonal states, and the health status of the individual.
[b]Unstimulated or stimulated.
[c]Whole gut lavage.

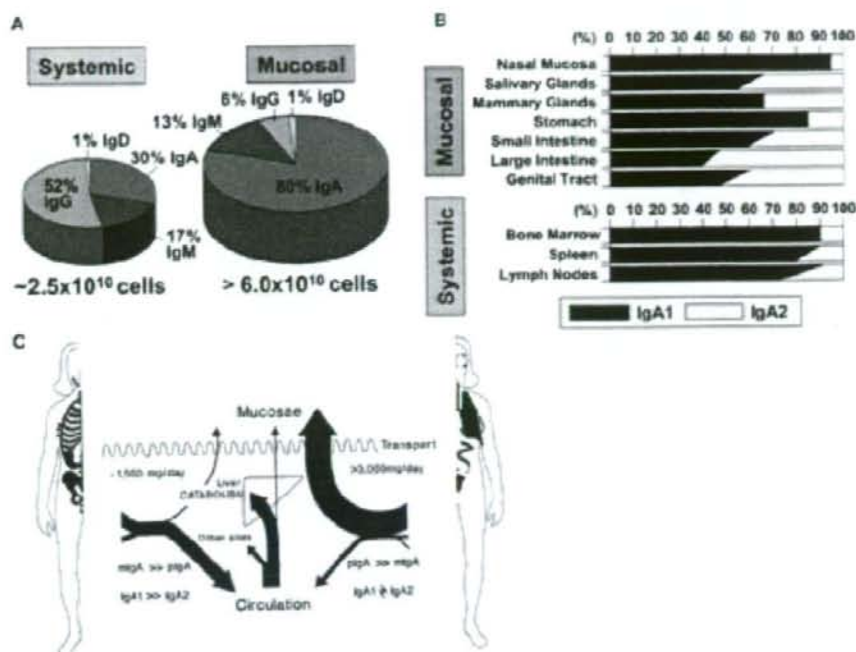

FIGURE 31.10 Comparative distribution and B cells in systemic and mucosal compartments. **A:** Quantitative distribution and Ig isotypes. **B:** Distribution of IgA subclasses. **C:** Distribution and properties of IgA produced in the systemic and mucosal compartments.

Fig. 31.10 IgA-producing cells in all such tissues (Figure 31.10A) (46). The only exception is the uterine cervix, where the numbers of IgG-producing cells are equal or slightly exceed IgA-producing cells (91). However, there are tissue-specific differences in the proportions of IgA-, IgG-, IgM-, and IgD-positive PCs. For example, nasal mucosa contains on average 69% IgA-, 17% IgG-, 6% IgD-, and 6% IgM-positive cells, while in the large intestine 90% of cells are positive for IgA, 6% for IgM, and 4% for IgG (46). Moreover, cells producing IgA1 or IgA2 also display a characteristic tissue distribution (Figure 31.10B) (46,91). Systemic lymphoid tissues (e.g., spleen, tonsils, LNs, and bone marrow), as well as most of the mucosal tissues (nasal, gastric, and small intestinal mucosa and to a lesser degree glandular tissues) contain more IgA1- than IgA2-producing cells, while in the large intestine and the female genital tract tissues, IgA2-producing cells are more frequent than IgA1 cells (Figure 31.10B). Although direct experimental evidence is not available, it has been speculated that this tissue-specific distribution of IgA1- or IgA2-producing cells is related to the differences in the origin of IgA1 and IgA2 precursors and perhaps their distinct homing patterns. Alternatively, Ag-driven clonal expansion in various mucosal tissues may also be involved. For example, most of the naturally occurring S-IgA Abs to bacterial endotoxin is associated with the IgA2 isotype (91). Thus, it is likely that endotoxin abundantly present in the large intestine induces clonal expansion of IgA2-producing cells in this locale.

Distribution of Polymeric or Monomeric IgA-Producing Cells

Analyses of molecular forms of IgA in supernatants of cells and tissue explants obtained from systemic and mucosal compartments, tissue perfusates, and immunohistochemical studies of such tissue demonstrated that the separate populations of pIgA- and mIgA-secreting cells display a characteristic tissue distribution (46,91). Typically, almost all IgA-producing cells in the normal bone marrow produce mIgA (Figure 31.10C). The admixture of peripheral blood in the bone marrow specimens grossly influences the results because peripheral blood lymphocytes secrete, especially after stimulation, predominantly pIgA and little mIgA (92). Supernatants collected from *in vitro* cultured human LNs and spleen contained both forms, usually with the preponderance of mIgA. In contrast, such supernatants