

## REVIEW ARTICLE

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## Pathophysiology of Inflammatory Bowel Diseases

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THE INFLAMMATORY BOWEL DISEASES (IBDS) ARE CHRONIC INTESTINAL disorders that are typically categorized as one of two subtypes: Crohn's disease and ulcerative colitis.<sup>1</sup> Ulcerative colitis is limited to the colon, with superficial mucosal inflammation that extends proximally in a contiguous manner, and can lead to ulcerations, severe bleeding, toxic megacolon, and fulminant colitis. In contrast, Crohn's disease can affect any part of the digestive tract, often in a noncontiguous manner, and is characterized by transmural inflammation, which can lead to complications such as fibrotic strictures, fistulas, and abscesses.

Although potentially important differences between ulcerative colitis and Crohn's disease have been observed, such as immune-cell subpopulations differentially enriched<sup>2</sup> and genetic variants (e.g., *NOD* and *PTPN22*) that increase the risk of Crohn's disease but may be protective against ulcerative colitis,<sup>3</sup> a comprehensive understanding of the underlying pathophysiological mechanisms resulting in these divergent clinical manifestations is still lacking. Moreover, additional heterogeneity beyond these two IBD subtypes is likely; for example, ileal and colonic Crohn's disease may represent distinct entities, and colonic Crohn's disease can be further classified into subtypes on the basis of gene expression profiles.<sup>4</sup>

The IBD armamentarium (see Table S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org) includes untargeted therapies, such as aminosalicylates, glucocorticoids, and immunomodulators, as well as targeted biologic therapies that act through one of the following mechanisms: neutralization of cytokines that promote inflammation (e.g., anti-tumor necrosis factor [TNF] antibodies) or drive the differentiation and function of specialized immune subsets (e.g., anti-interleukin-12 and anti-interleukin-23 antibodies), blockage of signal transduction cascades downstream of these pathways (e.g., Janus kinase [JAK] inhibitors), or modulation of lymphocyte trafficking (e.g., anti- $\alpha 4\beta 7$  integrin antibodies). Biologic therapies are effective in many patients, but up to 30% of patients do not have a response to initial treatment, and in up to 50% of patients, the response is lost over time. Although inadequate drug levels and development of immunogenicity to drug treatments underlie some of these failures, additional heterogeneity of IBD beyond the classic Crohn's disease and ulcerative colitis subtypes is likely to be another important factor. The pathophysiology of IBD involves complex genetic, environmental, epithelial, microbial, and immune factors. This review does not cover all the breakthroughs in these diverse areas but instead highlights some recent advances.

## INTESTINAL EPITHELIUM

The intestinal epithelium comprises a single layer of epithelial cells linked by tight junctions and intercalated with immune cells (Fig. 1 and Table S1).<sup>6</sup> The small

intestinal epithelium is a highly dynamic tissue organized as a series of protrusions (villi) and invaginations (crypts of Lieberkühn). Major functions include facilitating nutrient absorption, acting as a physical barrier against gut luminal contents, and responding to signals from the intestinal microbiota and immune system. Secretory cells include goblet cells, which produce mucus and such antimicrobial peptides as trefoil factor and resistin-like molecule beta that limit luminal microbes. Early studies suggested that the mucus layer was denuded in Crohn's disease owing to a reduction in goblet cells,<sup>7</sup> and a recent single-cell RNA sequencing (scRNA-seq) study showed that down-regulation of a colonic goblet-cell-secreted protein, whey acidic protein four-disulfide core domain 2 (WFDC2), in active ulcerative colitis may lead to abnormalities in mucus layer formation, increased colonization and invasion of microbiota, and breakdown of the epithelial barrier.<sup>8</sup> These findings suggest that WFDC2 and other molecules produced by goblet cells might be protective in ulcerative colitis.

Stromal cells, which are nonhematopoietic mesenchymal cells that include fibroblasts, myofibroblasts, and perivascular pericytes, reside below the epithelium in the lamina propria and play important roles in fibrosis and wound healing. A recent report suggested a role for a previously unknown subpopulation of fibroblasts in exacerbating ulcerative colitis, owing to increased expression of immune-cell-attractant chemokines CCL19 and CCL21, as well as interleukin-33, which induces certain immune-cell subsets to produce type 2 cytokines.<sup>9</sup> Thus, approaches aimed at enhancing epithelial barrier function could lead to potential therapeutic strategies for IBD.

#### GENETICS, GENOMICS, AND EPIGENOMICS

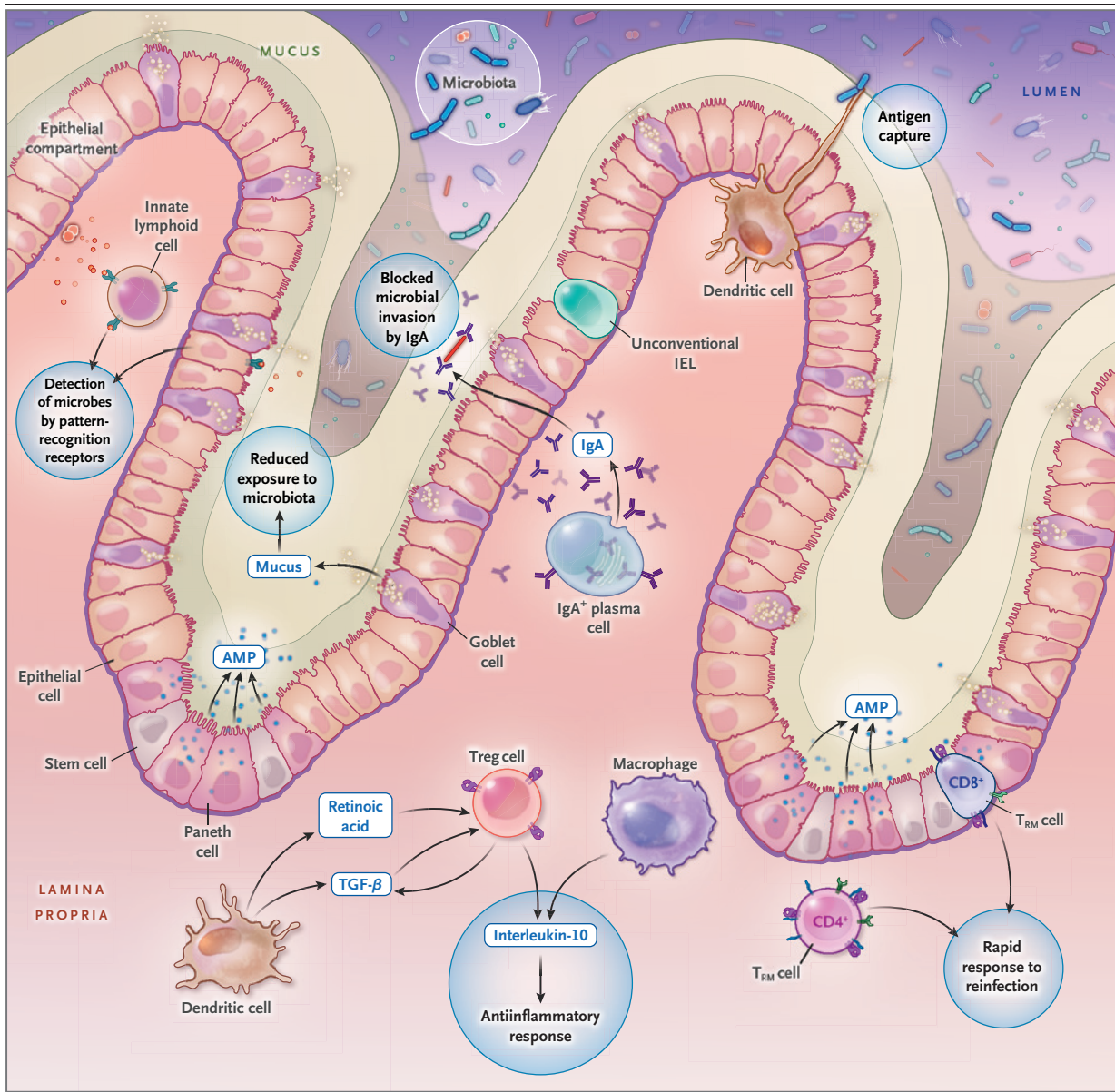
Early studies suggested a heritable risk that is greater for Crohn's disease than for ulcerative colitis and a higher incidence of IBD in first-degree relatives of patients with IBD than in the general population.<sup>1,10</sup> To date, genomewide association studies have identified more than 240 risk variants that affect intracellular pathways recognizing microbial products (e.g., *NOD2*); the autophagy pathway, which facilitates recycling

of intracellular organelles and removal of intracellular microorganisms (e.g., *ATG16L1*); genes regulating epithelial barrier function (e.g., *ECM1*); and pathways regulating innate and adaptive immunity (e.g., *IL23R* and *IL10*).<sup>1,10</sup> Only 8 to 13% of disease variance in Crohn's disease and 4 to 7% in ulcerative colitis can be explained by known IBD risk loci,<sup>3</sup> but genetic factors, such as variants in the antiinflammatory interleukin-10 signaling pathway, may play a more important role in children with very-early-onset IBD.<sup>11</sup> Moreover, genetic studies, recently reviewed in detail,<sup>1</sup> have greatly accelerated the identification of genes and pathways that may be critical for mucosal homeostasis and the development of IBD.

Genomewide profiling studies have focused on identifying molecular features, such as gene expression and epigenetic modifications, that distinguish additional subtypes within the canonical Crohn's disease or ulcerative colitis classifications, differentiate Crohn's disease from ulcerative colitis, or discriminate between IBD and a healthy state. Analyses of gene expression and chromatin accessibility in samples of colonic tissue have been used to identify two molecular subtypes of Crohn's disease that have differences in cellular metabolism (e.g., glucose and lipid metabolism pathways) and immune signaling pathways (e.g., interleukin receptors, G protein-coupled receptors, and toll-like receptors).<sup>4</sup> Other studies have identified genes that are more highly expressed in tissue from patients with IBD; for example, increased expression of the cytokine oncostatin M was observed in inflamed intestinal tissue from patients with IBD and was predictive of the subsequent failure of anti-TNF therapy.<sup>12</sup> A potential limitation of analyses using whole intestinal tissue, however, is the substantial heterogeneity of cell types contained within; thus, gene expression measurements may preferentially detect the most highly expressed messenger RNA (mRNA) transcripts in the most abundant cells and cannot be unequivocally linked to a specific cell type.

Technological advances (Table S2) enabling transcriptional profiling (e.g., scRNA-seq) and high-dimensional protein analyses (e.g., mass cytometry) at the single-cell level have resulted in the identification of IBD-associated signatures and the discovery of new subpopulations of fibroblasts,<sup>9</sup> epithelial cells,<sup>8</sup> and immune cells<sup>2,13-16</sup> that are enriched or depleted in IBD.





For example, a cellular module termed GIMATS (IgG-producing plasma cells, inflammatory mononuclear phagocytes, activated T cells, and stromal cells) was shown to be enriched in a subgroup of patients with ileal Crohn's disease and was associated with the lack of a durable remission in response to anti-TNF therapy.<sup>13</sup> Thus, genetic, genomic, and epigenomic studies have the potential to identify genes and pathways in specific cell subtypes that could represent future therapeutic targets or serve as biomarkers to aid in clinical decision making.

#### MICROBIOTA

Humans are colonized by trillions of viral, fungal, bacterial, and eukaryotic microbes, collectively referred to as the microbiome, which are present on all barrier surfaces.<sup>17</sup> The gastrointestinal tract, particularly the distal ileum and colon, contains the largest number and greatest diversity of bacteria. Gut microbes exist in a mutually beneficial relationship with humans, established over many millennia, and play an essential role in maintaining health by metabo-

**Figure 1 (facing page). Intestinal Mucosal Immune System in the Healthy State.**

Intestinal stem cells reside at the base of the crypts and give rise to all absorptive and secretory cells making up the epithelial layer. Goblet cells produce a mucus layer that reduces exposure of intestinal epithelial cells to microbiota. Paneth cells produce antimicrobial peptides (AMP), such as alpha-defensins, lysozyme, and secretory phospholipase A2. Plasma cells synthesize IgA, which binds to mucus, preventing invasion by pathogenic organisms and helping to maintain a homeostatic balance between the host and commensal microbiota. Epithelial cells and innate immune cells detect microbiota using pattern recognition receptors, including toll-like receptors and nucleotide oligodimerization domain proteins. Dendritic cells capture antigens directly, using membranous processes intercalated between intestinal tight junctions, or indirectly, by acquiring them from microfold cells. The mucosal immune system facilitates a predominantly antiinflammatory environment by virtue of active down-regulation of immune responses. For example, unlike macrophages in other parts of the body, intestinal macrophages do not produce inflammatory cytokines in response to phagocytosis or exposure to bacteria; instead, they produce large amounts of the antiinflammatory cytokine interleukin-10. Dendritic cells produce retinoic acid and transforming growth factor  $\beta$  (TGF- $\beta$ ) to promote the generation of regulatory T (Treg) cells, which in turn produce interleukin-10 and TGF- $\beta$ . Lymphocytes in the intestinal epithelial compartment are termed intraepithelial lymphocytes (IELs).<sup>5</sup> These lymphocytes can be divided into conventional IELs (e.g., TCR $\alpha\beta$ +CD8 $\alpha\beta$ + and TCR $\alpha\beta$ +CD4+ IELs), which acquire an effector program after encountering foreign antigens in the periphery, and unconventional IELs (e.g., TCR $\alpha\beta$ +CD8 $\alpha\alpha$ + and TCR $\gamma\delta$ +CD8 $\alpha\alpha$ + IELs), which are thought to recognize self-ligands in the thymus or periphery and acquire an effector program before exposure to infection or injury. CD4+ and CD8+ tissue-resident memory T (T<sub>RM</sub>) cells reside in the intestinal epithelial compartment and lamina propria, providing rapid responses against reinfection. ILC denotes innate lymphoid cell.

lizing dietary components such as cellulose. Moreover, gut microbes produce essential components such as vitamin K, an important cofactor in blood clotting, and short-chain fatty acids, an energy source for colonic epithelial cells. Commensal bacteria and their products also have an essential role in the normal development and functioning of the immune system. The presence of commensal bacteria interferes with the ability of pathogens to colonize and invade the gut, in part because of competition for space and nutrients. Thus, when the integrity of the mucosal barrier is compromised, normally innocuous commensal bacteria can become patho-

genic by crossing the epithelium and eliciting an immune response and intestinal inflammation.

Advances in next-generation sequencing have led to a number of studies that show changes in the composition of microbial communities, termed dysbiosis, in the context of myriad human diseases, including IBD.<sup>18,19</sup> Collectively, these studies have shown that both Crohn's disease and ulcerative colitis are associated with reductions in the total number, diversity, and richness of microbial species. Although these studies were unable to infer causal relationships between observed changes in the microbiome and IBD, they raised the possibility that microbiota-based interventions, such as fecal microbiota transplantation, which has been shown to be beneficial in *Clostridium difficile* infection,<sup>20</sup> might also be effective in IBD. In clinical trials, however, the efficacy of fecal transplants for the treatment of IBD has been variable,<sup>21-24</sup> perhaps owing to differences in trial design, including selection of stool donors, route of administration, number of infusions, and use of antibiotic pretreatment. The specific component of donor feces that is responsible for mediating a possible beneficial effect in IBD remains uncertain, and identification of that component is a necessary first step in the rational design of microbiota-based therapies.

Germ-free mice with no microbial colonization have increasingly served as valuable experimental models for investigating how microbiota can influence host physiology and pathology.<sup>17</sup> Studies have ranged from simple associations with individual microbes to transplantations of entire gut microbial communities. For example, fecal material from patients with IBD conferred increased susceptibility to colitis in germ-free mice, as compared with mice that received fecal material from healthy persons, in part by promoting increased numbers of inflammatory type 17 helper T (Th17) cells and reduced numbers of a subset of antiinflammatory regulatory T (Treg) cells.<sup>25</sup> Multiple groups of investigators are refining this approach to identify specific microbiota or microbial components that can induce specific immunomodulatory effects. As one example, Atarashi and colleagues developed a rational approach to isolating Treg-cell-inducing bacterial strains from human gut microbiota through iterative selection steps; they identified a consortium of 17 clostridia strains that attenu-

ated experimental colitis by facilitating a transforming growth factor  $\beta$  (TGF- $\beta$ )-rich environment that promoted Treg-cell differentiation, expansion, and expression of the antiinflammatory cytokine interleukin-10,<sup>26</sup> in part mediated through production of short-chain fatty acids such as butyrate and propionate.<sup>27</sup> The entire 17-strain consortium was required for these beneficial effects, since colonization with individual strains failed to recapitulate the Treg-cell-promoting effects, suggesting that this group acted synergistically in a microbial community-dependent manner. This concept is currently being tested in clinical trials for ulcerative colitis, and advances in bacterial genetic engineering<sup>28</sup> may enable even more precise tuning and enhancement of the native immunomodulatory activities of microbiota selected for therapy. Thus, studies of microbiota have the potential to identify specific microbes or groups of microbes that may promote or mitigate intestinal inflammation owing to effects on the mucosal immune system; this information can then be exploited for potential therapeutic benefit in IBD (Table S2).

#### MUCOSAL IMMUNITY

The immune system can be broadly compartmentalized on the basis of functional (innate vs. adaptive) and anatomical (systemic vs. mucosal) considerations.<sup>29</sup> Innate immune cells, which express invariant receptors that detect microbial products or patterns, include granulocytes, macrophages, and dendritic cells (Figs. 1 and 2 and Table S1). Adaptive immune cells include B cells and T cells, which express highly variable receptors that recognize specific antigens, and mucosal-associated invariant T (MAIT) cells, which express antigen receptors with more limited diversity. The mucosal immune system represents the largest component of the immune system, containing approximately 75% of all lymphocytes and producing the majority of immunoglobulin in healthy persons.<sup>29</sup> Unlike systemic immunity, mucosal immunity must simultaneously balance the opposing demands of providing protective immunity against pathogens while preventing excessive immune responses against innocuous food antigens and commensal microbes.

In the gut mucosa, immune cells can be found in organized secondary lymphoid structures, col-

lectively known as gut-associated lymphoid tissue (GALT), as well as in intestinal tissue-draining mesenteric lymph nodes, embedded between surface epithelial cells, and within the underlying connective tissue. Macrophages, which are positioned under the epithelium, engulf and kill invading microorganisms and dispose of pathogens and infected cells targeted by adaptive immune cells. Innate lymphoid cells (ILCs) regulate tissue homeostasis, repair, remodeling, and microbial defense, and subsets of these cells can be defined by their cytokine production (Fig. 3). Dendritic cells initiate and shape immune responses in mucosal tissues by acquiring antigen from microfold cells or by directly capturing and sampling luminal antigens, using membranous processes intercalated between epithelial cells. After acquiring antigens, dendritic cells remain in Peyer's patches or migrate to mesenteric lymph nodes to interact with naive T cells (i.e., T cells that have not yet encountered their cognate antigen). Naive B cells encounter antigens in the follicular areas of Peyer's patches and mesenteric lymph nodes and undergo activation and differentiation into antibody-secreting plasma cells, germinal center B cells, or memory B cells. Differentiating plasma cells can be short- or long-lived and can undergo IgM-to-IgA isotype switching. IgA is transported across the epithelium, where it neutralizes toxins and pathogens without causing inflammation, owing to its inability to fix complement. In contrast, the ability of IgG to fix and activate the complement cascade can result in cell lysis, inflammation, and tissue destruction.<sup>32</sup>

#### B CELLS

Antibodies targeting microbes or their products, such as *Saccharomyces cerevisiae*, *Escherichia coli* outer membrane protein C (OmpC), and bacterial flagellin (CBir1), are readily detectable in patients with IBD, but it remains uncertain whether these antibodies are directly involved in the pathogenesis of IBD. The observation that rituximab (anti-CD20 antibody) was not effective in inducing remission in active ulcerative colitis led to the conclusion that B cells were not involved in IBD.<sup>33</sup> However, other studies have shown that rituximab does not effectively target B cells in the tissue,<sup>34</sup> particularly antibody-producing plasma cells, which do not express high levels of CD20.

Moreover, several studies have confirmed the



long-standing observation<sup>35</sup> that inflamed IBD tissue has a pronounced IgG predominance, in contrast to the IgA predominance characteristic of healthy gut tissue,<sup>13,15</sup> raising the possibility that a paucity of IgA, an increase in IgG, or both might be pathogenic. Indeed, in mice, defects in IgA production or affinity maturation of certain IgA specificities can lead to reduced diversification of gut microbiota and intestinal inflammation.<sup>36</sup> In addition, an increase in commensal microbiota-specific IgG antibodies has been observed in the colonic mucosa of patients with ulcerative colitis, and the induction of anti-commensal microbiota IgG antibodies in a murine model resulted in intestinal inflammation owing to macrophage activation, recruitment of neutrophils, and type 17 immunity (i.e., all immune cells that are producing type 17 cytokines; these include ILC subsets [group 3 ILCs] and CD4 T-cell subsets [Th17]).<sup>37</sup> Thus, because the IgG predominance observed in IBD tissue could lead to intestinal inflammation through several mechanisms, including recruitment of inflammatory immune cells and activation of complement, resulting in cell lysis, approaches targeting IgG-producing plasma cells or shifting the IgG predominance in favor of IgA might represent potential therapeutic strategies in IBD.

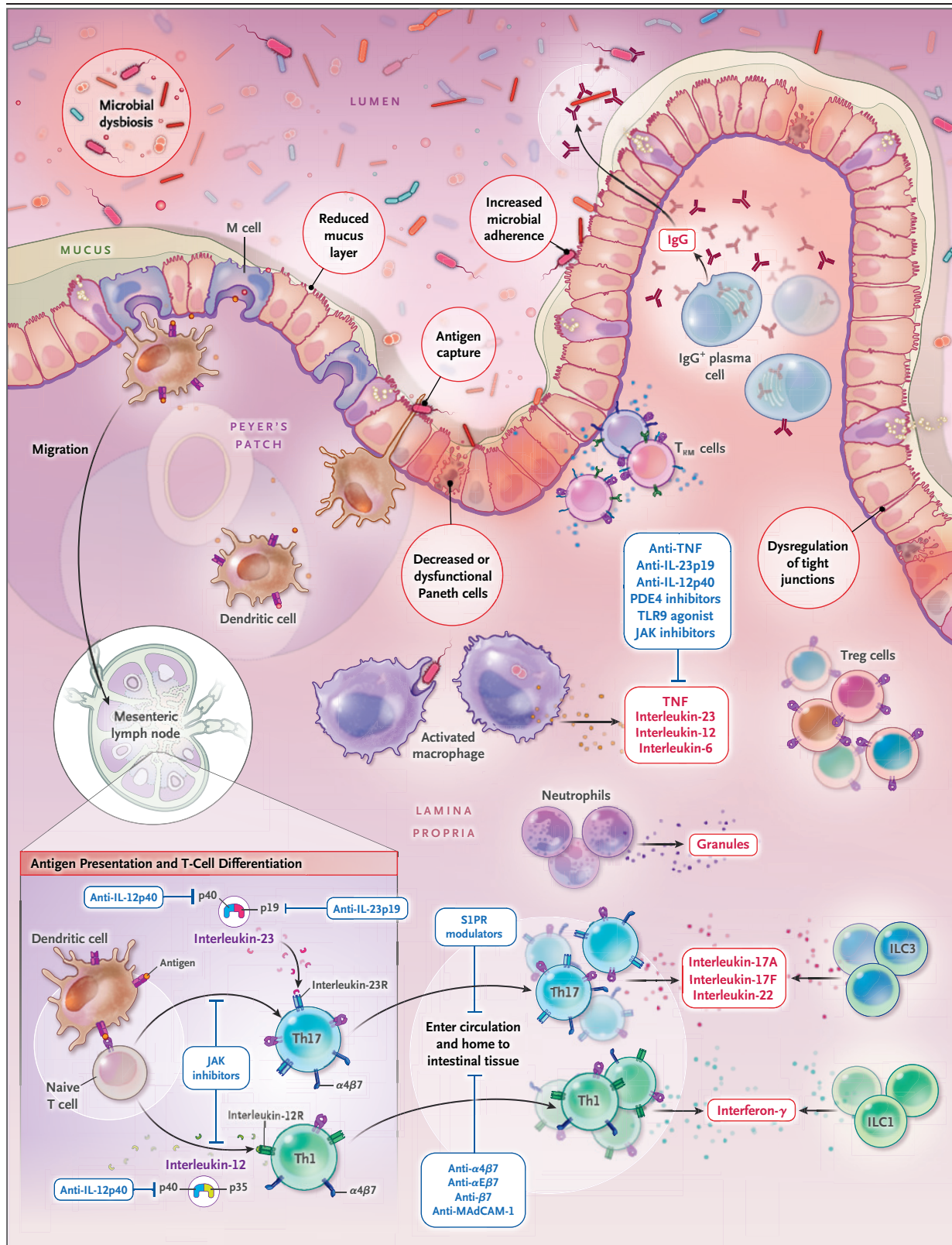
#### EFFECTOR T CELLS

Naive T cells undergo activation by antigen-bearing dendritic cells in the GALT or mesenteric lymph nodes and up-regulate specific homing receptors that allow for T-cell redistribution to mucosal surfaces. These include chemokine receptors (CCR9 in the small intestine and CCR10 in the colon) and  $\alpha 4\beta 7$  integrins that bind to mucosal addressin cell adhesion molecule 1 (MAdCAM-1) expressed on the endothelium of blood vessels in intestinal tissue. Lymphocytes migrating to the epithelial compartment up-regulate  $\alpha E\beta 7$  integrin (CD103), which interacts with E-cadherin on epithelial cells to promote retention of the lymphocytes. Therapies targeting integrins, which regulate lymphocyte trafficking to the intestine, or the sphingosine-1 phosphate receptor (S1PR) family, which mediates lymphocyte egress from lymph nodes, have been evaluated in the treatment of IBD (Fig. 2 and Table S1).

Activated T cells can differentiate into effector, regulatory, and memory subsets (Fig. 3).

Generally, effector cells produce inflammatory cytokines and provide immediate protection from microbial infection, regulatory cells dampen inflammation, and memory cells are long-lived and provide durable immunity.<sup>38</sup> Effector CD4+ T cells have additional heterogeneity, influenced by the cytokine microenvironment in which naive cells are activated (Fig. 3). For example, interleukin-12, a heterodimeric cytokine comprising interleukin-12p35 and interleukin-12p40 subunits, induces up-regulation of the transcription factor T-bet and promotes differentiation of type 1 helper T (Th1) cells, which produce interferon- $\gamma$  and recruit macrophages, natural killer cells, and CD8+ T cells. In contrast, interleukin-6, TGF- $\beta$ , and interleukin-1 induce up-regulation of interleukin-23R and a network of transcription factors, including retinoic acid-related orphan receptor gamma t (ROR $\gamma$ t). This enables responsiveness to interleukin-23, a heterodimeric cytokine made up of interleukin-23p19 and interleukin-12p40 subunits, facilitating differentiation of Th17 cells, which recruit neutrophils and produce interleukin-17A, interleukin-17F, and interleukin-22. Interleukin-17 is produced not only by T cells but also by several other type 17 immune cells, such as group 3 ILCs.<sup>39</sup>

Type 1 immunity (e.g., Th1 cells and group 1 ILCs) and type 17 immunity (e.g., Th17 cells and group 3 ILCs) have been implicated in murine models and in patients with IBD.<sup>39</sup> Accordingly, anti-interleukin-12p40 antibodies targeting both interleukin-12 and interleukin-23 (owing to their shared interleukin-12p40 subunit) have been shown to be effective in the treatment of Crohn's disease and ulcerative colitis.<sup>40-42</sup> Antibodies against the interleukin-23p19 subunit, which selectively target the interleukin-23 receptor, have also been shown to be effective in the treatment of Crohn's disease<sup>43,44</sup> and ulcerative colitis.<sup>45</sup> In addition, Janus kinase (JAK) inhibitors, such as tofacitinib, which block signals from interleukin-12, interleukin-23, and other cytokines by virtue of inhibiting downstream signaling pathways, are effective in ulcerative colitis.<sup>45</sup> In contrast, antibodies against interleukin-17A or the interleukin-17 receptor subunit interleukin-17RA have not been effective, at least in Crohn's disease.<sup>46</sup> These surprising results may be due to pleiotropic effects of interleukin-17A, which, in addition to inducing inflammation, promotes intestinal epithelial barrier function and repair.<sup>47,48</sup>





**Figure 2 (facing page). Intestinal Mucosal Immune System in Inflammatory Bowel Disease (IBD).**

In IBD, microbial dysbiosis occurs in association with disruption of the mucus layer, dysregulation of epithelial tight junctions, defects in the number and function of Paneth cells, and increased intestinal permeability, resulting in increased bacterial exposure. Activated macrophages engulf microbiota and produce increased levels of tumor necrosis factor (TNF), interleukin-6, interleukin-23, and interleukin-12, promoting inflammation. Neutrophils release preformed molecules stored in a variety of intracellular granules. Antigen-bearing dendritic cells remain in Peyer's patches or migrate to the mesenteric lymph nodes, where they present antigen to naive T cells. CD4<sup>+</sup> T cells undergo proliferation and differentiation into effector T-cell subsets, such as type 1 helper T (Th1) and type 17 helper T (Th17) cells. Differentiated Th1 and Th17 cells up-regulate chemokine receptors and integrins that enable them to enter the systemic circulation and home to the intestinal tissue, where they carry out inflammatory functions, such as production of interferon- $\gamma$  by Th1 cells and production of interleukin-17A, interleukin-17F, and interleukin-22 by Th17 cells. Interferon- $\gamma$ -producing group 1 innate lymphoid cells (ILC1), interleukin-17A-producing group 3 innate lymphoid cells (ILC3), and IgG-secreting plasma B cells are increased. T<sub>RM</sub> cells in the epithelial compartment and lamina propria undergo activation and expansion, producing inflammatory cytokines, killing infected cells, alerting innate cells, and recruiting additional immune cells. Approved or investigational therapeutic approaches (shown in blue) include neutralizing cytokines that promote inflammation (anti-TNF, anti-interleukin [IL]-12p40, and anti-IL-23p19 antibodies) or drive the differentiation of effector CD4<sup>+</sup> T-cell subsets (anti-interleukin-12p40 and anti-IL-23p19 antibodies); inhibiting signal transduction cascades downstream of inflammatory pathways (Janus kinase [JAK] inhibitors); blocking lymphocyte trafficking to the intestine (anti- $\alpha$ 4 $\beta$ 7, anti- $\beta$ 7, anti- $\alpha$ E $\beta$ 7, and anti-mucosal addressin-cell adhesion molecule 1 [MAdCAM-1] antibodies) or inhibiting lymph-node egress (S1PR modulators); decreasing production of inflammatory mediators (phosphodiesterase 4 [PDE4] inhibitors and toll-like receptor 9 [TLR9] agonists); or promoting wound healing (mesenchymal stem-cell [MSC]-based approaches). Anti-IL-12p40 antibodies block both interleukin-12 and interleukin-23 signaling by virtue of their shared p40 subunit.

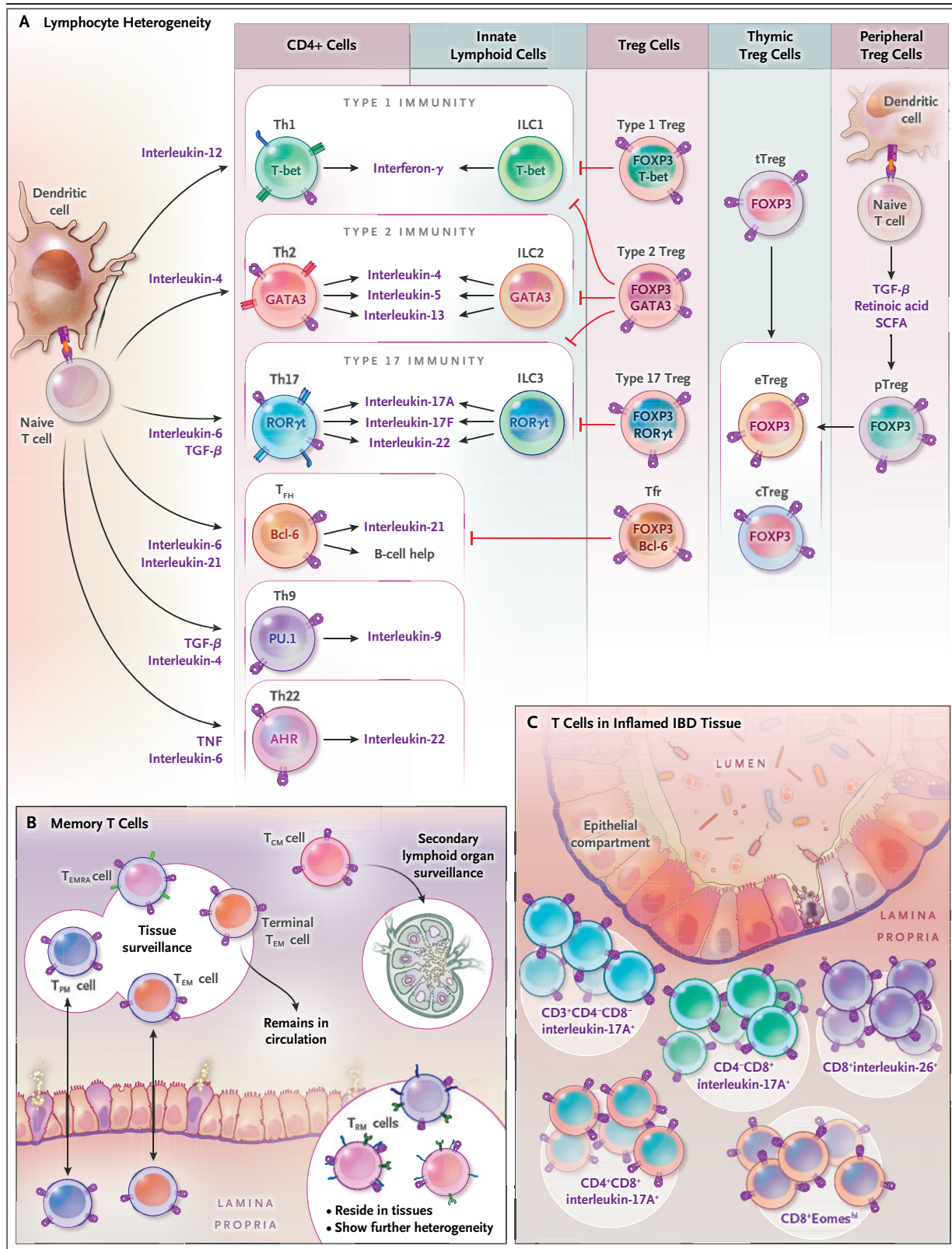
acts in an autoregulatory loop to limit Th17-cell pathogenicity,<sup>49</sup> and provides protection against commensal fungi.<sup>50</sup> These observations highlight the complexity of regulatory cytokine networks in maintaining gut mucosal homeostasis and health. Thus, therapeutic strategies targeting type 1 and type 17 immunity by virtue of lymphocyte trafficking (anti-integrin and S1PR modulators) or cytokine signaling (antibodies

against interleukin-12 and interleukin-23 and JAK inhibitors) are major components of the current IBD treatment armamentarium.

**REGULATORY T CELLS**

Treg cells expressing the transcription factor forkhead box P3 (FOXP3) maintain immune homeostasis owing to multiple mechanisms,<sup>51</sup> including expression of inhibitory molecules (e.g., cytotoxic T-lymphocyte antigen 4 [CTLA-4] and T-cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibition motif domains [TIGIT]) and production of anti-inflammatory cytokines (e.g., interleukin-10 and TGF- $\beta$ ). Moreover, Treg cells have nonimmunologic functions, such as mediating tissue repair through production of the growth factor amphiregulin.<sup>52</sup> Treg cells show additional heterogeneity (Fig. 3) with respect to the site where they develop (thymus vs. periphery); their anatomical localization and residency (circulation, lymphoid tissue, or nonlymphoid tissue)<sup>53</sup>; their activation state, which influences their trafficking and suppressive capabilities<sup>54</sup>; and their ability to up-regulate transcription factors and chemokine receptors associated with recognized effector subsets,<sup>55</sup> enabling Treg-cell subsets to migrate to the site of a specific type of inflammation and suppress the specific effector subset involved. In the gut mucosa, for example, Treg cells can up-regulate ROR $\gamma$ t, the transcription factor for Th17 cells and group 3 ILCs, enabling this Treg-cell subset to specifically suppress type 17 immune responses.<sup>56,57</sup>

Several studies have shown an increase in Treg cells in inflamed tissue from patients with IBD,<sup>58</sup> which would seem to imply that these cells are functionally deficient, though FOXP3, the transcription factor for Treg cells, can also be transiently expressed at low levels in activated conventional T cells.<sup>59</sup> However, most studies have not accounted for the possible heterogeneity of Treg cells, and it remains possible that Treg-cell subsets are differentially affected in IBD.<sup>58</sup> Indeed, one study showed enriched numbers of ROR $\gamma$ t+FOXP3<sup>+</sup> Treg cells in inflamed tissue from patients with Crohn's disease, which were capable of producing interleukin-17A and interferon- $\gamma$  while still retaining suppressive function.<sup>60</sup> A subsequent study showed a possibly analogous ROR $\gamma$ t+FOXP3<sup>lo</sup> population enriched in ulcerative colitis intestinal tissue.<sup>2</sup> It



**Figure 3 (facing page). Immune-Cell Heterogeneity.**

Naive CD4<sup>+</sup> T cells (Panel A, left side) interacting with dendritic cells bearing their cognate antigen can undergo differentiation into distinct effector subsets (e.g., types 1, 2, 9, 17, and 22 helper T [Th1, Th2, Th9, Th17, and Th22] cells and follicular helper T [T<sub>FH</sub>] cells), each producing a characteristic set of cytokines. Differentiation into an effector subset is controlled by the cytokine microenvironment in which the naive CD4<sup>+</sup> T cell is activated and results in up-regulation of lineage-determining transcription factors (e.g., T-bet for Th1 and RORγt [retinoic acid–related orphan receptor gamma t] for Th17). Innate lymphoid cells can be divided into ILC1, ILC2, and ILC3 subsets, analogous to CD4<sup>+</sup> Th1, Th2, and Th17 T-cell subsets. Treg cells (Panel A, right side) have substantial heterogeneity, with thymic Treg (tTreg) subsets that mature in the thymus and peripheral (pTreg) cells that differentiate from naive CD4<sup>+</sup> T cells interacting with antigen-bearing dendritic cells in the periphery in concert with factors such as TGF-β, retinoic acid, and short-chain fatty acids (SCFA). Some evidence suggests that tTreg cells recognize self-antigens, whereas pTreg cells may preferentially recognize microbe-derived antigens. Treg cells have additional heterogeneity according to their activation status and can be further subdivided into activated effector Treg (eTreg) and resting central (cTreg) cells. Finally, Treg cells have been shown to adopt the transcription factors associated with CD4<sup>+</sup> T-cell subsets. For example, some Treg cells can up-regulate T-bet, enabling them to migrate to sites containing Th1 cells and specifically suppress type 1 immune responses. Follicular regulatory T (T<sub>fr</sub>) cells repress differentiation of antibody-secreting cells in the germinal center, in part through their actions on T<sub>FH</sub> cells. Memory T cells can be broadly divided into circulating and tissue-resident subsets (Panel B). Circulating subsets include central memory T (T<sub>CM</sub>) cells, which survey lymph nodes, and peripheral memory T (T<sub>PM</sub>)<sup>30</sup> and effector memory T (T<sub>EM</sub>) cells, which can survey tissues. T<sub>EM</sub> cells have further heterogeneity, with additional subtypes comprising T<sub>EMRA</sub> (effector memory T cells that re-express CD45RA) and terminally differentiated T<sub>EM</sub> cells, which remain in the circulation.<sup>31</sup> Overlap is likely among the circulating memory T-cell subsets that are depicted; for example, T<sub>PM</sub> cells may represent a subset of T<sub>EM</sub> cells. Once formed, T<sub>RM</sub> cells reside in the tissue; these cells have additional molecular and functional heterogeneity. The relative contributions of each memory T-cell subset in IBD remain unknown. T cells in the intestinal epithelial compartment and lamina propria are heterogeneous (Panel C). Recent single-cell studies have shown increased numbers and proportions of various T-cell subpopulations in inflamed IBD tissue. The relative contributions and interrelationships of these T-cell subpopulations in IBD is unknown. Eomes denotes Eomesodermin.

remains unclear whether these cells represent a subset of Treg cells adapted to suppress type 17 immune responses or cells that are in the process of converting to a pathogenic effector phe-

notype by virtue of losing FOXP3 expression, adding to the uncertainty regarding Treg-cell plasticity and stability. Moreover, because Treg cells in these studies have been assessed in the context of active IBD, it is difficult to determine whether the Treg-cell phenotypes observed are a cause or consequence of intestinal inflammation.

These issues notwithstanding, strategies for boosting Treg-cell numbers, function, or both to ameliorate intestinal inflammation in IBD are being evaluated. These therapeutic approaches focus on rationally derived microbial consortia that promote Treg-cell differentiation and function (discussed above); low-dose interleukin-2, which, owing to constitutive expression of the high-affinity interleukin-2 receptor by Treg cells, selectively expands the number of Treg cells rather than effector T cells<sup>61</sup>; interleukin-2–anti-interleukin-2 antibody complexes that have been shown to be more stable *in vivo*<sup>62</sup>; engineered interleukin-2 variants, called muteins, that selectively bind to the high-affinity interleukin-2 receptor<sup>63</sup>; and adoptive cell therapy involving Treg cells derived from peripheral blood and increased in number *in vitro*.<sup>64</sup>

**MEMORY T CELLS**

In addition to producing heterogeneous subsets of effector and Treg cells, activated CD4<sup>+</sup> and CD8<sup>+</sup> T cells can give rise to functionally diverse circulating and tissue-resident subsets of memory cells (Fig. 3); tissue-resident memory T (T<sub>RM</sub>) cells are characterized by high expression of CD69 and CD103 in the gut mucosa.<sup>38</sup> Once formed in response to microbial challenges, T<sub>RM</sub> cells positioned at key barrier surfaces, such as the skin and intestinal, genital, and respiratory mucosa, function to diminish the microbial load in the earliest phase of infection by directly recognizing antigen, augmenting innate immunity, and recruiting circulating memory T cells.<sup>65</sup>

Tissue-resident immune cells, owing to their activated, poised state and anatomical location at barrier surfaces, may play a pathogenic role in organ-specific autoimmune and inflammatory diseases.<sup>66</sup> The observation that Crohn's disease is often manifested as skip lesions (areas of discrete inflammation separated by normal mucosa) is reminiscent of psoriasis, in which exacerbations tend to affect the same region of skin and have been linked to clonally related T<sub>RM</sub>-like cells.<sup>67</sup> Moreover, the tendency of Crohn's dis-



ease to recur at the site of surgical anastomosis after ileocelectomy<sup>68</sup> raises the possibility that  $T_{RM}$  cells are involved. Indeed, numbers of  $T_{RM}$ -like cells are increased in both ulcerative colitis and Crohn's disease. Increased numbers of intestinal interleukin-17A-producing, commensal microbiota-reactive CD4+ T cells with a CD154<sup>hi</sup> memory phenotype have been observed in both patients with ulcerative colitis and those with Crohn's disease, as compared with healthy controls.<sup>69</sup> Similarly, increased numbers of CD4+CD69+CD103+  $T_{RM}$  cells were observed in intestinal tissue from patients with ulcerative colitis and those with Crohn's disease, as compared with healthy controls.<sup>70</sup> In particular, CD8+  $T_{RM}$  cells may exist in several transcriptionally distinct states; in patients with ulcerative colitis, increased numbers of cells are found in an inflammatory state and are characterized by elevated expression of the transcription factor Eomesodermin (Eomes<sup>hi</sup>),<sup>15</sup> adding to the growing evidence for murine and human  $T_{RM}$ -cell heterogeneity.<sup>71-73</sup>

Genetic-deletion approaches have been used to show that  $T_{RM}$  cells play an important functional role in murine models of experimental colitis.<sup>70</sup> The recent finding that murine CD8+ T cells can mediate increased intestinal barrier permeability suggests one mechanism by which CD8+ T cells might be acting in the context of IBD.<sup>74</sup> Data showing that murine and human  $T_{RM}$  cells can exit tissue and recirculate<sup>75,76</sup> are consistent with the observation that patients with ulcerative colitis have increased numbers of CD8+ T cells in peripheral blood that are clonally related to intestinal CD8+  $T_{RM}$  cells in the inflammatory state,<sup>15</sup> raising the intriguing possibility that recirculation of CD8+  $T_{RM}$  cells that have exited the intestinal tissue may be related to the tendency of IBD to affect organ systems outside the gastrointestinal tract. The relationship of these Eomes<sup>hi</sup> CD8+  $T_{RM}$  cells with other T-cell subpopulations that have been found to be enriched in patients with ulcerative colitis, such

as CD8+interleukin-17A+,<sup>14</sup> CD4+CD8+interleukin-17A+,<sup>14</sup> CD3+CD4-CD8-interleukin-17A+,<sup>2</sup> and CD8+interleukin-26+<sup>16</sup> subtypes, as well as their functional involvement in IBD and antigen specificity, remains to be determined. Taken together, these data raise the possibility that long-lived memory T-cell populations, particularly tissue-resident subsets, may contribute to the chronicity of IBD and represent a potential target for therapy.

#### FUTURE DIRECTIONS

Technological innovations (Table S2), such as integrated epigenetic and gene expression profiling in the same single cells, spatial transcriptomics, and high-parameter protein profiling approaches, will continue to advance our understanding of the heterogeneity and complexity in the epithelial, stromal, and immune compartments of the gastrointestinal tract in a healthy state and in IBD. Use of model systems (Table S2) that may better recapitulate human biology, such as organoids derived from patient samples or mice housed in barrier-free conditions ("dirty" mice), may accelerate our understanding of factors that initiate and maintain IBD. Causal links between individual microbes or groups of microbes and functional effects on the mucosal immune system will be strengthened, leading to new microbiota-based therapies. Classification algorithms based on machine learning and incorporating increasingly "multi-omic" data may improve risk stratification, as well as predictions of disease progression and the likelihood of a response to a specific drug therapy. Together, these current and future research efforts may lead to paradigm-shifting discoveries, new therapies, and ultimately, improved care for patients with IBD.

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