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 $\beta 1$ 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?

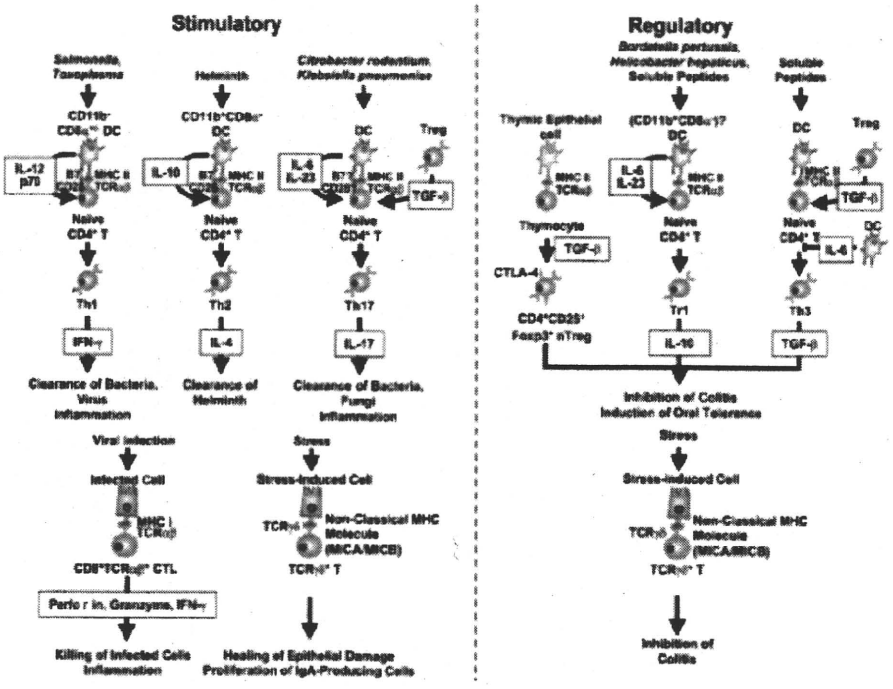

FIGURE 31.7 Various induction pathways for the generation of versatile mucosal T cells for the induction of productive and quiescent immune responses. Naïve T cells are primed and stimulated via a molecular network with the recognition of peptide Ag presented by MHC molecules, costimulatory molecules, 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

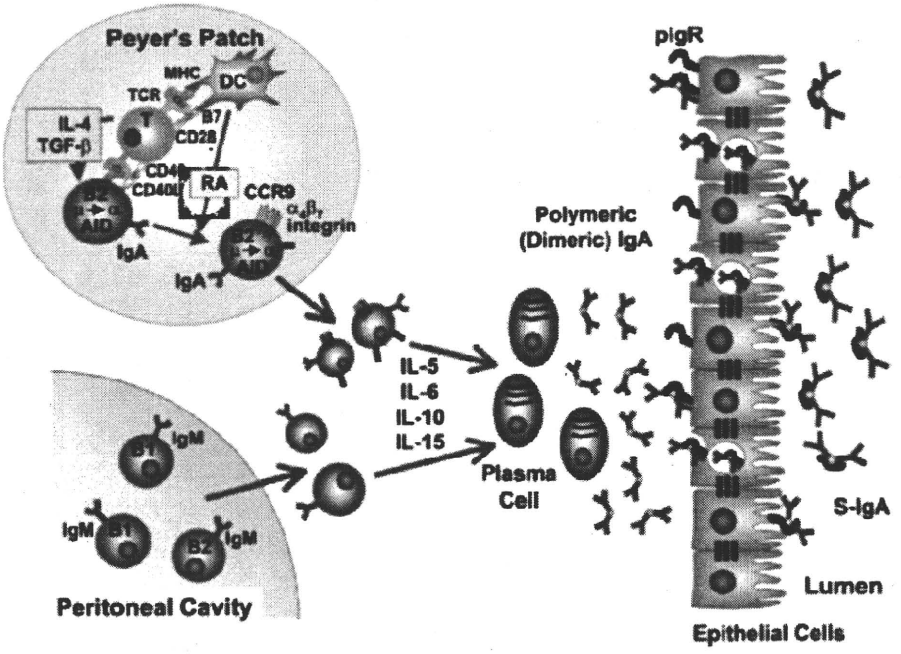

FIGURE 31.8 CMIS-dependent and alternative pathways for the production of intestinal S-IgA Abs. In the CMIS-dependent pathway, DCs take up luminal Ag via M cells, and then present the peptide to $CD4^+$ Th cells after intracellular processing of Ags in PPs. Ag-primed Th cells produce IL-4 and TGF-β, 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.

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

Fig. 31.9

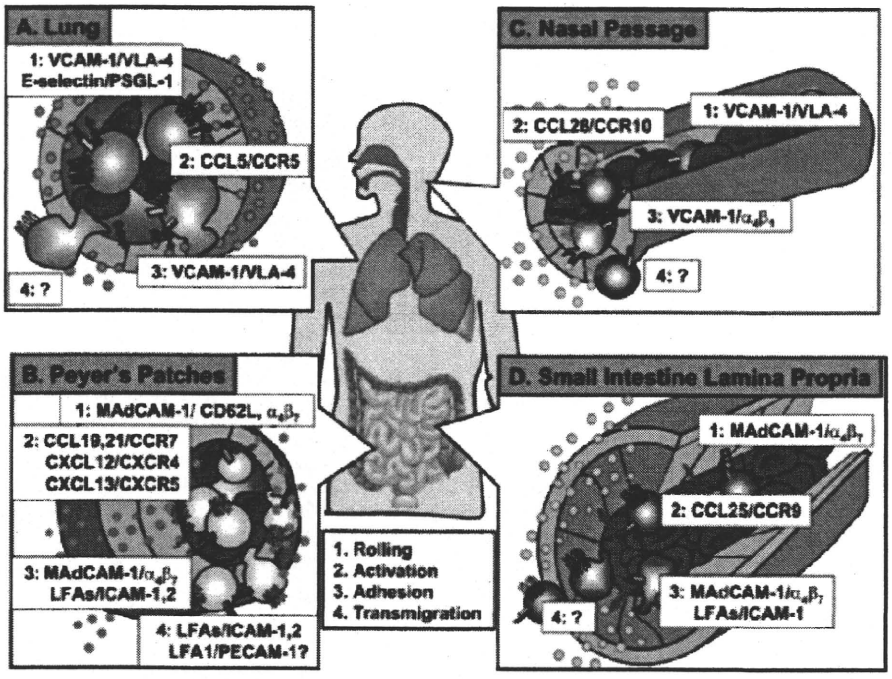

FIGURE 31.9 Mucosal lymphocyte migration-adhesion pathway. Lymphocyte migration is consistent with 1) rolling, 2) activation, 3) adhesion, and 4) transmigration or diapedesis. Effector/activated lymphocytes migrate to the mucosal effector sites, such as pulmonary tissue via the pulmonary vasculature (**A**), nasal passages (**C**), and intestinal LP (**D**) via endothelial venules. Naïve 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.

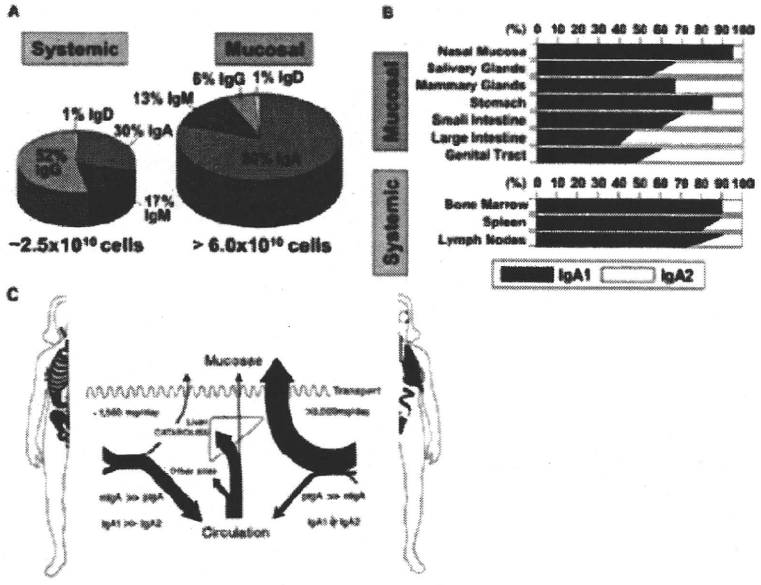

FIGURE 31.10 Comparative distribution and B cells in systemic and mucosal compartments. **A:** Quantitative distribution and Ig isotypes. **B:** Distribution of IgA subclasses. **C:** Distribution and properties of IgA produced in the systemic and mucosal compartments.

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

TABLE 31.2 Functions/Biological Properties of IgA

Protective Functions in External Secretions
Prevention of Ag absorption from mucosal surfaces due to the formation of Ag-IgA complexes
Mucus trapping (IgA-mucin complexes entrap microorganisms)
Virus neutralization (in some experiments nonneutralizing Abs may also be protective)
Enzyme and toxin neutralization
Enhancement of antimicrobial activities of innate factors (e.g., lysozyme, lactoperoxidase, and lactoferrin)
Biological Activities in Tissues
Inhibition of C activation in some experiments (polymeric IgA or glycan-altered IgA may activate complement by the alternative or lectin pathways)
Enhancement (opsonization) or inhibition of phagocytosis
Inhibition of type I and II hypersensitivity reactions (e.g., anaphylaxis and Arthus reaction)
Degranulation of eosinophils
Intracellular virus neutralization
Elimination of Ag-IgA immune complexes by ECs and hepatocytes expressing IgA receptors
Ab-dependent cell-mediated cytotoxicity
Inhibition of NK cell activity
Inhibition of the release of inflammatory cytokines

or perfusates of mucosal tissues, especially in the GI tract, contain pIgA as the dominant form (Figure 31.10C).

Au: Pls provide callout for Table 31.2.

Structure of Secretory IgA (S-IgA)

When compared to its serum counterpart, IgA in external secretions (called S-IgA) displays unique structural features with respect to its molecular form, chain composition, and IgA subclass distribution (91). Examinations of sera and mucosal secretions, culture supernatants, and cell lysates, and immunohistochemical studies of systemic and mucosal lymphoid tissues indicated that pIgA contains J chain as a typical component and that J chain–containing pIgA is capable of binding to pIgR and its extracellular region, SC (9). Consequently, the presence of intracellular J chain and the ability to bind SC have been taken as markers for pIgA- or IgM-producing cells. In humans, almost all serum IgA is present in a monomeric form (sedimentation constant 7S) and contains two heavy (α) and two light (either κ or λ) chains, and ~85% belongs to the IgA1 and ~15% to the IgA2 subclass. Only a small but variable fraction (1% to 10%) of serum IgA is found in a polymeric form and contains an additional polypeptide-joining (J) chain. In contrast, ~90% of S-IgA occurs in a polymeric form (dimers and tetramers with sedimentation constants 11S and 15.5S, respectively) and is associated with J chain acquired during the transepithelial transport via the pIgR. The structure of a typical dimeric IgA molecule is shown in Figure 31.11. Two mIgA molecules are mutually linked by disulfide bridges through their Fc regions; J chain is bound to the penultimate Cys residues of α chains. Although SC interacts noncovalently with the Fc regions of both monomers, it is attached by disulfide bridges to only one of them, and there are no covalent bonds formed between J chain and SC. Fig. 31.11

Mammalian α chains with molecular mass ~50 kDa contain one variable and three constant region domains. There are high numbers of Cys residues involved in the formation of intrachain and interchain disulfide bridges with another α chain, L and J chains, and SC (91). In addition, α chains can form complexes with a number of plasma and

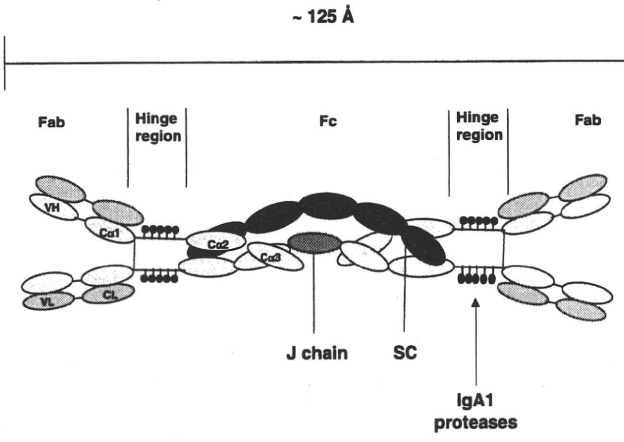

FIGURE 31.11 Molecular dimensions, proteolytic fragments, and domain structure of the human dimeric S-IgA1 molecule. Dimeric or polymeric IgA was formed by joining each IgA by J chain. The dimeric or polymeric forms of IgA are associated with SC, an extracellular domein of pIgR. Hinge region between Fab and Fc is a target site of IgA1 proteases.

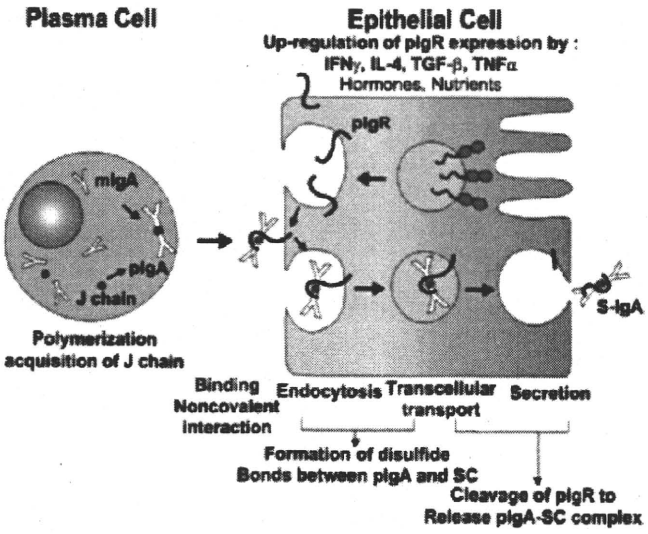

FIGURE 31.12 12 Transcellular transport of pIgA by the pIgR-mediated mechanism and regulation of pIgR expression. Subepithelial PCs produce J chain–associated pIgA that interacts with the epithelial pIgR, and the pIgA-pIgR complex is transcytosed through the epithelial cells and released, after the proteolytic clevage of pIgR, as S-IgA.

secretory proteins including albumin, amylase, lactoferrin, glycosyltransferases, and proteolytic enzymes. An unusual hinge region is present in the middle of the α chain of IgA1, between Cα1 and Cα2 domains (46,91). This 13 amino acid–long hinge region is reminiscent of mucin (high content of Pro, Ser, and Thr residues) and carries 3–5 *O*-linked oligosaccharide side chains (Figure 31.11). Although IgA is quite resistant to common proteolytic enzymes, the hinge region contains peptide bonds susceptible to the cleavage by highly substrate-specific IgA1 proteases of bacterial origin (e.g., *Streptococcus pneumoniae, Neisseria meningitidis, N. gonorrhoeae, Haemophilus influenzae,* and several other species of bacteria) (93). Comparative structural and genetic studies of IgA molecules from many species indicate that molecules of the IgA2 subclass represent phylogenetically older forms and that the IgA1 subclass arose in hominoid primates by insertion of a gene segment encoding the hinge region (91). The Fcα region, particularly its C terminus, displays a high degree of sequence homology to the μ chain of IgM, including the characteristic C terminae "tail" (an ~18 amino acid extension over the C terminus of γ, δ, and ε chains of corresponding Ig isotypes) involved in the polymerization and ability of α and μ chains to bind J chain. Both IgA1 and IgA2 contain 6% to 8% of glycans associated in the form of ~2 to 5 *N*-linked side chains within the Fc region; as described earlier, the hinge region of IgA1 also contains *O*-linked glycans (91).

J chain is a chacteristic polypeptide chain present in pIgA and IgM (94). It has a molecular mass of 15 kDa and a single *N*-linked glycan chain and displays an Ig domain folding pattern. Of eight Cys residues, six are involved in three intrachain disulfide bridges, and two participate in linkages to the penultimate Cys residues of α and μ chains. A very high degree of homology exists in the primary structures and antigenic cross-reactivities of mammalian and avian J chains, indicating that the basic properties have remained conserved throughout evolution (91). PCs in mucosal effector tissues and glands assemble pIgA intracellularly from mIgA and J chain as a last step before its externalization (Figure 31.12). Although the incorporation of J chain is not absolutely required for polymerization, the ability of pIgA and IgM to interact with pIgR expressed on ECs or SC depends on the presence of J chain as demonstrated in J chain knockout mice (95). **Fig. 31.12**

The pIgR specific for the J chain-containing pIgA and IgM is expressed on the basolateral surfaces of EC of the GI tract and endocervix, acinar, and ductal epithelia of the small and large secretory glands (e.g., lacrimal, mammary, and major and minor salivary glands) in humans (Figure 31.12) (9). In some other species (rats, mice, and rabbits, but not humans), pIgR is also expressed on hepatocytes (9). Structurally, pIgR comprises an extracellular region composed of five Ig domain-like structures, with ~560 amino acids, a 23 amino acid membrane-spanning region, and a cytoplasmic region with ~103 amino acids; the molecular mass of pIgR with attached glycans is ~110 kDa to 120 kDa. The similarity of the general structural features of pIgR from a number of mammalian species indicates

that this receptor and its ability to interact with pIg are conserved in phylogeny.

The N-terminal domain of pIgR interacts with Cα domains (9). The pIgA-pIgR complex is internalized, transcytosed, and finally released at the apical end of the ECs with the entire process taking ~30 minutes (Figure 31.12). Signals for basolateral targeting of pIgR, its endocytosis, and its transcytosis are encoded in the cytoplasmic region of pIgR as revealed by deletion mutants. In the final steps, pIgR is proteolytically cleaved, thus releasing the pIgA-SC complex; the intracellular and transmembrane regions are endocytosed and degraded or released from the apex. Unlike several other receptors, pIgR does not recycle; instead, it remains permanently associated with the ligand as bound SC (9). Therefore, the transport of pIgA and IgM is directly dependent on the availability of pIgR on EC (or hepatocyte) membranes. A number of substances of local and distant origin influence pIgR expression. Cytokines produced locally in mucosal tissues and glands (e.g., IFN-γ, IL-4, TNF-α, and TGF-β) up-regulate in an additive or synergistic pattern the expression of pIgR on established EC lines, usually of intestinal or endometrial origin (9). Similarly, ECs from the female genital tract and mammary gland express pIgR as a consequence of stimulation with hormones, particularly with estrogens, prolactin, and androgens.

Other IgA Receptors

The Fc region of IgA can interact with other receptors expressed on structurally and functionally diverse cell populations including monocytes/MΦ/mesangial cells, PMNs, granulocytes, ECs, hepatocytes, B and T cells, and PCs (96). Some of these receptors have been structurally defined and specific reagents are now available for their detection.

The best-characterized receptor expressed on monocytes, neutrophils, and eosinophils recognizes Fcα regions of both IgA1 and IgA2 with a certain degree of preference for pIgA, probably due to the presence of multiple binding sites on pIgA. This Fcα receptor, designated as FcαRI (CD89) and detectable by monoclonal Abs, occurs in several isoforms and is heavily glycosylated (96). Another study indicates that CD89 is present in minute quantities also in the circulation in complexes with high molecular mass IgA (96). Additionally, eosinophils, but not PMNs, express a receptor for SC, suggesting that S-IgA has the potential to stimulate eosinophils through an interaction with SC. Detailed analysis of the molecular properties of such complexes revealed that FcαRI and IgA were covalently linked, but the high molecular mass IgA complexes lacked J chain; it can be speculated that the soluble FcαRI is linked to the binding site occupied in pIgA by J chain.

Binding studies indicate that the sites of interactions include the first extracellular domain of FcαRI and the boundary between the Cα2 and Cα3 domains of IgA heavy chains (96). Cross-linking of FcαRI on cell surfaces triggers phagocytosis, superoxide generation, and release of inflammatory mediators from PMNs, eosinophils, and MΦ. A receptor specific for the Fc regions of IgA and IgM has been described and designated as Fcα/μR (97). However, the biological function of the receptor remains to be elucidated.

It has been previously reported that the transferrin receptor (CD71) is surprisingly effective in binding IgA1 molecules, especially in their monomeric form (98). Because the binding of IgA1 is inhibitable by transferrin, it appears that this novel receptor binds two structurally highly dissimilar ligands—transferrin as well as IgA1. Although the function of this receptor remains to be determined, its expression on intestinal ECs suggests it may be involved in the appearance of mIgA in GI tract secretions.

T Cell Help for IgA Production

Earlier studies revealed that addition of culture supernatants from DC-T cell clusters, T cell clones, or T cell hybridomas to cultures of PP or splenic B cells resulted in enhanced secretion of IgA (99). One factor responsible for this activity was subsequently shown to be IL-5 (Figure 31.8) (66,72). Removal of $sIgA^+$ B cells from PP B cell cultures abrogated IgA synthesis, demonstrating that this cytokine affected postswitched IgA-committed B cells (100). If splenic B cells were used, these cells required stimulation with LPS before increased IgA secretion occurred. Taken together, these results suggest that IL-5 induces $sIgA^+$ B cells that are in cell cycle (blasts) to differentiate into IgA-producing cells. Human IL-5 is thought to act mainly as an eosinophil differentiation factor and thus may have little effect on B cell isotype switching and differentiation. It has been reported, however, that human B cells, when stimulated with the bacterium *Moraxella (Branhamella) catarrhalis*, could be induced by IL-5 to secrete IgA, and also to possibly undergo isotype switching to IgA (99). This effect could not be demonstrated using other more conventional B cell mitogens, a finding that demonstrates the importance of the primary *in vitro* activation signal for B cell switching.

IL-6, when added to PP B cells in the absence of any *in vitro* stimulus, causes a marked increase in IgA secretion with little effect on either IgM or IgG synthesis (46,66). IL-6–induced two- to three-fold more IgA secretion than IL-5 (101). The removal of $sIgA^+$ B cells abolished the effect of IL-6, demonstrating that like IL-5, this cytokine also acted on postswitched B cells. In $IL\text{-}6^{-/-}$ mice, the numbers of IgA^+ B cells in the LP were markedly reduced, and Ab responses following mucosal challenge with OVA or vaccinia virus were greatly diminished (102). It was shown that human appendix $sIgA^+$ B cells express IL-6 receptors, while other B cell subsets do not. Further, appendix B cells were induced by IL-6 to secrete both IgA1 and IgA2 in the

absence of any *in vitro* activation (103). This effect was also shown in IgA-committed B cells, again demonstrating the importance of IL-6 for inducing the terminal differentiation of $sIgA^+$ B cells into IgA-producing PCs (Figure 31.8).

An additional Th2 cytokine, IL-10, has also been shown to play an important role in the induction of IgA synthesis in humans (46,66). Stimulation of human B cells with anti-CD40 and *Staphylococcus aureus* Cowan (SAC) resulted in B cell differentiation for IgM and IgG synthesis in patients with IgA deficiency. Further, naïve $sIgD^+$ B cells could be induced to produce IgA after co-culture with IL-10 in the presence of TGF-β and anti-CD40 (74). Taken together, these findings demonstrate that Th2 cytokines such as IL-5, IL-6, and IL-10 all play major roles in the induction of IgA responses by the generation of IgA-producing cells (Figure 31.8).

Since IL-2 has been shown to enhance IgA synthesis in LPS-stimulated B cell cultures, it would be too simplistic to conclude that Th2-type cells and their derived cytokines are the only elements important in the generation of IgA responses. IL-2 also synergistically augmented IgA synthesis in B cell cultures in the presence of LPS and TGF-β (46,66). Although IFN-γ is not directly involved in the enhancement of IgA B cell responses, this cytokine has been shown to enhance the expression of pIgR, an essential molecule for the transport of S-IgA (9). In summary, an optimal relationship between Th1- and Th2-derived cytokines is essential for the induction, regulation and maintenance of appropriate IgA responses in mucosa-associated tissues.

Further, a helper function of IELs has been proposed for support of IgA synthesis (104). Thus, IELs may be actively involved in the induction and regulation of S-IgA Ab responses at mucosal surfaces. It was shown that the numbers of IgA-producing cells in mucosa-associated tissues, such as the intestinal LP of $TCR\gamma\delta^{-/-}$ mice, was significantly lower than that observed in control ($TCR\gamma\delta^{+/+}$) mice of the same genetic background (104). In contrast, identical numbers of IgM- and IgG-producing cells were found in systemic compartments of $TCR\gamma\delta^{-/-}$ and $TCR\gamma\delta^{+/+}$ mice. Further, when $TCR\gamma\delta^{-/-}$ mice were orally immunized with tetanus toxoid (TT) plus cholera toxin (CT) as mucosal adjuvant, significantly lower IgA anti-TT Ab responses were induced in PPs and LP when compared with identically treated $TCR\gamma\delta^{+/+}$ mice. These findings indicate that $\gamma\delta$ T cells are involved in the induction and regulation of Ag-specific IgA Ab responses in both mucosal and systemic compartments.

ALTERNATIVE INDUCTION PATHWAY FOR MUCOSAL IMMUNITY

Although the CMIS-mediated pathway is a major mechanism for the induction of Ag-specific mucosal immune responses, an alternative way (or CMIS-independent pathway) exists for the induction of appropriate productive immunity at mucosa-associated tissues. This is supported by the fact that Ag-specific immune responses have been induced in PP- and/or ILF-null mice following oral immunization (41,42). Indeed, a number of those pathways have been identified, especially in the GI tract at the levels of Ag sampling and S-IgA Ab production.

Other Ag Sampling Systems in the Intestinal Epithelium

In the CMIS-independent acquired immune system, the epithelium is also likely to play an important role as alternative Ag-sampling routes. At least three different scenarios have been offered regarding the alternative Ag-sampling routes in the epithelium. First, M cells were identified on the intestinal villous epithelium (villous M cells), not in proximity to PPs (Figure 31.4) (105). Villous M cells developed in various PP/ILF-null mice and are capable of taking up bacteria, such as *Salmonella*, *Yersinia*, and invasin-expressing *Escherichia coli* (105). A recent study has suggested that villous M cells locate closer to the upper half of the villus and are preferentially observed in the terminal ileum when compared with other parts of the small intestine, suggesting that the microflora influences villous M cell development (106). In addition, villous M cells are present in mice lacking both B and T cells, indicating that villous M cell differentiation and maintenance does not require lymphocytes (106).

A second route for Ag uptake is the EC itself. As mentioned earlier, ECs are involved in innate immunity by expressing nonclassical MHC molecules that can be sensed by $\gamma\delta$ IEL and NK cells (25,27,37). In addition, there is evidence to suggest that ECs could process and then present Ags to T cells via MHC class I as well as class II molecules in humans and rodents (37). The presentation by MHC class II exhibits polarity with uptake of Ag primarily apical and presentation basal under normal noninflammatory conditions and in inflammatory conditions when MHC class II expression is enhanced through the action of the MHC class II transactivator, CIITA (37). Under normal circumstances, ECs do not express classical costimulatory molecules such as CD80 and CD86 but may do so, at least in the case of CD86, in the context of intestinal inflammation (37). However, intestinal ECs express a number of potential costimulatory molecules, which, in certain circumstances, are functional. For example, LFA-3 or CD58 is constitutively expressed on ECs *in vivo* and *in vitro*, is upregulated in response to inflammation, and may provide crucial costimulatory signals to mucosal T cells through its ligand, CD2, which is constitutively expressed on mucosal T cells (107).

Recent studies identified various DC populations in the unorganized intestine (Figure 31.4). Among them are DCs located between intestinal ECs. These intraepithelial

DCs are frequent in the terminal ileum and express TJ-associated proteins (e.g., occuludin, claudin 1, and *zona occuludens* 1), and thus are capable of extending their dendritic arms into the lumen via the TJ between ECs (108). A previous study had already demonstrated that CD18-expressing phagocytes were involved in an M cell–independent pathway for bacterial invasion (109). A recent study has revealed that these intraepithelial DCs are characterized by the expression of CX_3CR1 and that the interaction between CX_3CR1 and its ligand fractalkine/CX_3CL1 is required for extension of transepithelial dendrites into the epithelium (110). Once intraepithelial DCs take up luminal Ags, it is likely that they leave the epithelium and migrate into the LP or draining LNs for the presentation of Ag to T cells.

A recent study has demonstrated that Ag/IgG complexes from the intestinal lumen can be taken up into the LP across intestinal ECs through FcRn *in vivo* (10,37). These transported Ag/IgG complexes may be captured by LP DCs because they express FcRn (10). In addition to sampling a wide variety of foreign Ags, the mucosal immune system must contend with the high number of apoptotic ECs that result from the frequency with which the epithelium is replaced. Although most of these apoptotic ECs are shed by the epithelium to the lumen, some of these apoptotic ECs have been shown to be potentially immunogenic and transportable to T cell areas of MLNs by mucosal DCs (111).

Contribution of B1 Cells for Mucosal IgA Responses

In addition to conventional B cells (or B2 cells) located in MALTs (e.g., PPs), peritoneal B1 cells have been considered to be a precursor of intestinal IgA PCs (112113). B1- and B2 cells can be distinguished by their cell surface molecules (*e.g.*, B220, IgM, IgD, CD5, and Mac-1), origins, and growth properties (112). Further, B1 cells exhibit different V_H repertoires and Ig specificities, and they are thought to be specialized in responding to T cell–independent Ag conserved on common pathogens like DNA and phosphatidylcholine, while B2 cells recognize most T-dependent protein Ags (112). Consistent with this notion, IgA production from B1 cells was noted in MHC class II–deficient mice as well as TCR β and δ chain–deficient mice (114115). Of note, about 65% of fecal bacteria were reactive with B1 cell–derived IgA, and 30% of bacteria were bound with B2-derived IgA, indicating that S-IgA derived from B1 cells recognized a large population of commensal bacteria as well as pathogenic bacteria (112).

AU: Is this supposed to indicate "B one negative"? If not, cut the hyphen.

AU: "B one negative"? If no, cut the hyphen.

B1- and B2 cell responses have distinct IgA-associated cytokine requirements. It was shown that IL-5, a well-known IgA-enhancing cytokine, and IL-15 are also involved in the proliferation and differentiation of B1 but not of B2 cells into IgA-producing cells (113). Thus, mucosal EC-derived IL-15 promoted the proliferation and differentiation of B1 cells into IgA-producing cells. Based upon these findings, it appears that intestinal B1 cells migrate presumably from the peritoneal cavity into mucosal effector sites, where they further differentiate into IgA-producing PCs under the influence of IL-5 and IL-15 for IgA production against T-independent Ags and commensal microflora-associated Ags.

Although precisely where CSR might occur for B1 cells is an unresolved issue, several lines of evidence have demonstrated B1 cell migration into intestinal LP. Using *aly/aly* mice that carried a point mutation in the NIK, there was a complete absence of B cell populations in the intestinal LP, but elevated B cell levels were seen in the peritoneal cavity (54). In this context, another report proposed that the migratory pathway of B1 cells to the peritoneal cavity depended upon the BCL/CXCL13 produced by peritoneal MΦ (85). These results imply that the NIK-mediated pathway is involved in the B1 cell mucosal migration, which may be dependent on specific but not yet identified chemokine receptor(s). Additionally, a recent study showed that peritoneal B cells express comparable levels of the receptor for S1P, a lipid mediator, and that S1P plays an important role in the regulation of peritoneal B cell trafficking into the intestine (116). It was also previously reported that B1 cells existed in nasal passages (113), but the actual molecular machinery for B1 cell migration into nasal passages remains an open question. These findings suggested the presence of a unique migration mechanism for the continuous supply of B1 cells as a part of a CMIS-independent mucosal immunity.

MICROBIAL MUCOSAL IMMUNE SYSTEM

Protection

Mucosal Igs

Large amounts of Ig are delivered onto mucosal surfaces as a result of receptor-mediated transepithelial transport and passive transudation of plasma-derived Igs. Depending upon the species as well as type of external secretion, IgA, IgM, and IgG are present in variable proportions (Table 31.1). Igs of all of these isotypes provide, by different mechanisms, protection against pathogenic microorganisms, interact with commensal microbiota, and interfere with the absorption of undigested food Ags from the large surface area of the digestive tract.

The dominant presence of S-IgA has several important functional advantages that render Abs of this isotype and molecular form particularly suitable for functioning in the mucosal environment (117). Dimeric and tetrameric S-IgA and pentameric S-IgM display 4 to 10 Ag binding sites. Although of lower affinity than, for example, IgG Abs of the same specificity, this multivalency of pIg enhances their effectiveness over mIg by at least an order of magnitude. The

presence of such low-affinity IgA Abs that are also "polyreactive" and thus are capable of binding to a variety of bacterial Ags, and autoAgs have been shown in human external secretions. Further, the intrinsic resistance of pIgA to proteolysis, enhanced by association with SC, is of functional advantage in secretions, particularly those of the GI tract rich in proteolytic enzymes. Finally, due to the inability to activate complement (C) and thus generate C3 and C5 fragments, IgA displays strong anti-inflammatory properties. This fact is of special importance in the GI tract in which the external milieu rich in microbial and food Ags, and the internal milieu are separated by only a single layer of ECs.

As demonstrated in a number of studies, mucosal Ig inhibits the absorption of soluble and particulate Ags from mucosal surfaces by forming large immune complexes. Further, endogenous commensal microorganisms are coated *in vivo* with corresponding Abs that, in turn, prevent their adherence to epithelial receptors. In this respect, mucosal Abs and especially IgA may function by two independent mechanisms. Specific Abs interact with corresponding Ags through the Ag binding site. In addition, glycans that are abundant on the Fc region of IgA can aggregate bacteria based upon the interaction of bacterial glycan-binding lectins, with glycan side chains present on IgA molecules (117). Consequently, such IgA-coated bacteria are prevented from adhering to ECs expressing analogous mannose-rich glycans on their luminal surfaces without the need for Ag-specific Abs.

Biologically active Ags such as viruses, enzymes, and toxins can be effectively neutralized by mucosal Abs (117). The neutralization activity that is operational in a fluid phase may also extend to the intracellular compartment. In addition, it was demonstrated that virus-specific pIgA also exhibit their neutralization activity intracellularly (117). Apparently, the transcytosis route of pIgA intercepts the pathways involved in virus assembly, resulting in intracellular neutralization. Further, elimination of immune complexes composed of noninfectious Ags, absorbed by ECs and corresponding internalized pIgA Abs, has been demonstrated *in vitro*. Small circulating immune complexes containing soluble Ags and pIgA can be eliminated from the circulation into the bile by binding to hepatocytes that in some species (e.g., rats, mice, and rabbits) express pIgR (117). It appears that this mechanism of disposal of immune complexes is primarily restricted to species whose plasma IgA is dominated by pIgA, which in humans represent normally only a minor component (117). However, it is possible that immune complexes containing locally produced pIgA and absorbed Ags that may be formed within mucosal tissues are eliminated by this mechanism.

The noninflammatory nature of IgA is probably of considerable importance for the maintenance of the structural and functional integrity of mucosal tissues (117). The concept that IgA Abs are anti-inflammatory is exemplified by studies in which intact, native, and fully glycosylated human IgA Abs failed to activate C when complexed with Ags; actually in competition experiments, IgA effectively inhibited C activation by IgM and IgG Abs (117). Close examination of the frequently cited ability to activate C reveals that this may be largely due to artificial aggregation and conformational alterations caused by purification procedures and binding to hydrophobic surfaces in C activation assays and aberrancies in glycosylation frequently seen in IgA proteins. Indeed, specific IgA Abs with modified glycan moieties have been shown to activate the alternative and perhaps the lectin pathways of C activation.

Although phagocytic cells including monocytes/MΦ, PMNs, and eosinophils express receptors for the Fc region of IgA (96), the ability of IgA alone to effectively promote phagocytosis of bacteria remains controversial and depends upon the experimental system used in such studies. However, the binding of IgA and IgA-containing immune complexes to such receptors may provide transducing signals for cell activation, proliferation, and oxidative metabolism and prompt degranulation of eosinophils with local inflammatory consequences and extensive tissue damage (96).

The function of mucosal S-IgA also depends on the subclass distribution of specific Abs (46,91,117). Naturally occurring and immunization-induced Abs to protein and glycoprotein Ags are predominantly present in the IgA1 subclass, while Abs to polysaccharide Ags, LPS, and lipoteichoic acid are mainly of the IgA2 subclass. Because of its unique hinge region, IgA1 is susceptible to the cleavage by bacterial IgA1 proteases that are considered as one of the virulence factors produced by *S. pneumoniae, H. influenzae, N. gonorrhoeae, N. meningitides*, and other microorganisms (93). It was also shown that bacteria coated with Fabα are refractory to IgM- and IgG-mediated and C-dependent killing action due to blocking. The antibacterial activity of IgA may be further potentiated by cooperation with innate factors of immunity including the peroxidase system, mucin, lactoferrin, and lysozyme (117).

Although S-IgA is the dominant isotype in most external secretions, the protective effects of Abs of IgM and IgG isotypes are evident from many studies. In external secretions of some IgA-deficient individuals, S-IgM and IgG may functionally compensate for the absence of S-IgA (118). Further, systemic immunization, particularly with conjugated polysaccharide-protein vaccines induces, vigorous and long-lasting IgG immune responses that protect children from infections with upper respiratory tract pathogens causing otitis media and meningitis (*H. influenzae, N. meningitides*) (119). In animal species (e.g., horses, cows, and pigs) in which prenatal transplacental active transport of IgG is not operational, consumption of milk rich in IgG is of life-saving importance. Abs of this isotype are absorbed during the first 7 to 14 days of life from the gut into the circulation presumably by the action of FcRn (10).

Mucosal CTLs

In the mucosal setting, natural infection of the epithelium by enteric (rotavirus or reovirus) or by respiratory viral pathogens (influenza or RSV) leads to endogenous viral peptide processing that induces pCTLs to become effector (activated) and memory CTLs. Most virus-specific CTLs are $CD8^+$ $TCR\alpha\beta^+$, and recognition of viral peptides is associated with MHC class I presentation by infected cells. In this regard, high numbers of $CD8^+$ T cells reside in the mucosal epithelium as a subpopulation of IELs (25). These $CD8^+$ IELs are thought to represent an important cytotoxic effector population that can eliminate virus-infected ECs. When freshly isolated IELs were examined using a redirected cytotoxicity assay, these T lymphocytes were found to constitutively possess lytic activity (28).

Significant progress is being made in areas related to the roles of APCs for induction of pCTLs and for mechanisms of perforin-mediated or Fas-Fas ligand-associated killing of target cells (28). It should be noted that the same processes occur during host responses to intracellular bacteria, to tumor-associated antigens, and in certain mucosal parasite infections. Although this focus is on $CD8^+$ CTLs, cell- and Ab-mediated cytotoxicity and NK cell activity are major responses associated with IELs (28).

An obvious question is how a CTL immune response is initiated given that mucosal inductive sites, which harbor 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, the sigma protein of the reovirus enters the M cell in both NALT and GALT (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 (59). Further, it now established that administration of virus into the GI tract results in the induction of increased pCTL frequencies in PPs (28). These findings suggest that, after enteric infection or immunization, Ag-stimulated CTLs are disseminated from PPs into MLNs via the lymphatic drainage. Further, virus-specific CTLs were also found among LP lymphocytes, IELs, and spleen cells of mice mucosally immunized with reovirus or rotavirus (28). Although mucosal effector tissues such as intestinal epithelium contain high numbers of $\gamma\delta$ T cells in addition to $\alpha\beta$ T cells, virus-specific CTLs in IELs were associated with the latter T cell subset (25,28). These studies suggest that oral immunization with live virus can induce Ag-specific CTLs in both mucosal inductive and effector tissues and in systemic lymphoid tissues.

Detailed studies of immune responses after nasal infection with influenza virus have also revealed that both humoral and cellular pathways are involved in virus clearance (120). However, it was shown that using mice lacking $CD8^+$ T cells (β2-microglobulin knockout mice) or treating with anti-CD8 mAbs did not alter clearance of influenza (121). These results support the presence of multiple pathways for immunity and suggest that $CD4^+$ Th cell pathways are important for mucosal Ab responses and $CD8^+$ CTLs for respiratory tract immunity. Several studies have also established that effector CTLs protect mice from RSV infection. The murine RSV model was used to determine the relative importance of $CD4^+$ T cells, including Th1 and Th2 subsets, which resulted in inflammation versus immunity. These studies clearly suggest that $CD4^+$ IFN-γ-producing Th1 cells as well as $CD8^+$ T cells are associated with recovery, while $CD4^+$ Th2-type pathways are not (122). Interestingly, priming with inactivated RSV or F glycoprotein induced $CD4^+$ Th2 cells while live RSV elicited the Th1-type pathway. When one considers mucosal vaccine development for virus infections, these findings suggest that the outcome of Th1- (including induction of CTLs) and Th2-type immune responses could be regulated by the nature and form of viral Ag used for immunization.

CTLs also play an important role in the inhibition of HIV infection. It was shown that CTLs recognized Ags derived from gp120, p27, nef, gag, tat, and pol proteins (123,124). Thus, infection of rhesus macaques with simian immunodeficient virus (SIV) resulted in the induction of CTL responses in the vaginal mucosa, which played a crucial role in the control of viral replication in the acute phase of viral infection. CTLs killed HIV-infected cells in a granzyme, perforin, or FasL-dependent manner. It is interesting to note that chemokines (e.g., CCL3, 4, and 5, and CXCL12) were produced by CTLs, which prevented HIV infection by blocking CCR5 and CXCR4, which are specific receptors for HIV infection.

Symbiotic Interactions with the Mucosal Microbiota

Mucosal surfaces of the oral cavity; the GI, genital, and respiratory tracts; and conjunctiva are populated by a large number of bacteria of more than 200 species with a characteristic distribution (Figure 31.13) (125). The mucosal microbiota comprises some 10^{14} bacteria present mostly in the large intestine. Considering the relative numbers of host's eukaryotic cells and prokaryotic bacteria, it is estimated that the mucosal microbiota outnumbers mammalian host cells by a factor of at least 10. Mutually beneficial coexistence of the mucosal microbiota with the effective mucosal immune system is one of the most interesting problems in mucosal immunology. Although the innate and specific immune factors present in the mucosal secretions and tissues may limit the adherence of bacteria to mucosal ECs and prevent penetration of such bacteria into the mucosal tissues with subsequent systemic dissemination, the mucosal microbiota continues to survive with remarkable tenacity in the presence of an immune response

Fig. 31.13

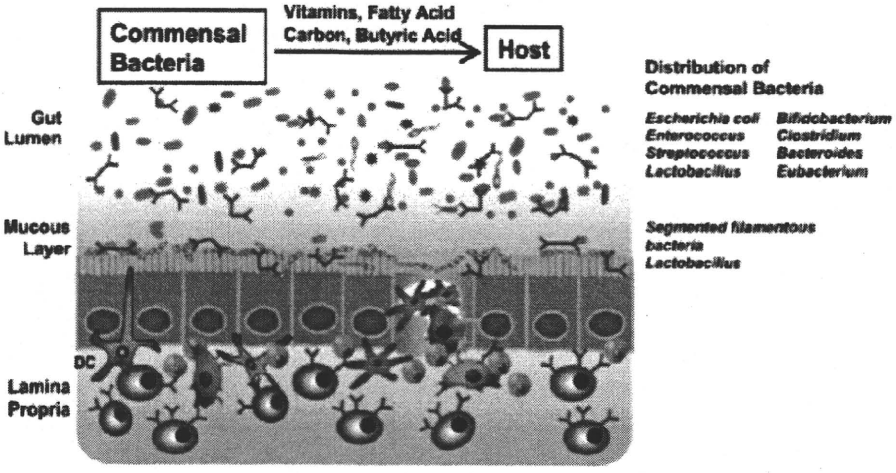

FIGURE 31.13 Symbiotic interactions between the mucosal immune system and commensal microbiota. Most microorganisms are present in the lumen and some preferably reside in the mucous layer. The host mucosal immune system regulates the diversity and quantity of intestinal microflora by secreting S-IgA Abs into the lumen. These Abs have been suggested to originate from B1 cells. These Abs may limit the adherence of commensal bacteria to ECs for an appropriate cohabitation environment and thus their ability to continuously produce beneficial bioactive molecules such as vitamins, fatty acids, carbon, and butyric acid for the host.

manifested by corresponding Abs (47). As a matter of fact, oral, intestinal, and probably other mucosal bacteria are coated *in vivo* with Abs, particularly of the IgA isotype, that may prevent their adherence to the epithelial receptors but do not significantly interfere with their elimination and metabolism (112). Therefore, products generated as a result of fermentation by mucosal bacteria such as butyric acid, fatty acids and vitamins can be important sources of energy and carbon for the human host, thus further stressing the immunological and physiological interdependence of the host on the mucosal microbiota (Figure 31.13) (125).

Quantitative and Qualitative Aspects of the Mucosal Microbiota

Quantitative data concerning the distribution of the indigenous microbiota on mucosal surfaces of the oral cavity, conjunctiva, and genital, GI, and respiratory tracts indicate that of approximately 10^{14} of bacteria, 99.9% are present in the large intestine (125). In this locale, bacteria are found free in the lumen and in feces, bound to the desquamated ECs, entrapped in the mucus layer, and deep in intestinal crypts (Figure 31.13). Despite the inherent difficulties with representative sampling, culture conditions, identification of cultured bacteria, as well as obvious host variables (e.g., hormonal status, diet, use of antibiotics, etc.), hundreds of species in 40 to 50 bacterial genera have been identified and described. These studies revealed that gram-negative and gram-positive, spore- and non–spore-forming, and aerobic as well as strictly anaerobic bacteria are present and are characteristically distributed in specific mucosal compartments. Although it is beyond the scope of this chapter to provide detailed information concerning the specific species distribution of indigenous microbiota in individual mucosal compartments, a brief summary of the colonic microbiota may illustrate the most important points. The intestinal microbiota is acquired shortly after birth and its composition is greatly influenced by the route of delivery (vaginal versus Cesarean section), the environment, and most profoundly by the diet (breastfeeding versus bottled formula and addition of solid food). Colonic microbiota changes from the dominant bifidobacteria at the initial stage to other species, particularly *Bacteroides* and anaerobic cocci with a significant presence of coliforms, streptococci and clostridia. Quantitative representation of bacteria in feces from adults indicates the dominance of bacteria of the genera *Bacteroides, Clostridium, Eubacterium, Lactobacillus, Streptococcus,* and *Bifidobacterium*;

E. coli constitutes only a minor contribution (~1%) of the colonic microbiota.

Regulation of Mucosal Immune Development and Immunological Homeostasis by Commensal Microbiota

The presence of the mucosal microbiota has a profound influence on the evolution and functionality of the immune system (47,126,127). As evidenced by a large number of studies performed on gnotobiotic (germ-free) animals, the development of both mucosal and systemic lymphoid tissues and the hosts' ensuing ability to respond to environmental Ags is, to a large extent, dependent on the previous exposure to a mucosal microbiota. Specifically, when compared to conventionally reared animals, lymphoid tissues of germ-free animals are hypotrophic, lack well-developed GCs, display minute numbers of mucosal PCs, and respond poorly to mitogens and polyclonal stimulants. Upon colonization with even a few representative species of mucosal microbiota, a prompt development of lymphoid tissues and restoration of responsiveness to a plethora of Ags and other stimuli ensues. Importantly, the development and responsiveness of both humoral and cell-mediated compartments of the immune system are profoundly affected by the mucosal microbiota as documented by the presence and numbers of B cells and ultimately Ab-forming cells (AFC) in mucosal and nonmucosal tissues, levels of mucosal and plasma Abs, and T cells of various phenotypes in the IEL and LP compartments of mucosal tissues, as well as in the systemic secondary lymphoid tissues (47,126,127).

The presence of the mucosal microbiota in the intestinal lumen induces S-IgA Ab synthesis. It was shown that induction of polyreactive S-IgA Ab responses to commensal bacteria is mainly derived from T cell–independent B1 cells (112). The T cell–independent IgA Abs originating from B1 cells possessed reactivity to conserved bacterial products (e.g., phosphorylcholine), which resulted in the nondiscriminating blockade of commensal bacterial attachment to mucosal surfaces. In this B1 cell Ab production pathway, IL-15 may be involved in IgA production facilitated by the intestinal microbiota, since it was previously reported that B1 cells proliferated in the LP when reacted with IL-15, an event induced by TLR-mediated signaling (113).

Another study revealed a unique pathway for specific Ab production against commensal microbiota in the intestine. It was shown that intestinal MΦ rapidly kill commensal bacteria, while intestinal DCs retain small numbers of live commensal bacteria and migrate only into MLNs but do not penetrate beyond MLNs (128). This function ensures a commensal bacteria-specific IgA Ab response that is specifically produced at the gut mucosa, but not in systemic immune compartments.

Mucosal microbiota-mediated innate immunity (e.g., TLR-mediated signaling) also plays an important role in the maintenance of mucosal homeostasis (47,126,127). For instance, the TLR-mediated cross-talk between the mucosal microbiota and IECs is biologically significant in the maintenance of epithelial homeostasis. EC cycles such as renewal, differentiation, and mitosis are significantly changed in germ-free or MyD88-deficient mice (22). In this regard, it was shown that TLR-mediated signals from the intestinal microbiota regulated the production of tissue protective factors such as IL-6, KC-1, and heat shock proteins (22). In addition to ECs, it was shown that TLRs were selectively expressed on $CD25^+$ $CD4^+$ Treg cells, which have been considered to be involved in the induction of oral tolerance, as discussed laer in this chapter (129). Thus, bacterial products such as LPS directly enhance their survival and proliferation. These products and host responses may explain why C3H/HeJ mice lacking TLR4-mediated signaling and germ-free mice show less sensitivity to oral tolerance induction (130).

Additional examples of molecules in the microbiota-dependent intestinal homeostasis are bacterial DNA. Bacterial DNA contains unmethylated CpG motifs within consensus sequences and is the ligand for TLR9 (11). Several lines of evidence have demonstrated that CpG targets DCs to induce the inhibitory environment (131), and it has been considered that some types of DCs are involved in these inhibitory immune responses (51,52); it appears that the intestinal microbiota stimulates intestinal DCs through the interactions between CpG and TLR9 for the maintenance of intestinal homeostasis. These inhibitory effects of bacterial DNA have led to the development of probiotics-mediated anti-inflammatory therapy, as discussed later in this chapter (131).

MUCOSAL TOLERANCE

Basic Concepts

In addition to the protective function of Ag-specific S-IgA and serum IgG Ab responses after mucosal immunization, the mucosal route of Ag delivery can also induce systemic unresponsiveness (132). Oral administration of a single high dose or repeated oral delivery of low doses of proteins has been shown to induce systemic unresponsiveness. Additional studies have shown that the nasal administration of proteins also induces systemic unresponsiveness and has led to the more general term mucosal tolerance to include nasal or oral Ag induction of unresponsiveness (132). The inhibition of Ag-specific immune responses in systemic compartments by mucosal Ag delivery is important for the prevention of overstimulation of responses and frequently encountered and hypersensitivity responses to food proteins and allergens. Further, this system could potentially be applied to the prevention and possibly treatment of autoimmune diseases by feeding relevant Ags.

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Role of PPs in Oral Tolerance

The precise site of Ag uptake in the GI tract during oral tolerance induction has not been firmly established. One possible route is that Ags may enter the GALT via M cells and lead to APC–T cell interactions that down-regulate T and B cell responses. Some investigators have suggested that organized lymphoid tissue in the GI tract was not required for oral tolerance to OVA, since B cell–defective mice, which contain poorly developed PPs, were fully tolerized at the level of T cells (133). The availability of mice without PPs has allowed reinvestigation of the notion that GALT may be involved in oral tolerance. In one study, it was shown that mice that lack GALT but retain MLNs could be orally tolerized to OVA (134). However, others found that mice that lack PPs but retain MLNs were resistant to oral tolerance to protein (135); however, these mice showed normal mucosal S-IgA Ab responses to oral protein given with CT as adjuvant (41,42). Although one cannot yet conclude whether GALT is a strict requirement for oral tolerance to proteins, it is plausible to suggest that the nature of the Ag itself may influence the site of entry into the host.

AU: First reference in chapter, or was this meant to be CTL?

$CD4^+$ T Cells in Oral Tolerance

The $\alpha\beta$ T cells appear to be the major players in down-regulation of systemic immune responses to orally administered Ags. It is generally agreed that the status of oral tolerance can be explained by: 1) clonal anergy or deletion of T cells, or 2) by active suppression by regulatory-type T cells through the secretion of inhibitory cytokines (132). Low doses of oral Ag tend to favor the latter form of inhibition, while higher doses of feeding induce clonal anergy of immunocompetent T cells. These two forms of oral tolerance are not mutually exclusive and may occur simultaneously following oral administration of Ags.

Anergy is defined as a state of T cell unresponsiveness characterized by the lack of proliferation and IL-2 synthesis and diminished IL-2R expression, a condition reversed by pre-culturing T cells with IL-2 (132). It was shown that Th1-type cells appear to be more sensitive to the induction of tolerance *in vitro* than Th2-type cells; *in vivo* evidence has demonstrated that Th1 cells are likely to be anergized in oral tolerance. This may be an oversimplification since it has been shown that oral tolerance can be induced in mice defective in Th1 ($STAT4^{-/-}$) or Th2 ($STAT6^{-/-}$) cells (136). Further, to identify which lymphocyte compartment (e.g., $CD4^+$ versus $CD8^+$ T cells) preferentially mediates the induction of oral tolerance, cell transfer experiments were performed using SCID and *nu/nu* mice, demonstrating that oral tolerance was induced by anergized $CD4^+$ but not $CD8^+$ T cells (137).

Clonal deletion has been considered as another pathway for the induction of oral tolerance. Clonal deletion of Ag-specific $CD4^+$ T cells was detected after oral feeding of high doses of Ag (132). Accumulating evidence has revealed that the clonal deletion induced by feeding high doses of Ag was due to an increase in the susceptibility of lymphocyte apoptosis via increased expression of caspase.

Mucosal Regulatory T Cell Networks

Regulatory- and suppressor-type T cells are crucial players in the induction of peripheral tolerance to self and foreign Ags. It is now accepted that several populations of T cells expressing CD4 or CD8 show regulatory or suppressive functions to other T cell-mediated mucosal immune responses. They can be classified as: 1) naïve, or those which have not yet encountered Ag; 2) activated (effector); and 3) memory, where both effector and memory T cells have engaged in the regulation of immune responses (48,132). $CD4^+$ T cells including $CD4^+CD25^+Foxp3^+$ Treg cells, Th3 cells (secreting TGF-β), and Tr1 cells (secreting IL-10) and $CD8^+$ T cells were shown to be key players for the creation of a mucosal regulatory T cell network in the establishment of quiescent immunity (or mucosally induced tolerance) (Figures 31.7 and 31.14).

Fig 31.14

$CD25^+$ $CD4^+$ Treg Cells

Recent evidence has revealed that naturally arising $CD25^+$ $CD4^+$ Treg cells play a pivotal role in the negative control of a variety of physiological and pathological immune responses (138). Naturally arising Treg cells specifically express a forkhead winged-helix transcription factor family member (Foxp3) for the process of Treg cell lineage commitment. Thus, Foxp3 deficiency results in early onset, fatal, systemic autoimmune disease. The $Foxp3^+$ $CD25^+$ $CD4^+$ Treg cells are present in the PPs, suggesting that $Foxp3^+$ $CD25^+$ $CD4^+$ Treg cells are involved in the maintenance of intestinal homeostasis.

In addition to inhibitory cytokines, such as IL-10 and TGF-β, several inhibitory molecules have been shown to be expressed on $CD25^+$ $CD4^+$ Treg cells (138). Although naïve T cells express CTLA-4 (or CD152) after activation, which competes with the cellular interaction between CD28 on effector T cells and CD80/CD86 on APCs, $CD25^+$ $CD4^+$ Treg cells constitutively express CTLA-4 (Figures 31.7 and 31.14). The CTLA-4 on Treg cells plays a pivotal role in the maintenance of mucosal homeostasis (139). Thus, anti-CTLA-4 mAb treatment inhibited the regulatory function of $CD25^+$ $CD4^+$ Treg cells and subsequently led to the development of IBD. Several mechanisms have been proposed for the inhibitory function of CTLA-4 on Treg cells, including the enhancement of Treg cell activity through the interaction with CD80/CD86 on APCs (138). Since ECs also express CD80 as mentioned earlier (37), it is possible that Treg cells recognize CD80 on the ECs exhibit their immunosuppressive function. Another possible mechanism is the induction of the inhibitory molecule, IDO (140). As

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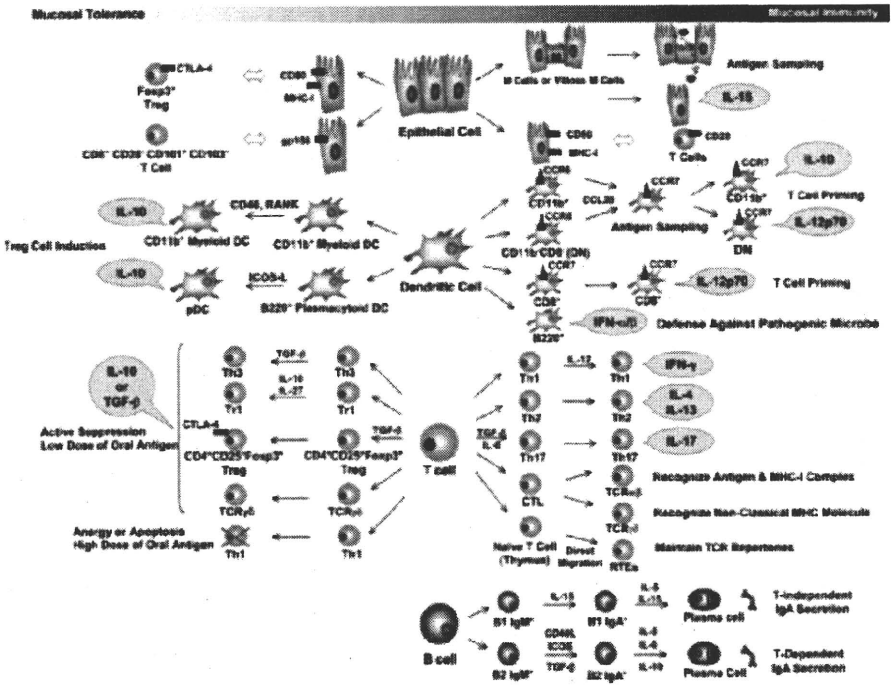

FIGURE 31.14 Cellular and molecular mechanisms for the induction and regulation of mucosal immunity and tolerance. A unique aspect of the mucosal immune system is the simultaneous presence of active (e.g., effector T cells and S-IgA Abs) and quiescent (e.g., Treg cells and mucosal tolerance) immunity. To smoothly operate two opposite types of immunity, ECs, DCs, and T cells play central roles by providing appropriate inflammatory and anti-inflammatory cytokine networks together with costimulatory and chemokine molecular families.

mentioned earlier, IDO exhibits immunosuppressive effects by catalyzing the catabolism of tryptophan, an essential amino acid for T cell proliferation (20). In addition to CTLA-4, Treg cells also express high levels of glucocorticoid-induced TNF-like receptor (GITR), another important suppressive function-associated molecule, that contributes to the maintenance of a quiescent condition in mucosal immune compartments (138). Thus, transfer of $GITR^{high}$ Treg cell–depleted spleen cells to athymic nude mice resulted in the development of autoimmune diseases including gastritis (141). GITR-mediated signaling triggers Treg cell proliferation, which has been considered to be one molecular mechanism for GITR-mediated suppressive function of Treg cells in the presence of IL-2, but detailed mechanisms remain unclear.

Although naturally arising $CD25^+$ $CD4^+$ Treg cells develop in the thymus and their survival in the periphery is dependent on IL-2 (138), accumulating evidence has demonstrated that $Foxp3^+$ Treg cells can be induced from $Foxp3^-$ precursors and, like naturally arising $CD25^+$ $CD4^+$ Treg cells, the induced Treg cells express CD25 and CTLA-4, and also produce IL-10 and TGF-β (142). It is interesting to note that cytokines such as TGF-β and IL-10 contribute to the differentiation of Treg cells (142). As mentioned earlier, TGF-β and IL-10 are predominantly produced in the intestinal compartments; thus, the GI tract seems to be an optimal environment for naturally inducing the differentiation of Treg cells for the creation of immunologic homeostasis in the otherwise harsh environment of the gut.