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# Intestinal macrophages and response to microbial encroachment

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Macrophages in the gastrointestinal mucosa represent the largest pool of tissue macrophages in the body. In order to maintain mucosal homeostasis, resident intestinal macrophages uniquely do not express the lipopolysaccharide (LPS) co-receptor CD14 or the IgA (CD89) and IgG (CD16, 32, and 64) receptors, yet prominently display Toll-like receptors (TLRs) 3–9. Remarkably, intestinal macrophages also do not produce proinflammatory cytokines in response to TLR ligands, likely because of extracellular matrix (stromal) transforming growth factor- $\beta$  (TGF- $\beta$ ) dysregulation of nuclear factor (NF)- $\kappa$ B signal proteins and, via Smad signaling, expression of I $\kappa$ B $\alpha$ , thereby inhibiting NF- $\kappa$ B-mediated activities. Thus, in noninflamed mucosa, resident macrophages are inflammation anergic but retain avid scavenger and host defense function, an ideal profile for macrophages in close proximity to gut microbiota. In the event of impaired epithelial integrity during intestinal infection or inflammation, however, blood monocytes also accumulate in the lamina propria and actively pursue invading microorganisms through uptake and degradation of the organism and release of inflammatory mediators. Consequently, resident intestinal macrophages are inflammation adverse, but when the need arises, they receive assistance from newly recruited circulating monocytes.

## INTRODUCTION

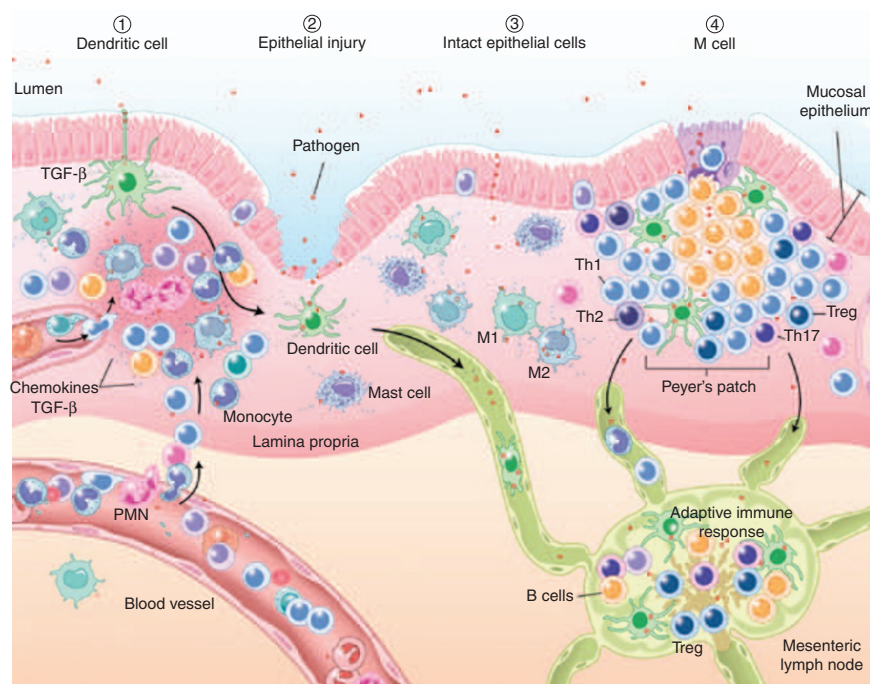
Intestinal and colonic macrophages represent the largest population of mononuclear phagocytes in the body.<sup>1</sup> Located strategically in the subepithelial lamina propria in close proximity to huge numbers of luminal bacteria and antigenic stimuli, gut macrophages regulate inflammatory responses to bacteria and antigens that breach the epithelium, protect the mucosa against harmful pathogens, and scavenge dead cells and foreign debris (**Figure 1**). In these effector roles, gut macrophages are part of the first-line defense mechanisms, referred to collectively as the innate immune system, with which the host responds to harmful microorganisms and their immunostimulatory products.<sup>2</sup> These first-line mechanisms constitute an ancient host defense system that arose an estimated billion years ago in early multicellular organisms and are still utilized by protozoa, insects, plants, and animals.<sup>3</sup> In animals, macrophages concentrate in the gastrointestinal mucosa, the body surface colonized shortly after birth by a multitude of microbial species, resulting in a microbe-mucosal ecosystem that contains over a hundred trillion bacteria in the adult gastrointestinal tract.<sup>4</sup>

In response to this crowded sea of microbes, innate host responses must distinguish nonharmful commensal organisms

from those that pose a danger to the host. The gut epithelium typically provides a structural barrier to both commensals and pathogens and secretes a protective coat of mucus that contains antimicrobial peptides, including defensins and serine leukocyte protease inhibitor<sup>5,6</sup> that restrict microbial translocation. When these impediments are breached through injury or infection, or when microorganisms undergo epithelial cell transcytosis or translocation via M cells or dendritic cells (DCs) (**Figure 1**), an array of subepithelial innate defense cells, especially mucosal macrophages, are poised for response. Such responses are rapid, initiated within minutes, and directed toward conserved structures in infectious agents that may cross the mucosal epithelium (**Figure 1**). Macrophage recognition of these patterns is mediated in part by pattern recognition receptors that include the germ-line-encoded transmembrane Toll-like receptors (TLRs) and the cytosolic NOD-like receptors,<sup>7,8</sup> predetermined repertoires of molecules that recognize foreign carbohydrate, lipid, and nucleic acid ligands (pathogen-associated molecular patterns) essential for microbe survival, thereby limiting the diversity of ligands to which macrophages can respond. Predetermination comes at a price, however, as the innate immune system, which also includes polymorphonuclear leukocytes, natural killer cells,

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**Figure 1** Mucosal exposure to pathogens and host response. The mucosal epithelium presents a formidable structural and chemical barrier to ill-intentioned microbes, which gain entry into the lamina propria (1) by dendritic cells, (2) through disrupted or injured epithelium, (3) between or through intact epithelial cells, and (4) through M cells. In noninflamed lamina propria, macrophages phagocytose and kill microbes without an inflammatory response. In the presence of inflammation and dysregulated mucosal homeostasis, newly recruited monocyte-derived macrophages (and other innate host defense cells) generate transforming growth factor- $\beta$  (TGF- $\beta$ ) and chemokines to recruit monocytes and other leukocyte populations to contain the infection. Microbes and/or their products are transported by dendritic cells in the lymphatics to draining lymph nodes where they induce an adaptive immune response. Failed clearance of the microbe and/or an inability to restore homeostatic regulation because of genetic and environmental factors can perpetuate inflammation locally and/or systemically (illustration by Lydia Kibiuk, NIH).

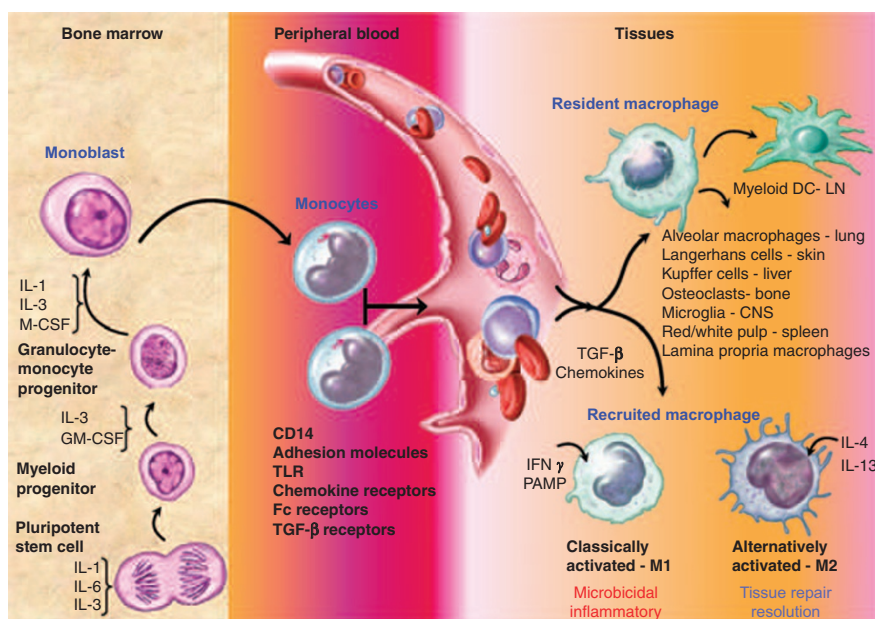
and DCs, cannot easily respond to novel molecules. Murine macrophages discriminate between pathogenic and non-pathogenic bacteria through a NOD-like receptor (NLR4) that detects the type III secretion apparatus used by Gram-negative bacteria to inoculate virulence factors into cells,<sup>9</sup> but whether human macrophages, and potentially mucosal macrophages, can distinguish between pathogenic and non-pathogenic bacteria is not known.

Complementing the innate system, but responding more slowly (hours to days), the acquired immune system of inducible defense mechanisms arose during the divergence of vertebrates from invertebrates, first in jawless vertebrates such as the lamprey and hagfish ~500 million years ago,<sup>10</sup> and then in jawed vertebrates ~400 million years ago.<sup>11</sup> Variations of this host defense system are present in fish, amphibians, reptiles, birds, and mammals, but not in invertebrates. The T and B lymphocytes of the acquired immune system utilize antigen-specific receptors and immunoglobulins (Igs), respectively, to recognize antigenic epitopes. In contrast to the evolutionarily conserved genes for innate response receptors,<sup>12</sup> the genes for antigen-specific receptors undergo somatic recombination, enhancing ligand diversity. Immunologic memory is a key feature of the adaptive, but not the innate, immune system. Macrophages, along with DCs, present foreign antigens to cells of the acquired immune system, thereby integrating the innate and acquired systems for quick, yet broad, responses to microorganisms and antigens. The role of gut macrophages in antigen presentation, however, has not

been thoroughly explored. Therefore, this review focuses on the host effector functions of gut macrophages.

### SOURCE OF MUCOSAL MACROPHAGES

All tissue macrophages are derived from bone marrow stem cells through a highly regulated cascade of differentiation events<sup>13,14</sup> (Figure 2). In the bone marrow, a combination of cytokines that include interleukin (IL)-1, IL-3, and IL-6 stimulates stem cell heteromitosis, resulting in a pluripotent granulocyte/erythrocyte/megakaryocyte/macrophage colony-forming unit. In the continued presence of IL-1 and IL-3, this precursor develops into the progenitor for both granulocytes and macrophages, referred to as a granulocyte-macrophage colony-forming unit. IL-3 and granulocyte/macrophage colony stimulating factor (GM-CSF) induce proliferation of both granulocyte and monocyte precursors, whereas IL-1, IL-3, and M-CSF induce the proliferation and differentiation of monocyte precursors. The continued presence of M-CSF, together with GM-CSF and IL-3, induces further differentiation of the monocyte precursor into a monoblast, then promonocyte, and finally a monocyte. Under homeostatic conditions, a single (CD117<sup>+</sup>CX<sub>3</sub>CR1<sup>+</sup>) murine precursor can develop into a monocyte or DC,<sup>15</sup> but differentiation can be redirected under appropriate circumstances, such as tissue inflammation. In addition to cytokine growth factors, transcription factors are involved in macrophage differentiation.<sup>16</sup> These factors include PU.1 and AML1, which control myeloid cell development.<sup>17</sup>



**Figure 2** Myeloid cell recruitment, activation, and regulation. Pluripotent stem cells differentiate into myeloid progenitors in the bone marrow where, under the influence of growth and colony stimulating factors, the cells differentiate into monoblasts. Monoblasts in turn develop into monocytes and migrate from the bone marrow into the blood where they circulate for several days and then are distributed to the tissues either as resident macrophages or as recruited macrophages at sites of infection and inflammation. Resident macrophages are phenotypically and functionally influenced by their environment, which varies with the anatomical location. During an inflammatory reaction, circulating monocytes adhere and migrate across the endothelium in response to chemoattractant gradients generated by stromal and inflammatory cells. Depending on the extracellular milieu, the cells may become polarized, exhibiting characteristics of M1 and/or M2 macrophages.

PU.1 is particularly important, because it regulates the expression of the receptor for M-CSF, which is critical for M-CSF-dependent differentiation.<sup>18</sup> The transcription factors GATA-2, SCL, and c-Myb regulate myeloid cell survival. Additional transcription factors, including NF-M/C/EBP $\alpha$  HOXB7 and c-Myc regulate the intermediate stages of myeloid differentiation, and C/EBP $\beta$ , EBR-1, IRF-1, NF-Y, and some Jun/Fos and Stat proteins regulate monocyte maturation.<sup>19,20</sup>

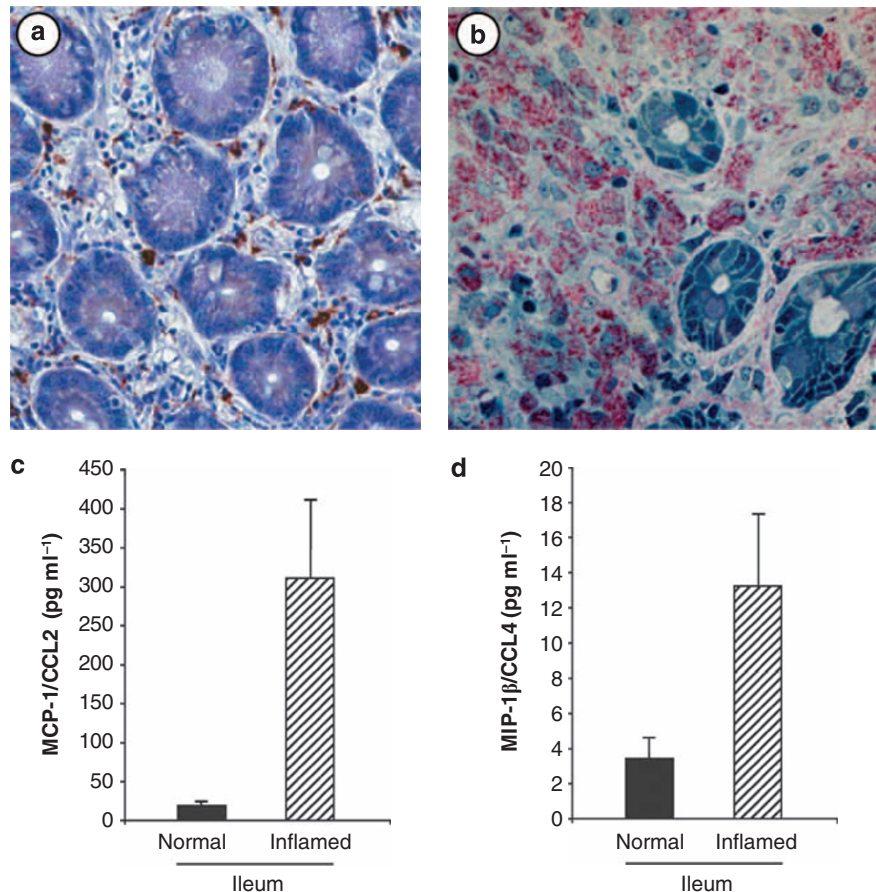
After leaving the bone marrow, monocytes enter the blood compartment, where they circulate for days before migrating into tissues,<sup>21,22</sup> including the mucosa (**Figure 2**). Circulating monocytes are typically CD14<sup>+</sup> and express Fc receptors, chemokine receptors, adhesion molecules, and TLRs, surface molecules that contribute to cell recruitment, retention, and pathogen-associated molecular pattern recognition at sites of infection and/or inflammation. After migration into the tissue, monocytes enlarge as they increase their content of lysosomal and hydrolytic enzymes, number and size of mitochondria, and energy requirements to become terminally differentiated resident macrophages. Distinct functional roles have been reported for subpopulations of macrophages. Referred to as M1 and M2 macrophages (**Figure 2**), these populations polarize generally along the Th1/Th2 paradigm based on cytokine release, chemokine elaboration, and enzyme production.<sup>23–25</sup> Such polarization is not well defined in human mucosal macrophages,<sup>26,27</sup> and we have not yet detected heterogeneity in macrophages isolated from normal human intestine, despite their functional proximity to M2 populations. After a period of weeks to possibly several months,<sup>21,22</sup> tissue macrophages undergo programmed cell death and are replaced by newly recruited blood monocytes.

## RECRUITMENT OF MONOCYTES TO INFLAMED AND NONINFLAMED GASTROINTESTINAL MUCOSA

Tissue mononuclear cells are replenished through the proliferation of resident cells, the migration of adjacent tissue cells, and the recruitment of circulating precursor cells. Intestinal macrophages, however, like many differentiated resident myeloid cells, do not proliferate and appear incapable of receptor-mediated chemotactic activity.<sup>28</sup> Thus, the recruitment of circulating precursor monocytes is the likely source of cells for the replacement of senescent and apoptotic macrophages in the gastrointestinal tract mucosa.

In noninflamed intestinal mucosa, transforming growth factor- $\beta$  (TGF- $\beta$ ) and IL-8 are constitutively produced by mucosal epithelial cells and mast cells and then bind to and are released by the lamina propria extracellular matrix (stroma).<sup>28–30</sup> In response to the potent chemokine activity of TGF- $\beta$  and IL-8, circulating blood monocytes localize to the lamina propria to become resident macrophages (**Figures 1, 2, and 4**). Supporting this concept, we have shown that intestinal stromal TGF- $\beta$  and IL-8 recruit blood monocytes,<sup>28</sup> an important finding because TGF- $\beta$  is the most potent natural chemokine for monocytes,<sup>31,32</sup> yet neither TGF- $\beta$  nor IL-8 induce chemotaxis by isolated intestinal macrophages. That chemokines released by the lamina propria extracellular matrix contribute to the recruitment of monocytes to noninflamed lamina propria is consistent with the gastrointestinal tract lamina propria being the largest reservoir of macrophages in the body. Thus, gut macrophages are derived exclusively from recruited blood monocytes, and, after terminal differentiation, they become resident macrophages in the mucosa of the small intestine and





**Figure 3** Influx of macrophages into the lamina propria in response to infection or inflammation. (a) Normal intestinal tissue (ileum) in which CD68<sup>+</sup> macrophages are distributed throughout the lamina propria. (b) Infection of an immunocompromised individual with *M. avium* (identified by red acid fast stain) results in striking increases in macrophage accumulation and phagocytosis of the bacteria in the lamina propria. (c, d) Conditioned medium obtained from normal/noninflamed intestinal tissues and from inflamed (inflammatory bowel disease (IBD)) tissues (ileum) reveals a significant elevation of monocyte-targeted chemokines including MCP-1/CCL2 (monocyte chemoattractant protein-1/chemokine (C-C motif) ligand 2) and MIP-1β/CCL4 (macrophage inflammatory protein-1/chemokine (C-C motif) ligand 4), which contribute to the recruitment of monocytes into inflamed or infected intestinal sites. Chemokine levels were determined using a bead-based multiplex cytokine assay (Biosource International, Camarillo, CA).

colon, where they survive for weeks to months, characteristic of macrophages in other tissues.<sup>20,21</sup>

In studies of macrophage accumulation in inflamed gastrointestinal mucosa, immunohistochemical analysis has shown that the endothelial cells lining small blood vessels in the mucosa of patients with Crohn's disease display high levels of CD34, a ligand that promotes the rolling of L-selectin<sup>+</sup> monocytes in high endothelial venules.<sup>33</sup> More recently, antibody blocking studies have shown that PSLG-1 (P-selectin glycoprotein ligand-1), P-selectin, and VCAM-1 (vascular cell adhesion molecule-1) promote CD14<sup>+</sup> monocyte rolling and adherence in the intestinal mucosa, particularly ileal mucosa, in a mouse model of spontaneous ileitis.<sup>34</sup> Finally, increased levels of ICAM-1 (intercellular adhesion molecule 1) and CD31, which facilitate transendothelial migration of monocytes, are also present in Crohn's disease lesions.<sup>33</sup> Thus, endothelial cells in mucosal vessels express molecules that promote the rolling, adherence, and subsequent transendothelial migration of circulating blood monocytes into inflamed gastrointestinal mucosa. In addition, recruitment factors, including CCL2 and CCL4 (chemokine (C-C motif) ligand 2 and 4; **Figure 3**) that selec-

tively induce monocyte transendothelial migration and accumulation, may be released in inflamed and/or infected mucosa, enhancing the migration of monocytes into the mucosa.

In the 1990s, macrophages in normal human colonic and intestinal mucosa were shown to lack CD14.<sup>35,36</sup> In contrast, the expression of this receptor on a substantial proportion of mucosal macrophages in patients with inflammatory bowel disease<sup>33,37–39</sup> suggested that significant numbers of newly recruited blood monocytes, which express CD14, are present in the inflamed mucosa. The absence of other features, including CD80 expression<sup>40</sup> and respiratory burst activity,<sup>41</sup> in macrophages in normal gut mucosa, but the presence of these features in macrophages in the inflamed mucosa of patients with Crohn's disease is consistent with blood monocyte recruitment to sites of mucosal inflammation. To explore experimentally the origin of CD14<sup>+</sup> macrophages in inflamed mucosa, Grimm *et al.*<sup>42</sup> showed that CD14<sup>+</sup> blood monocytes harvested from subjects with inflammatory bowel disease, labeled with <sup>99m</sup>technetium, and then inoculated back into the respective subjects migrated to the inflamed mucosa, confirming that the CD14<sup>+</sup> macrophages in the inflammatory lesions were newly recruited

proinflammatory blood monocytes. In this regard, the recently described<sup>43</sup> subset of macrophages isolated from the colon (not small intestine) of patients with inflammatory bowel disease that expressed CD14 and released proinflammatory cytokines likely represents newly recruited blood monocytes.

The recruitment of monocytes to the gastrointestinal mucosa is also accelerated during mucosal infection with organisms such as *Mycobacterium avium*, as shown in the infected human intestine in **Figure 3**. Compared with the prevalence of lamina propria macrophages in normal gut mucosa, the number of mucosal macrophages increases in response to bacterial and viral invasion,<sup>44,45</sup> as demonstrated by the increased number of lamina propria macrophages with internalized mycobacteria in *M. avium*-infected mucosa (**Figure 3**). Whether mucosal macrophages infected with opportunistic pathogens in immunosuppressed subjects exhibit a CD14<sup>+</sup> phenotype has not been defined, but the cells likely represent monocyte-derived macrophages that have exited the circulation at sites of infection and whose proinflammatory cytokine–chemokine profile has been dysregulated by the intracellular pathogen.<sup>46,47</sup>

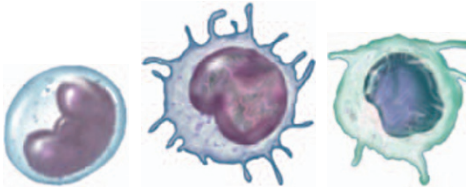
#### MUCOSAL MACROPHAGE RECOGNITION OF PATHOGENS

To distinguish between self and microorganisms, gut macrophages express an array of recognition receptors (**Table 1**), including TLRs 3–9, which are often present at higher levels than on autologous blood monocytes.<sup>48</sup> These receptors recognize conserved pathogen motifs such as lipopolysaccharide (LPS) and lipoteichoic acid on the surface of Gram-negative and Gram-positive bacteria, bacterial flagellins, and single-stranded RNA and DNA fragments, allowing the cells to detect infectious agents. Intestinal macrophages, like monocytes and monocyte-derived macrophages,<sup>49–52</sup> also express receptors for lipoprotein and phospholipid on apoptotic cells (CD36),<sup>29</sup> formyl C5aR peptides in bacteria (fMLPR), and complement component 5a (C5aR).<sup>28</sup> The presence of these innate receptors on intestinal macrophages facilitates the cells' efficient chemotaxis to and phagocytosis of bacteria, including *Escherichia coli* and *Salmonella*, and fungi such as the yeast *Candida albicans*.<sup>29,36</sup> The ability of intestinal macrophages to phagocytose inert material<sup>29,53</sup> and apoptotic mononuclear cells (L.E. Smythies, unpublished) is critical for the clearance of pathogen/cell debris and dead cells in the mucosal lamina propria. Intriguingly, intestinal macrophages lack the receptors for IgA and IgG<sup>29,53</sup> (**Table 1**), which mediate the recognition and uptake of IgA- and IgG-opsonized cells or particles, reducing mucosal macrophage integration of innate and adaptive immune responses. Thus, although resident intestinal macrophages lack some innate receptors, for example, Ig receptors and the LPS co-receptor (CD14) discussed below, they express receptors for conserved structures on bacteria, viruses, yeast, and certain parasites, which promote recognition of potentially harmful microbes and their subsequent phagocytosis.

Mutations in innate response genes that mediate bacterial recognition and processing have been identified as susceptibility loci for Crohn's disease. The first such gene to be identified was *NOD2/CARD15* (nucleotide-binding oligomerization domain 2/caspase activation and recruitment domain 15),<sup>54,55</sup> which

**Table 1 Phenotypic and functional differences between monocytes, macrophages, and intestinal macrophages**

	Monocyte	Inflammatory macrophage	Intestinal macrophage
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<b>Phenotype</b>			
CD4	+	+	Low
CD11a/LFA-1	+	+	–
CD11b/MAC-1	+	+	–
CD11c/integrin $\alpha$	+	+	–
CD13	+	+	+
CD14	+	Low	–
CD16/Fc $\gamma$ RIII	+	+	–
CD18/integrin $\beta$ 2	+	+	–
CD25/IL-2R $\alpha$	–	+	–
CD32/Fc $\gamma$ RII	+	+	–
CD33	+	–	+
CD36	+	+	+
CD40/TNFRSF5	+	+	–
CD64/Fc $\gamma$ RI	+	+	–
CD69	– (transient)	–	–
CD80/B7-1	–	+	–
CD86/B7-2	+	+	–
CD88/C5aR	+	+	Low
CD89/Fc $\alpha$ R	+	+	–
CD123/IL-3R $\alpha$	+	–	–
CD181/CXCR1 <sup>a</sup>	+	+	+
CD182/CXCR2 <sup>a</sup>	+	+	+
CD184/CXCR4	+	+	–
CD195/CCR5	+	+	–
HLA-DR	+	+	+
TGF- $\beta$ RI	+	+	+
TGF- $\beta$ RII	+	+	+
FPR1	+	+	Low
TREM-1	+	+	–
TLR1	+	+	–
TLR2	+	+	–
TLR4	+	+	+
TLR5	+	+	+

Table 1 continued on the following page

Table 1 (Continued)

	Monocyte	Inflammatory macrophage	Intestinal macrophage
TLR6	+	+	+
TLR10	+	+	?
TLR11	+	+	?
<b>Function</b>			
Phagocytosis	+	+	+
Killing	+	+	+
Chemotaxis	+	+ / -	-
Respiratory burst	+	+	-
Ag presentation	+	+	?
Cytokine production	+	+	-
Costimulation	+	+	-

Abbreviations: C5aR, complement component 5a receptor; CCR5, C-C chemokine receptor type 5; CXCR, chemokine (C-X-C motif) receptor; FPR1, formyl peptide receptor 1; IL-2R, interleukin-2 receptor; LFA-1, lymphocyte function-associated antigen 1; MAC-1, macrophage-1 antigen; TGF- $\beta$ , transforming growth factor- $\beta$ ; TLR, Toll-like receptor; TNFRSF5, tumor necrosis factor receptor superfamily, member 5; TREM-1, triggering receptor expression on myeloid cells 1; ?, not known.

<sup>a</sup>CD181/CXCR1 = IL-8R $\alpha$ ; CD182/CXCR2 = IL-8R $\beta$ .

encodes intracellular pattern recognition receptors for muramyl dipeptide, a component of the peptidoglycan cell wall in Gram-positive and Gram-negative bacteria. Mutations in the *ATG16L1* (autophagy-related like 1)<sup>56,57</sup> and *IRGM* (immunity-related GTPase family M)<sup>58,59</sup> genes, which encode proteins involved in autophagy, a process by which cell debris is directed into double-membrane autophagosomes for delivery into the lysosomal degradation pathway and by which intracellular bacterial growth is inhibited, have also been identified as genetic risk factors for Crohn's disease. These sets of genes appear functionally linked, as NOD1 and NOD2 were recently shown to recruit the autophagy protein ATG16L1 to the plasma membrane at the bacterial entry site to facilitate autophagosome formation.<sup>60</sup> NOD2 has been shown to mediate intracellular recognition of *Streptococcus pneumoniae*, *S. Typhimurium*, *Mycobacterium* spp., and *Listeria monocytogenes*, and potentially some viruses in experiments utilizing cell lines and murine models.<sup>61–63</sup> Also, Gram-negative and Gram-positive bacteria have been shown to induce monocyte-derived DCs via NOD2 recognition in order to polarize CD4<sup>+</sup> T cells for the production of IL-17,<sup>64</sup> a cytokine that is associated with the inflammatory response in Crohn's disease.<sup>65–68</sup>

Complementing the NOD2 studies, model systems have been used to show a crucial role for the *ATG16L1* gene product in intracellular clearance of *M. tuberculosis*, *S. Typhimurium*, and *Streptococcus pyogenes*.<sup>61</sup> Importantly, intracellular replication of adherent-invasive *E. coli*, which colonize the intestinal mucosa in high numbers in patients with Crohn's disease,<sup>69,70</sup> is inhibited by normal autophagy but enhanced in epithelial cell lines with defective autophagy.<sup>71</sup> As many of the bacteria used to study autophagy in these models infect and replicate in macro-

phages, it will be important to determine the role of autophagy in intestinal macrophage clearance of the organisms, particularly adherent-invasive *E. coli*. Genome-wide association studies will likely identify other genes that regulate intracellular pathogen recognition and clearance in mucosal macrophages.

The host response to gut microbes can have broad negative consequences, reflected in recent evidence linking the mucosal response to enteric bacteria and the development of autoimmune diseases.<sup>72</sup> For example, mice engineered to develop arthritis exhibit delayed and less severe arthritis in germ-free conditions, but the inoculation of a single enteric bacteria induces arthritis via Th17 cell activity.<sup>73</sup> Murine intestinal and colonic macrophages express IL-10, which together with retinoic acid and TGF- $\beta$  induce the differentiation of forkhead box p3<sup>+</sup> (Foxp3<sup>+</sup>) regulatory T (Treg) cells, promoting a tolerogenic mucosal microenvironment,<sup>74</sup> but human intestinal macrophages do not appear to produce detectable IL-10 or other immunoregulatory cytokines.<sup>29,48</sup> Polarization of CD4<sup>+</sup> T helper lymphocytes into cells of the Th17 lineage represents a response to intestinal microbe colonization, in the context of Th17-lineage promoting TGF- $\beta$ .<sup>75</sup> Additional local promoters of Th17 accumulation include IL-23 and IL-6, provided, in part, by recruited CD14<sup>+</sup> intestinal macrophages.<sup>43,76</sup> In turn, local Th17 populations may become enabling for distal autoimmune lesions, characterized by accumulation and generation of IL-17 and related factors.<sup>77</sup> Consequently, failure to regulate myeloid cell activation and responses to gut microbiota may precipitate or contribute to the development of intestinal and extraintestinal autoimmune inflammatory diseases.

#### MUCOSAL MACROPHAGE PHAGOCYTOSIS AND KILLING OF PATHOGENS

Receptor-mediated recognition is the first step in the interaction between mucosal macrophages and microorganisms. Consistent with their central role in host defense against microbes and noxious molecules that have reached the epithelium, recognition initiates engulfment, which leads to killing and degradation of the microbe. Intestinal macrophages exhibit greater phagocytic activity for inert material and stronger bacteriocidal activity than autologous blood monocytes.<sup>29</sup> However, intestinal macrophage phagocytosis does not induce cytokine release, irrespective of the internalized object, whereas monocyte phagocytosis of either inert material or bacteria results in strong proinflammatory cytokine release. Thus, in contrast to blood monocytes and macrophages in other tissues, resident intestinal macrophages perform host defense activities without inducing an inflammatory response, thereby contributing to the absence of inflammation during microbe encroachment. The mechanisms by which intestinal macrophages kill bacteria are not known, but the cells' bacteriocidal activity appears to be independent of reactive oxygen intermediates<sup>41</sup> (P.D. Smith, unpublished observations).

In immunocompromised conditions, newly recruited monocytes and resident macrophages avidly phagocytose certain pathogens, particularly opportunistic microorganisms such as *M. avium*, but internalization may not lead to degradation of the organism (Figure 3).<sup>45</sup> The mechanisms responsible for defective



macrophage clearance of such organisms are not known, but deficits in CD4<sup>+</sup> T cells and interferon- $\gamma$  triggering are likely contributing factors.<sup>45,46</sup> Besides *M. avium*, tissue macrophages, including mucosal macrophages, are capable of harboring other intracellular pathogens such as *M. tuberculosis*, *Histoplasma capsulatum*, and cytomegalovirus.<sup>78,79</sup>

### MECHANISMS THAT DAMPEN RESIDENT MUCOSAL MACROPHAGE INFLAMMATORY RESPONSES

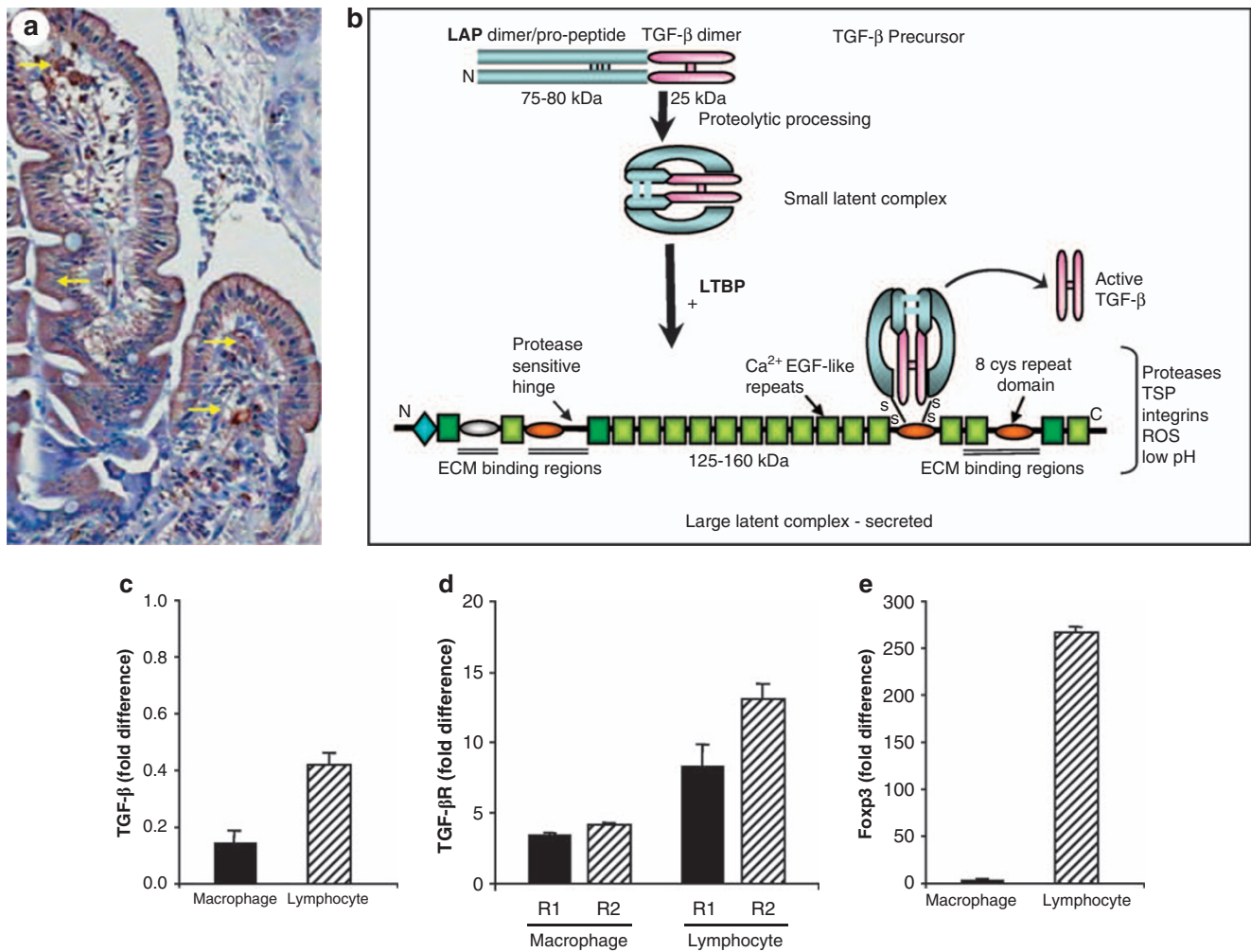
For millions of years, an astonishingly unhygienic external environment presented the evolving intestine of early vertebrates, then non-human primates, and eventually humans, a spectrum of colonized microorganisms and infectious pathogens likely far greater in complexity and numbers than the “typical” enteric microbiota of today. In such an inhospitable environment, a disrupted intestinal epithelium resulting from frequent infections was probably common. Because the exposure of myeloid effector cells to mucosal bacteria or their products can trigger a striking, and potentially life-threatening, proinflammatory transcriptional response,<sup>49</sup> the gastrointestinal mucosa co-evolved to downregulate inflammatory, but not antimicrobial, responses to luminal microorganisms that breach the disrupted epithelium. During this co-evolution, resident lamina propria macrophages emerged, at least in humans, unique for their capacity to phagocytose and digest microorganisms and innate material without an inflammatory response, a distinct selective advantage to the host. Extended to immune surveillance, our unpublished findings indicate that intestinal macrophages also scavenge apoptotic mononuclear cells without the release of proinflammatory cytokines. Today, the unique phenotype and functional profile of gut macrophages described below likely contributes to the absence of mucosal inflammation in the small intestine, despite a “cleaner” microbiota.

The ability of macrophages to elaborate over 100 secretory products contributes to the cells’ ability to perform proinflammatory, host defense, and tissue remodeling activities.<sup>49,80</sup> To reconcile this exuberant inflammatory potential and the absence of inflammation in the normal human small intestine, we have shown that intestinal macrophages are potently downregulated at both mRNA and protein levels for multiple innate response molecules (**Table 1**), including the receptors for LPS (CD14), Fc $\alpha$  (CD89), Fc $\gamma$  (CD64, CD32, CD16), CR3 (CD11b/CD18), and CR4 (CD11c/CD18); receptors for the growth factors IL-2 (CD25) and IL-3 (CD123); and the integrin LFA-1 (CD11a/CD18), the costimulatory molecules CD40, CD80, and CD86, and TREM-1 (triggering receptor expression on myeloid cells 1).<sup>29,36,48,53,81</sup> Intestinal macrophages are also downregulated for the production of TLR-inducible cytokines, including proinflammatory IL-1, IL-6, IL-8, tumor necrosis factor- $\alpha$ , IL-10, and RANTES, irrespective of the stimulus (bacterial products, cytokines, and phagocytosis).<sup>29,53</sup> The inability of most intestinal macrophages to elaborate proinflammatory cytokines is the consequence of near-absent or markedly reduced MyD88 (myeloid differentiation primary response gene (88)), Toll/IL-1 receptor (TIR)-domain containing adapter-inducing IFN  $\beta$  adapter

protein, and TRAF6 (tumor necrosis factor receptor-associated factor 6) signal protein.<sup>48</sup> As MyD88 is crucial for nuclear factor (NF)- $\kappa$ B activation of all TLRs except TLR3, and as TIR mediates TLR3-induced RANTES and interferon- $\beta$  production (as well as TLR4-mediated MyD88-independent signaling), the downregulated MyD88 and TIR predictably result in NF- $\kappa$ B inactivation. Moreover, as TREM-1 appears inducible by TLR2/4 ligands and requires TIR to signal an LPS response,<sup>82</sup> the compromised TLR signaling and downregulated TIR expression likely contribute to reduced TREM-1 responsiveness in intestinal macrophages.<sup>83–86</sup> In addition, active Smad signaling in the macrophages promotes constitutive expression of I $\kappa$ B $\alpha$ , the negative regulator of NF- $\kappa$ B, which together with the cells’ inability to phosphorylate NF- $\kappa$ B and I $\kappa$ B $\alpha$ , also blocks the cells’ ability to propagate an NF- $\kappa$ B signal, leading to inhibition of NF- $\kappa$ B-mediated activities and profound inflammation anergy. Consistent with these findings, TGF- $\beta$  has also been shown to block NF- $\kappa$ B activation in response to TLR2, 4, and 5 signaling by facilitating ubiquitination and proteosomal degradation of MyD88.<sup>87</sup> Remarkably, extracellular matrix (stroma) TGF- $\beta$  induces similar downregulation in NF- $\kappa$ B signaling and NF- $\kappa$ B-mediated function in blood monocytes,<sup>48</sup> implicating stromal TGF- $\beta$  in the inactivation of NF- $\kappa$ B that characterizes the differentiation of proinflammatory blood monocytes into noninflammatory intestinal macrophages. Importantly, in the mucosa of people with inflammatory bowel disease, intestinal macrophages may express high levels of NF- $\kappa$ B DNA-binding activity,<sup>88</sup> and NF- $\kappa$ B may be activated;<sup>89</sup> these cells likely represent newly recruited monocytes that have not been downregulated because of the loss of normal homeostatic controls.

In the mucosa, latent TGF- $\beta$  is produced by an array of different cells, including epithelial cells, mast cells, and stromal cells (**Figures 1 and 4**). Under homeostatic conditions, T cells undergoing apoptosis are not only a target for phagocytic cells but also release TGF- $\beta$  from their intracellular membrane-bound compartment<sup>90</sup> and thus constitute another important source of TGF- $\beta$  in the mucosal lamina propria (**Figures 1 and 4**). This protective response facilitates noninflammatory clearance of dead cells, as well as the noninflammatory engulfment and clearance of invading intestinal microorganisms by resident macrophages. In the setting of inflammation, the recognition and uptake of apoptotic mucosal cells by newly recruited monocytes provides another source of immunoregulatory cytokines, including TGF- $\beta$ .<sup>91,92</sup> The subsequent binding of the TGF- $\beta$  to lamina propria extracellular matrix (stroma) via extracellular binding domains (**Figure 4b**) establishes a “TGF- $\beta$  reservoir.”<sup>30,32,93</sup> Activation and release of TGF- $\beta$  from the reservoir leads to engagement of cognate receptors TGF- $\beta$ R1 and TGF- $\beta$ R2 expressed on intestinal macrophages,<sup>28</sup> and newly recruited monocytes (**Figure 4d**), promoting cell recruitment, noninflammatory host defense, and noninflammatory immune surveillance of apoptotic cells.

Another influence on macrophage behavior is the population of Foxp3<sup>+</sup> Treg cells. In addition to thymus-derived Tregs,



**Figure 4** Constitutive and inducible expression of transforming growth factor- $\beta$  (TGF- $\beta$ ). **(a)** Section of intestinal mucosa shows TGF- $\beta$  staining in epithelial cells (arrows) and cells morphologically consistent with lymphocytes, and mast cells (arrows), and the lamina propria matrix. **(b)** TGF- $\beta$  is secreted as a large latent complex that contains extracellular matrix (ECM) binding domains and is retained in a latent state while attached to ECM. Once TGF- $\beta$  is activated and released from the ECM, it can interact with TGF- $\beta$  receptors on target cells to initiate Smad-dependent and -independent signaling (adapted from ref. 133). **(c–e)** Lymphocytes and macrophages were isolated from noninflamed intestinal mucosa and RNA prepared for reverse transcriptase PCR (RT-PCR) for **(c)** TGF- $\beta$ , **(d)** TGF- $\beta$ R1 and TGF- $\beta$ R2, and **(e)** forkhead box p3 (Foxp3). Results were obtained using the  $2^{-\Delta\Delta Ct}$  method and expressed as fold difference relative to isolated peripheral blood monocyte populations.

peripheral CD4<sup>+</sup> T cells, under the influence of antigen-specific receptor stimulation in the presence of TGF- $\beta$ , are induced to express the transcription factor Foxp3 and orchestrate suppression of both pathologic and healthy immune responses.<sup>94–96</sup> In support of these tolerogenic pathways, lymphocytes isolated from the lamina propria represent an abundant source of Foxp3 expression (**Figure 4e**), consistent with their important role in providing additional protection from the untoward consequences of an unrestricted inflammatory response to the intestinal microbiota. Indeed, the prevalence of these important immunoregulatory cells is increased in the lamina propria of the intestine in people with Crohn's disease<sup>97</sup> and the stomach in children with *Helicobacter pylori* infection.<sup>98</sup>

Peripheral tolerance to nonpathogenic microbes and/or environmental antigens is also coordinated via intestinal lamina propria DCs that require activation of the  $\beta$ -catenin signaling pathway to secrete immunosuppressive cytokines that drive

Treg cell induction.<sup>99</sup> Intimately linked to intestinal homeostasis, altered expression of TGF- $\beta$  and/or expression of other co-regulatory cytokines (i.e., IL-6, IL-1, and IL-10) sets in motion a cascade of immunopathogenic events. As failure to limit innate pathways is linked to the development of intestinal disease, multiple non-redundant pathways are in place to control these events. Continued elucidation of these complex pathways will enhance our understanding of how innate responses protect the host from commensal and pathogenic microbes, providing rationale for the design of new approaches to intervention, with an emphasis on intestinal macrophages and their return to homeostasis and regenerative functions.

#### MUCOSAL MACROPHAGES AND DEFENSE AGAINST HIV-1

The mucosal surfaces of the gastrointestinal tract are the route by which human immunodeficiency virus-1 (HIV-1) enters the host in most mother-to-child and male homosexual transmissions.



Routes of entry into the lamina propria include direct inoculation through an epithelium disrupted by trauma or infection, uptake by intraepithelial DC processes, M-cell translocation, and epithelial cell transcytosis<sup>100–103</sup> (Figure 1). In the lamina propria, HIV-1 encounters permissive intestinal CD4<sup>+</sup>CCR5<sup>+</sup> T cells,<sup>104</sup> which support productive viral infection and undergo early, rapid, and profound depletion during primary HIV-1 and simian immunodeficiency virus infection.<sup>105–112</sup> Resident macrophages in the human small intestine, however, are incapable of supporting productive HIV-1 infection,<sup>113–115</sup> although extraintestinal macrophages have crucial roles in the establishment, pathogenesis, and latency of HIV-1 infection.<sup>116–123</sup> The non-permissiveness of intestinal macrophages to HIV-1 appears to be the consequence of marked downregulation of surface CD4/CCR5 expression and NF- $\kappa$ B activation (R. Shen, unpublished data), requirements for HIV-1 entry and transcription, respectively. However, after CD4<sup>+</sup> T-cell depletion in late-stage disease, HIV-1-infected monocytes circulating in the blood may be recruited to inflamed mucosa deficient in normal downregulatory mechanisms, allowing viral replication to continue after the cells take up residence in the lamina propria, as we have reported for esophageal macrophages in patients with AIDS and opportunistic mucosal infections.<sup>124</sup> Indeed, tissue macrophages infected with opportunistic pathogens are particularly vulnerable to productive infection and can represent formidable cellular factories of HIV-1 production.<sup>45,119</sup>

Differential expression of host cell antiviral mechanisms in monocytes compared with mature macrophages contribute to their discordant HIV-1 permissiveness.<sup>125–128</sup> Such restrictions include cytoplasmic APOBEC (apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like) subunits, particularly APOBEC3G (hA3G) and hA3A,<sup>129,130</sup> which become incorporated into virions and cause dC-to-dU mutations in nascent HIV-1 DNA during reverse transcription and lead to subsequent viral degradation.<sup>130–132</sup> However, resident intestinal macrophages appear to have a post-entry block in the capacity to replicate virus<sup>115</sup> not readily attributable to APOBEC (R. Shen, unpublished data). Elucidation of the mechanisms responsible for the inability of intestinal macrophages to support HIV-1 infection are currently receiving intense investigative attention in an attempt to inform development of novel therapeutic strategies based on endogenous restriction.

## CONCLUDING REMARKS

Monocytes exit the circulation and take up residence in tissues throughout the body. Of these populations, lamina propria macrophages in the distinct microenvironment of the gastrointestinal mucosa acquire a unique phenotypic and functional profile, mediated at least in part by matrix-bound TGF- $\beta$  and Treg cells. In the homeostatic conditions of noninflamed mucosa, many pattern recognition receptors are downregulated, along with their pathogen-associated molecular pattern-engaged signal transduction pathways, resulting in inflammation anergy. In the event of an epithelial breach and/or microbial invasion, however, the suppressive milieu is challenged by newly recruited circulating monocytes and lymphocytes, which generate proinflammatory

mediators and engage a protective response. Failure to resolve this antimicrobial response and return the mucosa to steady-state conditions can promote chronic inflammation in an array of mucosal diseases.

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## DISCLOSURE

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