There are more T cells in the gut-associated lymphoid tissue (GALT) and intestinal lamina propria and epithelium than in the rest of the body. That they are absolutely essential for health is demonstrated when they are not present, as in children with severe combined immunodeficiency or adults with untreated human immunodeficiency virus-1 (HIV-1) infection: intestinal infections caused by cryptosporidia, isospora, cytomegalovirus, and other low-grade pathogens result in chronic diarrhea, wasting, and eventually death (Table 7.1). The advent of highly active antiretroviral therapy that helps maintain gut T cell numbers has seen gut infections and the enteropathy of HIV-1 infection diminish in clinical significance. Gut T cells are also at the forefront of protective responses to more aggressive pathogens such as Salmonella and Shigella. Identifying the pathways that allow gut T cells to react appropriately to commensal microbiota, yet respond to gut pathogens, remains one of the major challenges in biology. At the same time, over-reactivity of gut T cells to harmless foods, or the commensal microbiota, underpins food-sensitive enteropathies and inflammatory bowel disease (IBD).

In healthy individuals, T lymphocytes are a natural component of the cell types present at mucosal surfaces. The largest numbers are found in the small intestinal mucosa, primarily in the epithelium, the lamina propria cores of the

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<td>Bacterial pneumonia</td>
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Table 7.1 Mucosal infections in T-cell-deficient patients
villi, Peyer’s patches (PPs), and isolated lymphoid follicles (Figure 7.1). They are somewhat less common in normal colon epithelium and lamina propria, but abundant in isolated colonic lymphoid follicles. There are also many T cells in the appendix. Normal gastric mucosa only contains a few T cells. At other mucosal surfaces, such as the upper airways and the upper respiratory tract, T cells are rather sparse in the connective tissue, but are abundant in nasopharynx-associated lymphoid tissue in rodents, and tonsils and adenoids in humans. It is important to emphasize that the organized mucosa-associated lymphoid tissues (MALT) are the inductive sites for mucosal immune responses, and the connective tissue between MALT is the site of expression of the mucosal immune response (Chapter 1). The majority of T cells in MALT are small non-activated T cells, largely part of the recirculating pool, whereas T cells in the lamina propria are activated cells, the progeny of T cells responding to antigen in the MALT.

The origin and phenotype of mucosal T cells.

In all mammalian species studied so far, CD4 T cells predominate in the lamina propria and CD8 cells in the gut epithelium. Intraepithelial lymphocytes are discussed in detail in Chapter 6; here we will focus on CD4 T cells. Embedded in the connective tissue matrix, lamina propria T cells receive signals from epithelial cells, stromal cells, and the matrix itself via integrin receptors, and are closely associated with dendritic cells and macrophages. These cells and signals likely control the lifespan and function of T cells in the gut, but there is also cross-talk between the T cells and other cell types; and, importantly, the presence of the microbiota, signaling via pathogen-associated molecular patterns and producing short-chain fatty acids in the distal bowel, produces an extremely complex series of interactions, which are only now being unraveled.
7-1  **Mucosal T cells traffic from MALT to mucosal lamina propria.**

It has been known for 40 years that the gut-homing patterns of small lymphocytes and immunoblasts differ. Small resting lymphocytes from mesenteric lymph nodes (MLNs) do not migrate to the lamina propria when transferred into normal recipients. However, in animals, T and B blasts from the mesenteric nodes or dividing cells in thoracic duct lymph readily migrate to the lamina propria. While this is more difficult to show in humans, analysis of T-cell receptor rearrangements in GALT and nearby lamina propria has shown the same clones of cells at each site, strongly supporting the notion that in humans, GALT T cells traffic via blood to lamina propria. The molecular basis for gut homing was initially elucidated 20 years ago when it was shown that gut homing of blasts was conferred by the expression of the $\alpha_4\beta_7$ integrin on T- and B-cell blasts and mucosal addressin cell adhesion molecule-1 (MAdCAM-1) on intestinal vascular endothelium. Later studies showed that chemokines were also involved. The small bowel epithelium secretes CCL25 and gut-homing cells express the CCL25 receptor CCR9. CCL25 is also tethered to vascular endothelium in the gut. In contrast, CCR9 and CCL25 interactions do not seem to be important in T-cell migration to the colon and in fact colonic epithelium does not express CCL25. Instead, colon homing may be controlled by CCL28 made by colon epithelium, $\alpha_4\beta_7$, integrin, and perhaps CCR10. It was, however, only relatively recently that the molecular basis for imprinting of gut homing by Peyer’s patch T cells was elucidated, and it came from an unexpected angle, namely vitamin A.

Vitamin A is stored in the liver, so to make vitamin A deficient mice, pregnant mice are given a vitamin A deficient diet. There is enough vitamin A in the mother to allow the mouse pups to be born. However, maintaining the pups on a vitamin A deficient diet for 3 months has dramatic effects on mucosal T cells, in that these mice have marked depletion of small intestinal CD4 T cells and CD8+ intraepithelial lymphocytes. Subsequent studies showed that in the Peyer’s patches and mesenteric nodes, dendritic cells (DCs) express a retinal dehydrogenase (RALDH), which can convert all-trans-retinol to all-trans-retinoic acid. When added to activated T cells and B cells all-trans-retinoic acid is a potent inducer of both CCR9 and $\alpha_4\beta_7$. Thus the preferential expression of RALDH by antigen-presenting cells (APCs) in PPs and MLNs accounts for gut homing.

Interestingly, vitamin A deficiency had no effect on T cells in the airways, an observation of relevance to a notion which has gone out of fashion, namely the common mucosal immune system. This hypothesis suggested that immunization in the gut could protect other mucosal sites due to activated B cells and perhaps T cells migrating into all mucosal sites after enteric immunization. However, since respiratory tract vascular endothelium does not express MAdCAM, there is no longer a physiological basis for the hypothesis. A possible explanation for the homing of mesenteric lymph node cells to the airways may lie in the fact that the rules that govern homing become disrupted during inflammation. For example, gut-homing cells lodge in inflamed skin, but not normal skin. In the 1970s when the common mucosal immune system was suggested, virtually all mouse and rat colonies were infected with *Mycoplasma pulmonis*, a low-grade pathogen that induces lung inflammation.

7-2  **Lamina propria T cells have the characteristics of activated lymphocytes.**

Lamina propria mononuclear cells can be isolated relatively easily from human and mouse small bowel and colon. Many studies in humans have confirmed that the CD4 cells in normal gut lamina propria have the phenotype of activated effector T cells. Thus they are CD45RO+, CD62low, CD69high, CD25+, Fas+, and FasL+, and as expected from above, $\alpha_4\beta_7^+$ and CCR9+.
It has also been established for many years that MALT are on the major route of lymphocyte recirculation. Small recirculating naive T cells (CD45RA⁺, L-selectinhigh) can enter PPs because glycosylation of the mucin-like domains of the gut-specific addressin MAdCAM-1 allows it to bind L-selectin on naive T cells to allow them to cross high endothelial venules into the tissue. If these small naive T cells become activated by gut antigen in PPs, they lose L-selectin and express CD45RO. The presence of CD45RO⁺ cells co-expressing high levels of the αβ integrin and L-selectinlow near microlymphatics in PPs suggests that these cells are leaving the PPs and are on their way to the lamina propria via the blood. The other site in PPs at which activated T cells are seen is next to M cells. These cells are also CD45RO⁺, L-selectinlow, CD69⁺ and are dividing.

All of these data are consistent with the idea that the gut-associated lymphoid tissues are the inductive sites of mucosal CD4 immune responses and that cells traffic via the thoracic duct and blood toward the lamina propria.

7-3 Cytokine production by mucosal T cells in healthy animals is T\(_{h}1/T_{h}17\) dominated.

Germ-free mice have virtually no T cells in their gut lamina propria and the Peyer’s patches in these mice only have primary follicles. Following colonization with bacteria, there is rapid immune activation and mucosal T cells rapidly reach the same levels as normal mice. Thus we can conclude that mucosal T cells are activated by gut microbial antigens and it would be expected that as lamina propria T cells are activated effector cells, they would be actively secreting cytokines. In humans, enzyme-linked immunospot (ELISPOT) analysis of freshly isolated PP and small intestinal lamina propria T cells shows a response dominated by interferon-γ (IFN-γ), with very few T cells secreting interleukin-4 (IL-4), IL-5, or IL-10. When human PP T cells are activated with mitogens or food antigens, the response is again IFN-γ dominated. These studies, however, pre-dated the discovery that IL-17A and IL-17F, made largely by a specialized helper T cell (T\(_{h}17\)) subset (T\(_{h}17\) cells), seem to be particularly highly expressed at mucosal surfaces. Thus CD4 T cells from healthy gut secrete equivalent amounts of IL-17A and IFN-γ when activated with anti-CD3/CD28. Mucosal T cells are therefore directed toward effector T\(_{h}1\) and T\(_{h}17\) responses; and in mice, in the absence of intestinal helminths, there are virtually no T\(_{h}2\) cells in the small intestine. The ability of CD4 cells in the gut to make IFN-γ is probably very important in the control of gut infections such as cytomegalovirus and cryptosporidia, because, in the latter case, there is compelling evidence that immunity to this parasite depends on the ability of IFN-γ to render gut epithelial cells resistant to infection.

7-4 IL-17 and IL-22 may play an important role in protecting mucosal surfaces.

The strategic positioning of T\(_{h}17\) cells at barrier surfaces reflects their importance in the neutralization of pathogens and commensal microbiota alike, both by coordination of neutrophilic inflammation and by maintenance or restitution of epithelial barrier integrity. Mucosal epithelia express receptors for IL-17 and/or IL-22, T\(_{h}17\) cytokines that promote tight junction formation, mucus production, antimicrobial peptide production, and epithelial regeneration following injury. Elevation of IL-17 and IL-22 is characteristic of intestinal inflammation in murine colitis and IBD. Whereas IL-17 contributes to both neutrophilic inflammation and aspects of epithelial barrier function, IL-22 functions appear more limited to epithelial barrier function in the gut. Accordingly, IL-22-deficient mice have more severe intestinal inflammation and epithelial injury in mouse models of colitis, and are highly susceptible to colonic infection with the attaching-effacing natural mouse pathogen, Citrobacter rodentium.
The origin and phenotype of mucosal T cells

Unlike the genes that encode IL-17A and IL-17F; expression of the gene encoding IL-22 is strictly dependent on the aryl hydrocarbon receptor, a ligand-activated transcription factor that binds a wide array of environmental and endogenous aromatic hydrocarbons, including tryptophan metabolites. Since tryptophan makes up 1–2% of the total protein in many foods, there is the intriguing possibility that dietary proteins may regulate immunity by the AroA pathway. In humans, CD4 T cells that express IL-22 in the absence of IL-17 cytokines have been identified, supporting the possibility that these cells are regulatory in nature and are dedicated to the maintenance and repair of epithelial integrity. However, their role in normal and inflamed gut has not been established.

7-5 Different types of gut microbiota appear to induce different cytokine responses in mucosal T cells.

In animals, where it is possible to experimentally control the gut flora, remarkable progress has been made on the texture of cytokine responses and their regulation by specific members of the microbiota (Figure 7.2). Germ-free mice monocolonized with the human gut bacterium, Bacteroides fragilis, show an expansion of T-cell numbers in the spleen and a shift in T-cell cytokine responses from a predominantly Th2 response, to a Th1 response. Remarkably, this effect is not seen if mice are mono-associated with B. fragilis lacking polysaccharide A, a highly unusual capsular polysaccharide which can be processed by APCs and induce CD4 T-cell activation. Polysaccharide A itself seems to induce IL-10-secreting regulatory T cells (Treg) in the colon which can limit the extent of chemical colitis, both prophylactically and therapeutically.

Further support for the idea that specific types of bacteria can induce markedly different types of T-cell responses comes from the observations that mice from different suppliers have markedly different numbers of Th17 cells in their small intestine, despite having identical numbers of T cells in their gut. It was shown that adult germ-free mice had virtually no Th17 cells in their gut; however, on colonization with a microbial flora, Th17 cells became abundant. If adult normal mice were treated with antibiotics to lower the flora, then Th17 cell numbers were reduced by 50%. When mice from different vendors were examined, most had abundant Th17 cells in their intestine, apart from mice from the Jackson Laboratory, where there were essentially none. Likewise, reconstitution of germ-free mice with the altered Schaedler flora, for many years considered to be the gold-standard specific pathogen free (SPF) flora, also did not result in mucosal Th17 cells. Detailed analysis of the components of the microflora in mice from different vendors showed that the key organism that drives Th17 responses was an unculturable segmented filamentous bacterium (SFB), related to clostridia. Mice harboring SFB are somewhat more resistant to C. rodentium infection than mice lacking SFB.

The final example of commensals which appear to induce regulatory responses are gut clostridial species. Colonization of mice with a flora restricted to clostridia induces somewhat more IL-10-secreting Foxp3+ inducible Treg cells in the colonic lamina propria than in mice colonized with standard SPF flora. Increase in Treg cells in the colon is associated with increased resistance to chemical colitis.

These studies are conceptually important because they indicate that different types of microbes may influence the generation of helper T cell lineages. They are also interesting in a historical context because the components of the Schaedler flora were chosen by Russell Schaedler in the 1960s on the basis of their lack of immunogenicity, and so a whole generation of immunologists who used animals colonized with the Schaedler flora were working on highly unusual and atypical mice. It will be intriguing to identify which bacterial components skew immune responses. At the same time, however, care has to
be taken in extrapolating from a mouse monocolonized with a single bacterial strain to the complex microbiota (1000 species) of humans.

**7-6 The developmental pathways of TH17 cells are not well defined, especially at mucosal surfaces.**

The developmental origins of TH17 cells in mice and humans have stirred considerable controversy and a clear understanding of potential differences between the species is not resolved. Akin to the earlier debate over the presence of TH1 and TH2 subsets in humans after their discovery in mice, it may be that many of the apparent species differences in TH17 biology reflect more the differences in the cell types investigated (blood T cells in humans, spleen T cells in mice), and experimental conditions, than intrinsic species differences, although this remains to be determined. In fact there is no a priori
reason why different species should not adopt different strategies to generate effector T-cell responses appropriate for the lifestyle of the species, and the pathogens they face in their particular environments.

Whereas there is agreement over the requirement for pro-inflammatory cytokines in the differentiation of TH17 cells, whether or not the same cytokines act similarly in both species is contentious. Even more contentious is the role of transforming growth factor-beta (TGF-β). In mice, where TH17 cells were first described, IL-6 is indispensible for the induction of TH17 cells and acts in concert with TGF-β to initiate TH17 cell development from antigen-stimulated naive CD4 T cells. IL-23 acts downstream of IL-6 and TGF-β to reinforce TH17 development in mice, but is not needed for the development of IL-17-producing T cells in mouse intestinal mucosa, despite the requirement for IL-23 in some mouse models of intestinal inflammation. IL-1β has been found to amplify TH17 development in mouse T cells, and along with IL-23, elicits the production of TH17 cytokines from mature TH17 cells without a requirement for T-cell receptor stimulation, providing an antigen-independent mechanism for TH17 cell responses.

In humans, initial studies that examined the generation of the TH17 cells using naive (CD45RA+) CD4 T cells derived from peripheral blood found that IL-1β and IL-23 induced production of TH17 cells without a requirement for IL-6 or TGF-β, contrasting greatly with studies from mice. However, it was subsequently found, using naive CD4 T cells derived from human umbilical cord blood, that TH17 development did require low levels of TGF-β as well as IL-1β and a STAT3-inducing cytokine—IL-6, IL-21, or IL-23—more closely resembling findings from mice. These studies emphasize that differences in the previous activation history of T-cell precursors in human and mouse studies may influence TH17 cell development, although fundamental differences in the development of human and mouse TH17 cells cannot be excluded. Despite these differences, there are major similarities in some aspects of TH17 cells in both species, such as the expression of the transcription factor retinoic acid-related orphan receptor RORγt, IL-23 receptor, and CCR6.

In mice, TH17 cells can be derived from naive precursors by the concerted actions of IL-6, IL-1β, and IL-23 in the absence of TGF-β, in agreement with some human studies. Importantly, the TH17 cells generated under these conditions have a gene expression profile distinct from TH17 cells generated in the presence of TGF-β, raising the possibility of further TH17 subsets that might have different functions. While further studies will be needed, there appears to be different pathways involved in the generation of subtypes of TH17 cells, the precise functions of which are yet to be defined. It is plausible that cells derived via the TGF-β-dependent pathway of TH17 development may play a more important role in maintenance of barrier integrity at mucosal sites, particularly in the intestine where there is abundant active TGF-β made by epithelial cells and stromal cells and the greatest number of TH17 cells are found in health; whereas a TGF-β-independent pathway plays a more prominent role under conditions of pathogen-induced or autoimmune inflammation.

7-7 It is unclear if TH1 and TH17 responses in the gut are due to local differentiation or selective migration.

As for other CD4 T-cell subsets, the priming of TH17 cell development is thought to occur in the GALT, where on exposure to antigen there is rapid downregulation in T cells of L-selectin and expression of CCR6 and CCR9, which favors trafficking to the intestinal lamina propria. As for TH1 and TH2 cells, the transfer of TH17 cells into lymphopenic hosts has established that, at least in the setting of an ‘empty’ T-cell compartment, migration of TH17 cells into the intestinal lamina propria can readily be seen. Whether this occurs similarly in a T cell-replete repertoire is not known. In fact, there is remarkably
little known about the factors which control effector CD4 polarization in the MALT of experimental animals, and essentially no data for humans.

There is no doubt that the gut lamina propria is enriched for effector T\(_{h1}\) cells, T\(_{h17}\) cells, and T cells that make both IFN-\(\gamma\) and IL-17A. The critical question is whether the imprinting of gut homing in GALT following T-cell activation has another layer of complexity, with differences in homing of different CD4 cell lineages. Added to this is the issue of how plastic development in the lamina propria is; in other words, is lineage commitment settled in the lamina propria or is it pre-committed prior to extravasation, perhaps imprinted in GALT. At the heart of this debate is whether there is a defined T\(_{h17}\) cell precursor, separate from virgin CD4 T cells that can become T\(_{h1}\), T\(_{h2}\), or T\(_{reg}\) cells. In humans it has been suggested that CD161 along with IL-23R, CCR6, and the transcription factor retinoic acid-related orphan receptor C isoform 2 are markers of T\(_{h17}\) cells. CCR6 is of particular interest in the gut because it is the only receptor for CCL20. Normal colonic epithelial cells express CCL20, but expression is highest in inflamed epithelium and, constitutively, in the follicle-associated epithelium overlying Peyer’s patches and cryptopatches. CCR6 is also expressed on some lymphoid tissue inducer (LTi) cells and mice lacking CCR6 have small Peyer’s patches. Further experiments are therefore needed to determine if T\(_{h17}\) and T\(_{h1}\) cells differ in their ability to migrate into the gut. It is also unclear if the effects that different types of bacteria have on mucosal T\(_{reg}\), T\(_{h1}\), and T\(_{h17}\) responses are controlled at inductive sites or the lamina propria.

On the other hand, when a T cell extravasates into the lamina propria, the local environment contains molecules known to affect T\(_{h17}\) cell development. Epithelial cells produce active TGF-\(\beta\) and latent TGF-\(\beta\) is abundant in the matrix. Lamina propria DCs also secrete retinoic acid which may inhibit T\(_{h17}\) generation and promote T\(_{reg}\) development. Mice lacking TGF-\(\beta\) have few T\(_{h17}\) cells in their gut and spleen, suggesting that TGF-\(\beta\) has a global effect on T\(_{h17}\) cell generation rather than a local effect in the gut lamina propria. A slightly different mouse which can make TGF-\(\beta\) but which cannot activate the cytokine in the matrix also has a reduced number of lamina propria T\(_{h17}\) cells. Confounders in these experiments, however, are that mice lacking TGF-\(\beta\) have extremely strong T\(_{h1}\) responses that inhibit T\(_{h17}\) cell generation and there is the possibility that even at inductive sites, matrix bound TGF-\(\beta\) may be needed for T\(_{h17}\) cell generation.

7-8  There appears to be considerable flexibility in the T\(_{h17}\) lineage.

With the emergence of better technical approaches to track the fate of defined T-cell populations \textit{in vivo}, it has become apparent that there is substantial flexibility, or plasticity, in the developmental programs of regulatory and effector T-cell subsets. CD4\(^+\) T cells that co-express lineage markers of regulatory (Foxp3) and T\(_{h17}\) (ROR\(\gamma\)t) T cells have been detected in the normal gut of both mice and humans, and the frequencies of these cells increase in the setting of inflammation. At present, it is unclear whether these ‘dual-expressors’ represent early precursors from which T\(_{h17}\) or T\(_{reg}\) cells emerge, represent Foxp3\(^+\) T\(_{reg}\) cells that are in transition to T\(_{h17}\) cells in response to pro-inflammatory cytokines, are a functionally distinct T-cell subset, or a combination thereof. However, there are growing data from studies in murine models of infectious disease that T\(_{reg}\) cells can transition to progeny with effector properties, such as T\(_{h17}\) or T\(_{h1}\), under the influence of inflammatory cytokines. Reciprocal programming (i.e., effector T cells into T\(_{reg}\) cells) has not been well demonstrated, suggesting that the conversion of T\(_{reg}\) cells to T effectors might represent an evolutionary adaptation to override the dominance of homeostatic T\(_{reg}\) function at mucosal sites in the face of a pathogenic threat. It is currently unclear to what extent similar transitions might occur in the context of immune dysregulation during the evolution of IBD. However, the possibility that T\(_{reg}\) cells
may have the potential to become effector T cells needs to be considered when thinking of adoptive T_{reg} therapy for chronic inflammatory conditions, or even strategies to boost T_{reg} numbers in vivo.

T-cell subset plasticity is not, however, limited to T_{reg} cells. The transition of CD4+ T cells of one effector subset to progeny with features of a distinct effector subset has been increasingly reported. This has been most clearly demonstrated for T_{H17} cells, which can give rise to T_{H1}-like cells contingent upon the hierarchy of cytokine signals received following T_{H17} cell differentiation. In particular, T_{H17} cells exposed to the T_{H1}-polarizing cytokine IL-12 extinguish expression of genes that encode IL-17 cytokines in favor of IFN-γ. The transition of T_{H17} cells into IFN-γ-producing T_{H1} cells is dependent on STAT4 signaling and induction of the T_{H1} lineage transcription factor, T-bet. Similarly to the transition of T_{reg} cells to T effector cells, T_{H17} to T_{H1} transitions appear to be unidirectional. That is, T_{H17} cells can transition to T_{H1} cells, but T_{H1} cells do not transition to T_{H17} cells. In intestinal inflammation in mice, the transition of T_{H17} cells into T_{H1}-type cells is associated with the development of colitis, suggesting that the mix of T_{H17} and T_{H1} cells typically found in the intestinal lamina propria, both at homeostasis and in IBD, might be due to diversion of some T_{H17} precursors locally in the intestines (Figure 7.3).

The plasticity of T_{H17} cells appears to be shared in humans. CD4+ T-cell isolates from the intestine of patients with Crohn’s disease contain a mix of distinct subsets of T_{H17} and T_{H1} cells, as well as IL-17+ IFN-γ+ T cells (Figure 7.4). Similar to findings in mice, T_{H17} and T_{H17/T_{H1}} cells cloned from intestinal T-cell isolates can be diverted to a T_{H1} phenotype in response to IL-12. Interestingly, CD161, which was initially proposed as a marker for human T_{H17} cells (mice do not express a CD161 homolog) is also expressed by a substantial fraction of IFN-γ+ T cells in normal and inflamed intestines. Further,
CD161+ human T cells give rise to a distinct subpopulation of IFN-γ+ cells under Th17-polarizing conditions. Thus, as in studies in mice, human Th17 cells may be phenotypically unstable and give rise to Th1-like cells, whereas the converse has not been demonstrated. The implications of Th17 plasticity for normal intestinal immune regulation and the development of IBD remain to be defined.

Regulatory T cells and the control of effector T-cell responses.

It is very well established that reconstitution of mice lacking T or B cells with small numbers of naive CD4+ T cells leads to colitis. If the recipients are germ-free, colitis does not develop. It is also well known that co-injection of natural CD4+ Treg cells ameliorates the colitis induced by naive T cells. Thus it is often claimed that pathogenic effector T-cell responses against the gut microflora are controlled or dampened by Treg cells, and a corollary of this statement, namely that IBD is somehow due to a failure of T-cell regulation, is often mooted. Suppression appears to be mediated by TGF-β, although other cytokines such as IL-10 and even IL-17A may be suppressive in the correct context. In reality, control of mucosal effector and regulatory T cells is likely to be multilayered and multifaceted, and there are almost certainly many different mechanisms that control immune responses in the gut, including the accessibility of antigen to the lamina propria. For example, patchy defects in the intestinal barrier leading to increased local intestinal permeability cause a patchy Crohn’s-like lesion in mice with a normal immune system. The colonic epithelial cells in mice with a gut epithelial knockout of nuclear factor κB essential modulator (NEMO) undergo apoptosis and the barrier is broken, leading to colitis. Finally, in patients with neutrophil defects such as chronic granulomatous disease or glycogen storage disease Type 1b, the normal flora enters the lamina propria and persists and elicits a disease almost identical to Crohn’s disease. In normal lamina propria, there is abundant active TGF-β made by epithelial cells and stromal cells and as relatively little gut antigen crosses the intact epithelial/mucus barrier, it is entirely possible that effector T-cell regulation in health is due to ‘neglect,’ rather than active suppression (Figure 7.5).

7-9 Human diseases due to single gene defects are informative in understanding gut inflammation and immune regulation.

Immunodysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome is a very rare X-linked disease where mutations in Foxp3 lead to defective regulatory T-cell activity. It is often used as an example of how
regulatory T-cell defects cause colitis. Indeed, virtually all of these children have small bowel inflammation and some have colitis. The gut manifestations, however, go alongside chronic inflammation in the pancreas and other endocrine organs, kidneys, liver, and skin; there is often anemia and/or thrombocytopenia, autoantibodies, and a generalized lymphadenopathy. It is therefore possible that children with IPEX have a general autoreactivity to self-antigens, with the gut tissue as a target of autoimmune attack because the gut is also an endocrine organ. Indeed before IPEX and Foxp3 were identified, the disease was often referred to as autoimmune enteropathy. The scurfy mouse also has mutations in Foxp3, but does not appear to have colitis; instead, the mouse dies at about 3–4 weeks of age showing lymphohistiocytic infiltration of many tissues and lymphadenopathy. Both of these situations differ markedly from human IBD where, especially for Crohn’s disease, pathological manifestations in tissues other than the gut are generally thought to be a consequence of the gut lesions. It is also worth noting that prior to the development of the transfer model of colitis in lymphopenic mice, there was an extensive literature in the rat where transfer of naive T cells into athymic animals caused generalized autoimmune disease, including colitis.

Almost 20 years ago it was shown that IL-10 null mice developed spontaneous enteritis, dependent on the microbiota. Mice with T-cell deficiency of IL-10 also develop a spontaneous colitis. Mice lacking IL-10 in the T\textsubscript{reg} population also develop spontaneous colitis, but it is less severe than in mice with global loss of IL-10, suggesting that other sources of IL-10 may be important. Consistent with this, IL-10-producing Foxp3\textsuperscript{+} cells are present in the intestinal lamina propria, and there are also data to suggest that T\textsubscript{H}1 cells, as they differentiate, also begin to make IL-10. Extremely compelling evidence that IL-10 controls responses to the flora in humans comes from rare cases where there are defects in IL-10R. Very young children with IL-10R loss-of-function mutations develop a severe early onset inflammatory bowel disease with many characteristics of Crohn’s disease. Successful therapy has been achieved using bone marrow transplantation.

**7-10 Regulation of mucosal immune responses is complex and depends on whether the effector T cell can be regulated.**

It is worth considering whether potentially pathogenic T\textsubscript{H}1 or T\textsubscript{H}17 responses in the gut are controlled by regulatory T cells. First, in Peyer’s patches and mesenteric lymph nodes there is evidence that feeding protein antigens in intact mice leads to conversion of naive T cells into Foxp3\textsuperscript{+} T\textsubscript{reg} cells. However, the largest numbers of antigen-specific Foxp3\textsuperscript{+} T\textsubscript{reg} cells are found in the small bowel lamina propria. CD103\textsuperscript{+} DCs from lamina propria can also convert antigen-specific T cells into T\textsubscript{reg} cells and this process is dependent on retinoic acid production. In mice, where Foxp3 is a good marker for T\textsubscript{reg} cells, these experiments suggest that there is generation of T\textsubscript{reg} cells in response to gut protein antigens in GALT and cells then migrate to the lamina propria. In the small intestine there is evidence for heterogeneity in the lamina propria in that macrophages appear to be able to induce T\textsubscript{reg} cells by secreting retinoic acid, IL-10, and TGF-\beta, whereas dendritic cells activate T\textsubscript{H}17 cells. It is not known, however, whether the same type of response occurs to the antigens of the microbiota.

In humans there are difficulties in using markers such as Foxp3 and CD25 to examine T\textsubscript{reg} cells in the gut. Both markers are expressed on activated effector T cells, so they are not particularly good tools to analyze T\textsubscript{reg} function. In humans there are few data on GALT; however, there have been some functional studies on lamina propria mononuclear cells. CD4\textsuperscript{+}CD25\textsuperscript{high} cells from normal lamina propria are less proliferative and produce fewer cytokines than CD4\textsuperscript{+}CD25\textsuperscript{−} lamina propria mononuclear cells. When the former population is added to mitogen-activated CD4\textsuperscript{+}CD25\textsuperscript{−} blood T cells, there is a marked
reduction in proliferation. This suggests that there are regulatory T cells in the lamina propria; however, regulatory activity is partially abolished by exogenous IL-2, which does suggest that at least some of the effect is due to cytokine deprivation, rather than active suppression. There does not appear to be any reduction in $T_{reg}$ numbers in the gut in IBD.

An alternative approach to understanding CD4 $T_{h}1$ or $T_{h}17$ responses in the gut in idiopathic inflammation is to assume that the problem is an antipathogen response gone awry, directed at harmless microbes. In pathogen responses, it is important to prevent $T_{reg}$ cells dampening effector cell function until the pathogen has been eliminated. Thus there is an advantage for effector CD4 cells in the gut to be resistant to suppression, regardless of the numbers of $T_{reg}$ cells in the tissue. One way that effector T cells achieve this is by overexpressing Smad7, the intracellular inhibitor of TGF-β signaling. Smad7 functions in two main ways: by blocking the binding of Smad2/3 to ligand-activated TGF-β receptor; and by targeting the receptor itself for ubiquitination and degradation. As might be expected, $T_{reg}$ cells and TGF-β protein are ineffective in dampening pathogenic T-cell responses from inflamed IBD tissues. However, knocking down Smad7 with an anti-sense oligonucleotide allows endogenous TGF-β to dampen T-cell responses. Overexpression of Smad7 in CD4 T cells makes animals more susceptible to experimental colitis because endogenous TGF-β and $T_{reg}$ cells cannot dampen inflammation. Similarly, in the transfer model of colitis, T cells overexpressing Smad7 cannot be controlled by transferred $T_{reg}$ cells. Paradoxically, however, in mouse models of colon cancer, overexpression of Smad7 in T cells is highly beneficial because the exaggerated T-cell responses translate into exaggerated anti-tumor responses, even though the mucosa remains highly inflamed (Figure 7.6).

One of the main difficulties in this area, especially when looking at the effects of the microflora, is the difficulty in generating T-cell responses to the flora. Although it was demonstrated many years ago that T cells from normal gut responded to microbial antigens from the flora of different individuals but were tolerant to their own flora, and that in IBD autologous gut T cells responded vigorously to an individual’s own microflora, these results have not been followed up. In mice, T-cell lines reactive to gut bacterial antigens cause colitis when transferred into lymphopenic mice, and the colitogenic response can be inhibited in vivo by in vitro-generated $T_{reg}$ cells with the same specificity.

**Figure 7.6 Smad7 overexpression in activated T cells in Crohn’s disease (CD) and ulcerative colitis (UC) renders mucosal T cells resistant to TGF-β.** In humans, addition of exogenous TGF-β rapidly phosphorylates Smad3 (p-SMAD3) in lamina propria mononuclear cells (LPMC) from normal human gut, but has no effect on lamina propria mononuclear cells from IBD patients. Consistent with this, TGF-β can inhibit the production of tumor necrosis factor (TNF) transcripts by superantigen-activated normal lamina propria mononuclear cells, but has no effect on lamina propria mononuclear cells from Crohn’s patients. Collectively these data show that expression of Smad7 in effector T cells regulates whether T cells are susceptible to regulation. (Adapted from G. Monteleone et al., J. Clin. Invest. 108:601–609, 2001.)

**T cell-mediated gut diseases.**

Although IBD and celiac disease will be covered in other chapters, it is worth considering these conditions as situations where the ‘balance’ between CD4 T-cell activity in the gut and luminal antigens becomes skewed and chronic T-cell activation causes disease.

Celiac disease is of interest because there is no doubt that most, if not all, of the pathology is driven by CD4 T cells in the lamina propria responding to gliadin peptides presented in the context of HLA-DQ2 or DQ8. However, DQ2 is an extremely common haplotype in the general population and it is still not known what additional factors lead to only a few percent of DQ2+ individuals becoming gluten reactive. Environmental factors are clearly important. There are cases in the literature where identical twins have developed celiac disease years apart, and when the proband was diagnosed, the healthy sibling has been investigated thoroughly and shown to be normal. One notion is that viral infections in the gut not only break the barrier to allow antigen to enter the lamina propria, but can induce the local production of type I interferons, which are potent stimulators of $T_{h}1$ responses. Once sensitized, however, celiac disease is a lifelong condition, which implies T-cell memory. It is
highly unlikely that long-lived gluten-specific memory T cells persist in the lamina propria; instead they are probably part of the recirculating memory T-cell pool, in which case they may encounter gluten in GALT. One feature of celiac disease, however, that has not been explained is the time to relapse when returning to a gluten-containing diet. This is highly variable between individuals and does imply that environmental factors may also be important at all stages of the disease.

Recently, genome-wide association studies have attempted to fill the gap between the high frequency of DQ2+ individuals and the low numbers of these who develop disease. Virtually all of the polymorphisms identified are associated with genes that control the tone and texture of the T-cell responses, such as IL-2 and IL-21.

In Crohn’s disease there is overwhelming evidence that the disease is mediated by activated CD4 T_{H1} and T_{H17} cells. Genome-wide association studies have identified many dozens of polymorphisms related to innate immune function as being important risk factors (Chapter 32). It needs to be remembered, however, that the penetrance of Crohn’s disease is almost completely environmental. Thus Crohn’s disease is very uncommon in India, but when families migrate to westernized countries with high incidences of Crohn’s disease, the incidence in the children of immigrants is the same as that of the indigenous population.

The unifying factor in both celiac disease and Crohn’s disease is the persistence of activated CD4 T cells in the intestinal lamina propria. In celiac disease, the T cells are antigen driven, as shown by a gluten-free diet restoring normality of gut structure. In Crohn’s disease, however, the situation is much more complex. The contribution of antigen is not known, and there are very many cytokines overexpressed in Crohn’s disease mucosa, such as IL-1, IL-2, IL-6, IL-7, IL-12, IL-15, IL-18, IL-21, and IL-23, which can signal to activated T cells, prevent apoptosis, and allow the cells to continue to produce IFN-γ, IL-17A, and tumor necrosis factor-α (TNF-α). Identifying which, if any, of these cytokines are critical, and thus worthy of the expense of therapeutic intervention, represents an extremely significant challenge. It may well be that so many factors are involved in driving CD4 T-cell survival in Crohn’s disease that targeting cytokines may not be the best way to go, and that strategies to target the T cells themselves may be more productive. The caveat, however, remains that because CD4 T cells in the gut and airways are vital to maintain freedom from low-grade infections, any T-cell directed therapy has to be effectively monitored.

The role of CD4 T cells in ulcerative colitis remains opaque. There are some data which suggest overexpression of IL-13; however, in absolute terms IL-13, or IL-5 for that matter, is dominated by IL-17A and IFN-γ, which are present in at least tenfold excess compared with IL-13. Severe ulcerative colitis responds well to cyclosporin A, which clearly suggests a role for T cell-mediated damage in fulminant disease; however, trials of tolerizing anti-CD3 antibodies in ulcerative colitis have not been a success.

**Innate lymphoid cells.**

Although there is much focus on cytokines in the gut made by T cells, one of the most interesting recent observations in mucosal immunology has been on the pro-inflammatory and regulatory cytokines made by innate lymphoid cells (ILC). The prototypic ILC is the natural killer (NK) cell, originally identified through their ability to kill tumor cells, but which also recognize and kill cells expressing pathogen-associated and stress-associated ligands. NK cells are also hard-wired to secrete cytokines when activated, typically IFN-γ, TNF-α,
and granulocyte–macrophage colony-stimulating factor (GM-CSF). NK cells display heterogeneity and can be subdivided into different functional subsets in humans by the expression of CD56 and CD16. Likewise, in mice different NK subsets based on, for example, CD27, CD127, and CD11b have different functional properties. Despite much investigation, however, a clear role for NK cells in mucosal health and disease, especially in the gut, has yet to be identified.

A second type of ILC is represented by lymphoid tissue inducer (LTi) cells. These cells were originally described as being key for lymphoid tissue organogenesis through their ability to activate stromal cells to express adhesion molecules and secrete chemokines needed to attract dendritic cells, T cells, and B cells into developing lymph nodes and Peyer’s patches. Like T\(_{17}\) cells, LTi cells depend on ROR\(_{γt}\), and produce IL-17A and IL-22. IL-22 is thought to play an important role in epithelial homeostasis and induces production of defensins. IL-22-deficient mice are highly susceptible to *Citrobacter rodentium* infection, and there is now evidence that IL-22 production by LTi cells may be more important in resistance to *Citrobacter* than T cell-derived IL-22.

However, matters are complicated by the fact that a third type of ILC has also been identified that may be an intermediate between NK cells and LTi cells. In mice they express NKp46, but in humans they express NKp44 and are CD56\(^+\). These cells produce very little IFN-\(γ\), and are not cytotoxic, but contain abundant IL-22 and have been termed ILC22 cells; they have also been implicated in resistance to *Citrobacter* infection. Other ILC have been identified in the gut, termed natural helper cells or nuocytes, which make T\(_{H2}\)-type cytokines. Unraveling the complexity of ILC will be a major challenge in the next few years.

**Summary.**

The mucosal surfaces are the battleground between the immune system, pathogens, and dietary and environmental antigens. Terms such as ‘balance’ and ‘homeostasis’ are used to describe the situation in health. These words, however, are descriptors and give no insight into mechanisms. The fact that many different transgenic and knockout mice, many with normal numbers of T cells and B cells, develop chronic gut inflammation, mediated in most cases by CD4 T cells, strongly suggests that control of mucosal T cells is complex. In health it would appear that activated mucosal T cells are generated in GALT (hence the large numbers of T\(_{H1}\)1 and T\(_{H1}\)17 cells in normal gut) in response to, and perhaps controlled by, the microbiota and perhaps even diet. When effector cells migrate to the lamina propria in health they are short lived and die by apoptosis. An unpredictable coalition of antigen(s) (perhaps due to a break in the epithelial barrier or a genetically determined inability to break down the cell walls of the microbiota) and perhaps infection changing the local cytokine milieu, may result in the rescue of CD4 T cells from the apoptotic path, and drive them toward survival. The inflammation generated by the activated CD4\(^+\) T cells then tips the microenvironment into self-perpetuating inflammatory milieu, since the barrier is then broken by T cell-derived cytokines, and inflammatory cells producing survival cytokines move into the tissues. Nonspecific agents such as corticosteroids may dampen the inflammation, and anti-cytokine agents such as anti-TNF may also produce transient relief. However, disease persists at low levels or returns, presumably because of CD4 T-cell memory, about which we know very little in mucosae. Exciting recent developments concerning different subsets of ILC in the gut add a new dimension to the complexity of gut immunity and inflammation and it will be interesting to determine the role of ILC at mucosal surfaces in humans.


