Intestinal Inflammation and Mucosal Barrier Function

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Abstract: Intestinal mucosal barrier function is the capacity of the intestine to provide adequate containment of luminal microorganisms and molecules while preserving the ability to absorb nutrients. The central element is the epithelial layer, which physically separates the lumen and the internal milieu and is in charge of vectorial transport of ions, nutrients, and other substances. The secretion of mucus-forming mucins, sIgA, and antimicrobial peptides reinforces the mucosal barrier on the extraepithelial side, while a variety of immune cells contributes to mucosal defense in the inner side. Thus, the mucosal barrier is of physical, biochemical, and immune nature. In addition, the microbiota may be viewed as part of this system because of the mutual influence occurring between the host and the luminal microorganisms. Alteration of the mucosal barrier function with accompanying increased permeability and/or bacterial translocation has been linked with a variety of conditions, including inflammatory bowel disease. Genetic and environmental factors may converge to evoke a defective function of the barrier, which in turn may lead to overt inflammation of the intestine as a result of an exacerbated immune reaction toward the microbiota. According to this hypothesis, inflammatory bowel disease may be both precipitated and treated by either stimulation or downregulation of the different elements of the mucosal barrier, with the outcome depending on timing, the cell type affected, and other factors. In this review, we cover briefly the elements of the barrier and their involvement in functional defects and the resulting phenotype.

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Key Words: tight junction, epithelial permeability, pattern recognition receptor, inflammatory bowel disease, microbiota

The intestine serves the essential function of providing the organism with water and nutrients for the sustainment of life. To achieve this goal, transport of these substances must be finely regulated to maintain homeostasis. The intestinal epithelium is in charge of these essential transport tasks and constitutes a physical barrier that regulates the selective paracellular permeability to ions and molecules. At the same time, it coexists with the intestinal microbiota tolerating commensal bacteria while fighting pathogens to preserve homeostasis.[1] Mucosal barrier function (MBF) is the capacity of the intestine to provide adequate containment of luminal microorganisms and molecules while preserving the ability to absorb nutrients.

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MBF is built upon the main physical barrier, i.e., the epithelium, which is a monolayer mainly composed of 4 types of intestinal epithelial cells (IECs): absorptive enterocytes, mucus-producing goblet cells, antimicrobial peptide (AMP)-producing Paneth cells, and hormone-producing enteroendocrine cells. The epithelium is protected by a mucus layer, AMP, and secretory IgA (sIgA). In addition, the microbiota itself can be considered to be part of the intestinal barrier, in as much as it can enhance or weaken MBF. Underneath the intestinal epithelium, the lamina propria contains dendritic cells, macrophages, innate lymphoid cells, T lymphocytes, and plasma cells. Both epithelial and lamina propria cells are main mediators of the innate and adaptive immune responses, that are also components of MBF (Fig. 1).

Alterations of MBF are increasingly being related to a wide variety of disorders directly or indirectly linked to the intestine, including inflammatory bowel disease (IBD), irritable bowel syndrome, metabolic syndrome, allergy, hepatic inflammation, septic shock, etc.[2] IBD comprises ulcerative colitis (UC) and Crohn's disease (CD), which are chronic inflammatory conditions of the intestine that are of undetermined origin and share a number of characteristics but also have distinct features. As implied by the name, UC affects only the large intestine, and colectomy is curative; it affects the rectus and/or anus and progresses proximally, causing superficial inflammation. CD is characterized by transmural inflammation and can affect any part of the gut, although most often, the ileocecal region is involved. Here, we review the role and importance of the main elements of the MBF and discuss their relevant role in intestinal inflammation, particularly IBD.

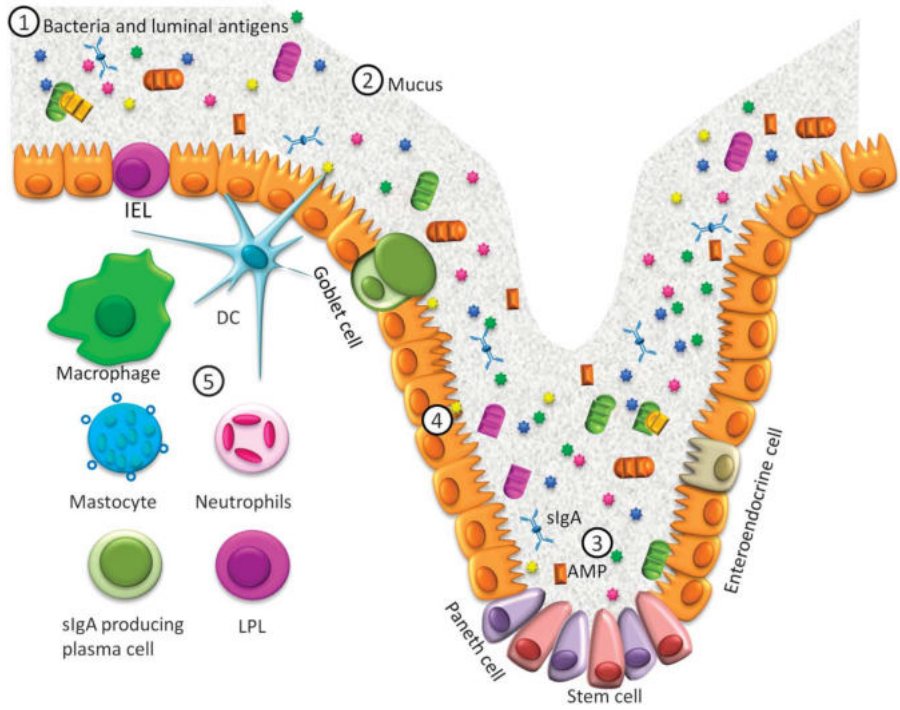

FIGURE 1. Elements of the MBF. 1, the microbiota; 2, the mucus layer; 3, AMP and sIgA; 4, the epithelium; 5, the mucosal immune system. The mucosal immune system also involves epithelial cells.

COMPONENTS OF THE INTESTINAL BARRIER

Microbiota

The microbiota colonizes the gastrointestinal tract immediately after birth and contributes to MBF maturation, a process that is delayed by antibiotic treatment.[3] Although the microbiota is nonessential, there is a mutual benefit relationship between luminal microbes and the host.[1] The microbiota greatly influences MBF through both direct and indirect modulation of the epithelial layer and mucosa. For example, the microbiota stimulates epithelial proliferation and secretion of IL-8, one of the main chemokines, through Toll-like receptor (TLR)-5 ligation and IL-22 production (see below).[4] The composition of the microbiota critically influences the relationship with the host, and is modulated by the environment, diet, and genetic factors. Alteration of the normal microbiota (dysbiosis) is a known feature of IBD and also other conditions related to abnormal MBF. These alterations include, for instance, a decreased level of *Bacteroides*, *Lactobacilli*, *Bifidobacteria*, and *Clostridium* IXa and IV groups, and an increase in *Escherichia coli*, among others. Dysbiosis may result at least partly from defects in innate or adaptive immunity. For instance, in nucleotide-binding oligomerization domain (NOD)-like receptor family pyrin domain–containing (NLRP)-6-deficient mice, there is an increase in Bacteroidetes and TM7 bacteria,[4] and sIgA-deficient mice show an increase in segmented filamentous bacteria.[5]

It is not clear how dysbiosis may lead to intestinal inflammation. Because IBD has not been linked with any particular microorganism, carrying a host unfriendly microbiota must be considered one of the multiple risk factors potentially leading to the inflammatory reaction. Thus, a number of pathogenic bacteria disrupt MBF as part of their deleterious actions resulting in clinically relevant gastroenteritis episodes, but they have also been related to reactivation of IBD by weakening MBF.[6] In turn, host-friendly bacteria (i.e., probiotics) tend to have the opposite effect, enhancing MBF, in addition to their microbiota-balancing properties. The mechanism has been investigated and includes increased expression and improved relocation of tight junction (TJ) proteins, induction of heat shock protein (HSP) 27, and even immunostimulation.[7,8] In many cases, these effects are reproduced by bacterial lysates, killed bacteria, or isolated molecules,[3,9] pointing at a direct action. In addition, host-friendly bacteria digest dietary fiber and provide the host with short-chain fatty acids, which are an important source of energy for the colonic epithelium and have anti-inflammatory and cancer preventing effects. In addition, short-chain fatty acids are determinant in the regulation of mucosal T regulatory cells and play a role in intestinal repair.[10,11] Butyrate lowers bacterial translocation in an in vitro model, without changes in molecular permeability, associated with decreased nuclear factor κB activation.[12] Some clinical evidence of MBF enhancement by probiotics is also available.[13]

At the same time, ample evidence obtained chiefly in animal models indicates that the microbiota also drives intestinal inflammation, because experimental colitis is virtually impossible to elicit in germ-free conditions.[14] Furthermore, depletion of the normal microbiota with antibiotics has similar anti-inflammatory effects in mice, and it produces clinical benefit in some patients

with IBD. This has led to the current view of IBD as an uncontrolled reaction to the normal microbiota in susceptible individuals. In turn, mucosal healing mechanisms seem to be greatly depressed in germ-free conditions and in microbiota-depleted animals, resulting in enhanced blood loss, weight reduction, and death in models of epithelial stress.[15,16] Thus, the microbiota plays an important role by modulating MBF at several levels, depending on its composition.

Mucus Layer

The mucus layer constitutes a physical/biochemical barrier that maintains bacteria apart from IECs while allowing nutrient absorption. Mucus is mainly composed of mucins (MUCs) produced largely by goblet cells. Mucins are heavily O-glycosylated proteins that form polymeric nets. They can be divided in 2 groups: secretory, gel-forming MUCs (MUC2, 5AC, 5B, and 6) produced by goblet cells, and membrane-associated MUCs (MUC1, 3, 4, 13, and 17), expressed by both goblet and absorptive cells in their apical membrane.[17] MUC2 is the main mucus component and the key to produce an effective mucus layer. Muc2 knockout (KO) mice develop colitis spontaneously and have decreased resistance against enteral pathogens (Table 1). Consistent with this notion, the mucolytic *N*-acetylcysteine weakens MBF, an action that is enhanced by proteases.[34]

The mucus barrier is divided into an outer and an inner layer. Colonization by commensal intestinal microbiota is normally limited to the outer "loose" mucus layer, which is formed by proteolytic and glycosidic degradation of MUC2, whereas the "inner" adherent mucus layer is largely devoid of bacteria and physically separates them from the IECs.[17,35,36] Recent studies have shown evidence of bacterial penetration through the inner layer to contact IECs in colitic mice (dextran sulfate sodium [DSS] colitis and also C1galt glycosyltransferase, Nhe3, Tlr5, and Il10 KO mice).[35] This has been also shown for patients with UC who, in addition, present a thinner mucus layer and a reduced number of goblet cells with a smaller size.[35]

Antimicrobial Peptides and sIgA

AMPs are secreted by Paneth cells located at the base of crypts in the small intestine, as well as by regular enterocytes and immune cells. They include defensins, cathelicidin, regenerating islet-derived protein (Reg) IIIγ, elafin, secretory leukocyte protease inhibitor, bactericidal/permeability-increasing protein, lysozyme, lactoferrin, and even chemokines such as chemokine (C–C motif) ligand (CCL) 14 and CCL15.[37] AMPs are present at the crypt space and also in the mucus layer, where they contribute to the local containment of bacteria, fungi, enveloped viruses, and protozoa. They are also part of antimicrobial defense within the mucosa, especially by neutrophils. AMPs typically exhibit additional biological properties. For instance, defensin alpha 5 induces IL-8 secretion, whereas cathelicidin has antiapoptotic and wound-healing effects in IECs and elastin is a protease inhibitor.[37]

Dysfunctional AMP production has been linked to dysbiosis as noted above. In addition, immune defense may be compromised. Thus, defective expression of both α- and β-defensins is associated with reduced killing of certain microorganisms. Consistent with this, spontaneous colitis developing in mice with

TABLE 1. Phenotype of Mice with Genetic Deletion of Various Genes Involved in IBF

Gene	Phenotype	Reference
MUC2	Spontaneous colitis	Van der Sluis et al[18]
TFF3	Increased sensitivity to DSS colitis	Podolsky et al[19]
IL-22	Increased sensitivity to lymphocyte transfer colitis	Zenewicz et al[20]
TNF	Increased sensitivity to DSS colitis	Naito et al[21]
TNFRI	Increased sensitivity to DSS colitis	Mizoguchi et al[22]
IFN-γ	Increased sensitivity to DSS colitis	Jin et al[23]
IL-1β	Increased sensitivity to DSS colitis	Bersudsky et al[24]
IL-1α, IL-1α$^{\Delta IEC}$	Decreased sensitivity to DSS colitis	Bersudsky et al[24]
IL-1R	Increased sensitivity to DSS colitis	Gonzalez-Navajas et al[25]
IL-18/IL-18R	Increased sensitivity to DSS colitis	Takagi et al[26]
MyD88	Increased sensitivity to DSS colitis	Rakoff-Nahoum et al[15]
MyD88$^{\Delta IEC}$	Spontaneous intestinal inflammation	Gong et al[27]
Caspase 1	Increased sensitivity to DSS colitis	Zaki et al[28]
NEMO$^{\Delta IEC}$	Spontaneous colitis	Nenci et al[29]
IKK2$^{\Delta IEC}$	Increased sensitivity to DSS colitis	Greten et al[30]
p65$^{\Delta IEC}$	Increased sensitivity to DSS colitis	Steinbrecher et al[31]
TAK1$^{\Delta IEC}$	Spontaneous colitis	Kajino-Sakamoto et al[32]
IKK2ca[a]	Mild intestinal inflammation and increased sensitivity to DSS colitis	Vlantis et al[33]

[a]Mice with constitutively active form.

intestinal epithelial-specific deletion of MyD88 has been ascribed to decreased production of alpha defensins and RegIIIγ rather than to increased epithelial apoptosis or permeability. Of note, this is associated with increased commensal bacterial translocation.[27] MyD88 is also pivotal for sIgA and MUC2 release by the intestinal mucosa. Levels of the α-defensins 5 and 6 are reduced in CD, particularly in patients carrying NOD2 mutations (see below). Interestingly, reduced expression of human defensin 5 has been noted in children with CD at the age of onset, suggesting a causative role by weakening MBF.[38]

In line with the above findings, cathelicidin KO mice are more susceptible to DSS colitis, whereas intracolonic cathelicidin administration has protective effects against experimental colitis. Elafin overexpression has similar colitis protective effects and enhances MBF in IECs in vitro, whereas treatment with either lysozyme or lactoferrin dampens experimental colitis. Thus, at least in some cases, protection from inflammation has been obtained with AMPs.[37] It should be noted that AMP downregulation is far from being the rule in active IBD, and there are also differences between patients with UC and CD.[37]

sIgA is the main antibody isotype in the intestinal mucosa. It is released by B cells and then actively secreted by epithelial cells into the lumen as dimers. This contributes significantly to the biochemical component of MBF by blocking bacterial proteins involved in epithelial attachment, by inducing bacterial agglutination and by facilitating containment at the mucus layer. In addition, as mentioned above, sIgA modulates the composition of the microbiota and contributes to the maintenance of homeostasis, so that the absence of sIgA may lead to inflammation. In turn, the microbiota also regulates sIgA production.[39,40]

Epithelium

Epithelial cells are held together by the apical junctional complex, consisting of adherens junctions and TJs, and by desmosomes. Adherens junctions and desmosomes provide mechanical adhesive strength between IECs, but do not determine paracellular permeability. In turn, TJs regulate the selective paracellular permeability to solutes by allowing the passage of water, ions, and solutes through pores. TJs are multiprotein complexes located at the apical ends of the lateral membrane of IECs, just apical to the adherens junctions (Fig. 2).[41] TJs are composed of transmembrane proteins (claudins, occludin, junctional adhesion molecules [JAM], and tricellulin) and cytosolic scaffold proteins (zonulae occludens [ZO] 1–3, AF-6, and cingulin). Claudins 1, 3, 4, 5, 8, 9, 11, and 14 decrease paracellular permeability and increase barrier function, whereas claudins 2, 7, 12, and 15 are linked to pore formation and increase paracellular permeability.[41] ZO proteins interact with many transmembrane proteins through the N-terminal region while the C-terminal end interacts with the actin cytoskeleton and cytoskeleton-associated proteins.[41]

TJs are highly dynamic and tightly regulated; their protein expression is regulated at transcriptional and postranscriptional levels, and there is a rapid movement of individual proteins within the junctions. Indeed, some TJ proteins are continuously being endocytosed into actin-coated vacuoles and recycled back to the plasma membrane.[41,42]

Alterations of the TJ as in inflammatory conditions result in significant disturbance of MBF. Thus, occludin, claudins 1 and 4 and JAM-A are internalized in response to interferon (IFN)-γ in T84 cells, thereby increasing permeability. Proinflammatory cytokines tumor necrosis factor (TNF)-α, IL-4, IL-6, and IL-13

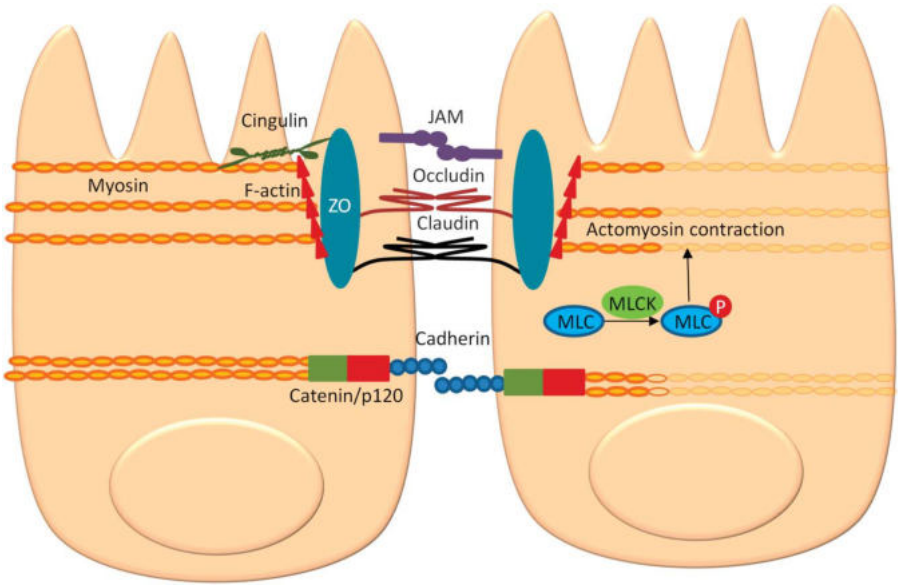

FIGURE 2. Structure of the apical junctional complex. The basic structure of the TJ and adherens junction with the main components is shown, as well as the association with the actomyosin cytoskeleton and the regulatory mechanism mediated by MLCK.

are known to increase the permeability of IEC monolayers, and this effect has been related to an increased expression of claudin 2.[43,44] Similar alterations have been documented in animal models, for instance, IL-10 KO mice show a decreased expression and redistribution of claudin 1, ZO-1, and occludin.[45] In patients with CD and UC, redistribution and low expression of occludin and JAM-A together with an increase in claudin 2 expression has been observed.[46] Claudin 3, 5, and 8 are also decreased in CD, whereas claudin 4 and tricellulin have been found to be overexpressed in UC.[47,48] Internalization of claudins 3, 5, and 8, occludin, and JAMA-A has also been observed in biopsies from patients with UC.[49]

An important regulator of TJ is myosin light-chain (MLC) phosphorylation by MLC kinase (MLCK) through actomyosin contraction, which tends to open the junctional gap (Fig. 2), and it mediates part of the TJ-disrupting effects of proinflammatory cytokines.[42] Mice expressing a continuously active MLCK are prone to experimental colitis. A role for MLC phosphorylation and MLCK expression has also been observed in both UC and CD.[48]

An additional mechanism for TJ regulation is based on modulation of the actin cytoskeleton through activation of protein kinase C and Rho, which is exploited by some pathogenic bacteria. Alterations of intercellular adhesions at sites other than the TJ have also been involved in MBF defects. For instance, inactivation of E-cadherin produces augmented epithelial apoptosis and cell shedding, altered maturation of Paneth and goblet cells, decreased AMP release, and bloody diarrhea.[50]

In addition to epithelial permeability, MBF depends exquisitely on an adequate balance of IEC proliferation, migration, and differentiation, i.e., epithelial dynamics. Epithelial cells constantly move from the bottom of the crypts to the crypt surface or villous tip in the colon and small intestine, respectively, resulting in total renewal in approximately 1 week. Epithelial cells are terminated by apoptosis and/or shedding, which are not accompanied by inflammation or apparent MBF loss in homeostatic conditions. IEC apoptosis is markedly increased in IBD. In contrast, IEC necrosis and caspase-1-mediated pyroptosis do result in functionally relevant gap formation,[51] which may lead to inflammation.

Challenges to the epithelial monolayer by microorganisms, inflammation, toxic luminal substances, inflammation, and so forth, impose the need for appropriate mechanisms to preserve MBF despite the occurrence of gaps. The initial response to injury (minutes to hours) in the epithelium is restitution, whereby surrounding cells flatten and quickly migrate to cover the gap. The second mechanism is increased proliferation, which takes hours to days. Restitution is enhanced by certain defensins and chemokines, trefoil peptide, prostaglandin E_2, and various cytokines including granulocyte-macrophage colony-stimulating factor (GM-CSF), transforming growth factor-β, epidermal growth factor, or IL-1β, among other factors.[52] For instance, IL-1β protects against DSS colitis partly by promoting epithelial healing (Table 1). Many of these agents modulate wound-healing indirectly by the release of transforming growth factor-β.

Epithelial dynamics is greatly influenced by the microbiota, as mentioned above. In germ-free animals, epithelial turnover is reduced and cells show enhanced expression of differentiation markers. Sensing of microbiota molecular components by pattern recognition receptors (PRRs) seems to mediate this regulation.[15,53] This extends also to apoptosis inhibitory effects, which are mediated chiefly by activation of the NFκB pathway (see below). Nenci et al observed that conditional suppression of intestinal epithelial expression of inhibitor of kappa B kinase (IKK)-γ (also known as NFκB essential modulator [NEMO]) or of IKKα/β, resulting in reduced activation of the NFκB pathway, produced a severe inflammatory response (Table 1). Inflammation was not only associated with increased epithelial cell death but also with lower defensin production and increased bacterial mucosal invasion. In addition, epithelial NFκB activation probably contributes to loss of fluid and diarrhea in the inflamed intestine.[54]

Mucosal Immune System

The mucosal immune system of the intestine features various immune cell types such as neutrophils, monocyte/macrophages, dendritic cells, mast cells, innate lymphoid cells, B cells, and T cells. In addition, epithelial cells are increasingly viewed as an integral part of the system. Neutrophils are efficiently recruited to inflammatory sites in active intestinal inflammation both in humans and in experimental models, where they contribute to the inflammatory reaction. However, neutrophils also limit bacterial invasion of the mucosa and translocation. The latter factor may predominate in experimental colitis because neutrophil depletion aggravates colitis.[55] Similarly, lack of expression of CXCL1, which is considered the main chemokine responsible for neutrophil recruitment in the colon, is associated with augmented colitis.[56] Consistent with this concept, mice deficient in peptidoglycan recognition proteins 1 to 4, which are part of the antimicrobicidal machinery of neutrophils, display enhanced sensitivity to DSS colitis, associated with dysbiosis.[57] In humans (CD), impaired neutrophil recruitment has been reported,[58] although this may be a consequence of inflammation rather than a cause.[59] In contrast with these studies, Berndt et al[60] found no change in DSS colitis after neutrophil depletion, and Buell and Berin reported a similar unchanged phenotype in 3 different models of experimental colitis 20 years ago.[61] Only 1 study has reported amelioration of experimental colitis (rat trinitrobenzenesulfonic acid—TNBS—colitis) by neutrophil depletion or treatment with an inhibitor of neutrophil activation.[62]

Macrophages and dendritic cells are also important players in intestinal inflammation. Intestinal mucosal macrophages are unique in that they do not normally elicit inflammatory responses while maintaining intact their phagocytic and microbial killing capacity.[63] In addition, mucosal macrophages and dendritic cells are involved in tissue repair and immune tolerance in the gut. However, during an inflammatory bout, proinflammatory monocytes are recruited and probably contribute to the overall intensity of the inflammatory response.[64] Mucosal macrophage depletion has been shown to suppress colitis in IL-10 KO mice. Dendritic cell ablation is also protective in DSS colitis, whereas adoptive transfer of dendritic cells exacerbates inflammation.[60,64] In

contrast, apoptosis-mediated depletion of macrophages and dendritic cells was found to aggravate colitis in another study.[65] Moreover, localized depletion of mucosal mononuclear phagocytes with clodronate liposomes[65] and isolated dendritic cell depletion[66] had a similar outcome. In humans, CD has been related to reduced/altered macrophage cytokine and bacterial clearance responses.[58,67] It is important to note that disorders of phagocyte function often result in chronic colitis that resembles CD.[68,69]

A number of diseases associated with impaired lymphocyte function feature the development of IBD such as colitis or small intestinal inflammation, including WASP (Wiskott–Aldrich syndrome protein) mutations, sIgA deficiency, common variable immunodeficiency, or X-linked agammaglobulinemia. Of note, WASP KO mice reproduce the human phenotype to a large extent. It should be noted however that there are also substantial differences between these presentations and idiopathic IBD, and that some of the associations may be simply casual. In contrast, no such association has been found for patients with isolated T-cell disorders.[70] The fact that experimental colitis induced by DSS or TNBS is similar between mice that are devoid of lymphocytes and normal mice further suggests that protracted intestinal inflammation may develop independently of B and T cells.

Despite some conflicting results, the available evidence suggests that efficient microbe handling by the immune system provides protection against inflammation, probably because translocation is prevented. Interestingly, commensal bacteria may aid in immune response to pathogens in certain cases. Thus, in mice infected with *Clostridium difficile*, NLRP3 dependent IL-1β secretion is required to prevent massive translocation, which in turn depends on the translocation of nonpathogenic bacteria.[71] In addition, epithelial dynamics is regulated by subepithelial cells and by the microbiota, and therefore the intestinal immune system is also relevant in this regard. Because the immune system also affects AMP and sIgA production, it modulates the composition of the microbiota. Therefore, the intestinal immune system regulates MBF at least by 4 mechanisms: (1) by regulating epithelial dynamics, (2) by regulating AMP production, (3) by influencing the microbiota, and (4) by responding to invading molecules and microorganisms.

ROLE OF PRRs IN THE REGULATION OF MBF

PRRs bind molecules with structural characteristics that are different from those of the host. In addition, PRRs bind a number of host molecules, such as calprotectin, high-mobility group protein B1, or some AMPs, which act as endogenous damage–associated ligands or alarmins. The PRR family includes the TLRs, NOD-like receptors (NLR), and retinoid acid inducible gene-I-like receptors. TLRs and NLRs have received most attention to date. TLR-mediated recognition of intestinal microflora by IECs is important in general for TJ preservation, production of chemokines, and cell survival. Of the TLRs, TLR3, TLR7, and TLR8 are located exclusively in endosomes and TLR5 at the basolateral membrane, and therefore have no direct contact with the luminal microbiota. TLR9 is unique in that it is expressed at the apical and basolateral membrane (and in endosomes) and the effects of receptor ligation are different depending on the expression site.[53] The responses to the natural ligands are normally attenuated in vivo, consistent with the status of tolerance prevailing in the intestine.

A great deal of information about the role of PRR in intestinal inflammation has been gathered in the last few years with the use of KO mice and experimental colitis models (Table 2). Thus, TLR5 KO mice exhibit spontaneous colitis, whereas TLR2, TLR4, and TLR9 have a normal phenotype but are more sensitive to DSS colitis (conflicting results have been obtained with TLR9 KO mice[4]). Contrary to these results, TLR3 KO mice have attenuated colitis in response to DSS, although TLR ligands ameliorate experimental colitis, as shown in the same study.[72] TLR2-mediated protection depends on the production of TFF3, which works as a mucus-associated protective factor secreted by goblet cells,[19] and on increased ZO-1 and reduced permeability. In contrast, the role of TLR4 seems to be more complex. In fact, lipopolysaccharide (LPS) disrupts TJs, induces epithelial cell apoptosis/shedding, attenuates restitution, and increases permeability by actions dependent at least partly on the TNF receptor 1.[74,75] These effects correspond to engagement of TLR4 at the basolateral membrane of IECs or on subepithelial cells. In fact, TLR4 epithelial overexpression is proinflammatory. When LPS reaches the bloodstream causing endotoxemia, further MBF derangement may occur, which is higher in the ileum than in the colon, both in terms of inflammation and bacterial translocation.[76] However, TLR4 is also associated with epithelial repair. TLR4 in IECs and mucosal macrophages induces prostaglandin E_2 synthesis and upregulates epidermal growth factor levels, thereby increasing epithelial survival and proliferation. Thus, although TLR4 blockers display anti-inflammatory effects in experimental colitis, they also delay mucosal healing.[77]

Consistent with the results in TLR KO mice, treatment with TLR ligands is protective against experimental colitis, including

TABLE 2. Phenotype of Mice with Genetic Deletion of PRRs[4,72,73]

PRR	Phenotype
TLR2	Increased sensitivity to DSS colitis
TLR3	Decreased sensitivity to DSS colitis
TLR4	Increased sensitivity to DSS colitis; variable response in IL-10 KO colitis
TLR5	Spontaneous colitis and increased sensitivity to DSS colitis
TLR9	Increased sensitivity to DSS colitis (but resistance to chronic DSS colitis)
NOD1	Increased sensitivity to DSS colitis
NOD2	Increased sensitivity to DSS and TNBS colitis
NLRP3	Increase/decreased sensitivity to DSS and TNBS colitis
NLRP6	Spontaneous intestinal inflammation
NLRC4	No change
RIG-I	Spontaneous intestinal inflammation

TLR2, 3, 4, 5, 7, and 9 (although discrepancies have been observed regarding TLR9).[4] For instance, the TLR7 agonist imiquimod lessens the severity of DSS colitis by inducing a type I IFN response and presumably augmenting AMP release. Exogenous LPS inhibits colitis acting on dendritic cells to upregulate IL-22 and enhance MBF in Lyn tyrosine kinase KO mice, which are highly sensitive to DSS colitis.[15,78] In fact, feeding mice with a "bacterial meal" (featuring TLR ligands) also ameliorates DSS colitis and improves MBF.[79]

NLRs include NOD1 and NOD2, NLR family CARD domain-containing protein (NLRC) 4, NLRP3, and NLRP6, among others. NOD2 was the first IBD polymorphic gene with established risk variants. It acts as an intracellular sensor for muramyl dipeptide; and upon ligation, it drives proinflammatory cytokine and AMP secretion. In addition, NOD2 is involved in antiviral responses mediated by IFN-β, and in autophagy, augmenting bacterial clearance. Nod2 KO mice exhibit exacerbated colitis with increased translocation, augmented paracellular permeability, decreased E-cadherin, and lower colonic antimicrobial RegIIIγ expression.[4] Interestingly, NOD2 overexpression in MHC-II-bearing cells protects against TNBS colitis. Similar to Nod2, Nod1 KO mice exhibit increased sensitivity to experimental colitis (Table 2).

As mentioned above, NLRP6 KO mice display spontaneous colitis and increased susceptibility to DSS colitis, which is attributed to lack of expression in nonhematopoietic cells and linked to impaired IL-18 production and increased CCL5.[80] Alterations in the microbiota are pivotal in this phenotype. NLRP3 KO mice have also been shown to be more sensitive to colitis induction by either DSS or TNBS, and again the cells involved are nonhematopoietic. The same phenotype is observed in apoptosis-associated speck-like protein (ASC) or caspase 1 KO mice (both part of the inflammasome), which, interestingly, are ameliorated by IL-18 treatment.[4] In fact, both IL-18 and IL-18 receptor KO mice show enhanced sensitivity to experimental colitis (Table 1). However, opposite results have been obtained in other studies.[81] The inflammasome is involved in the synthesis and release of IL-1β and IL-18 by macrophages, and patients with CD reportedly have defective IL-1β production ex vivo. Notably, NLRP3 KO mice display lower epithelial proliferation, which suggests that repair mechanisms are compromised in the absence of this receptor. Reduced AMP production has also been reported. In contrast, NLRC4 KO mice have no increased susceptibility to colitis.[4]

Thus, there are several instances where conflicting phenotypes are described after genetic manipulation of PRRs. This may be partly explained by facility-related changes in the microbiota because the balance of bacterial populations in the intestinal lumen is significantly shifted by alterations in the PRRs. Other confounding factors are the relative contribution of PRR signaling in different cell types, such as epithelial cells and immune cells, and the relative weight of PRRs in different phases of colitis. PRRs are classically considered as positive regulators of immune/inflammatory responses, but their influence is more complex as noted, because they also regulate epithelial proliferation, permeability/translocation, bacterial clearance, and even the composition of the microbiota.

ROLE OF CYTOKINES IN THE REGULATION OF MBF

Cytokines have been implicated in the regulation of MBF at various levels, including the modulation of epithelial integrity and dynamics and modulation of the immune response. TNFα is a major player in IBD and animal models of colitis. TNFα promotes removal of claudin 1 from TJ, increases claudin 2 expression, enhances occludin degradation, and promotes MLCK phosphorylation, thus augmenting paracellular permeability.[82,83] In addition, TNFα increases apoptosis and cell shedding.[84] In vivo, anti-TNFα antibodies improve MBF and promote mucosal healing. However, both TNFα and TNF receptor 1 KO mice have increased sensitivity to colitis (Table 1). Furthermore, TNFα has been implicated in mucosal healing as explained above.[8]

IFN-γ is one of the predominant cytokines in CD- and Th1-driven models of colitis, and it has well-documented negative effects on epithelial permeability, which are mediated in part by hypoxia-inducible factor-1α and NFκB.[85] However, IFN-γ KO mice are also more sensitive to colitis (Table 1), and CD has been shown to be resistant to treatment with IFN-γ-blocking antibodies. This may be related to the recently reported MBF-enhancing effect of this cytokine by induction of apical IL-10R expression in IECs,[86] because IL-10 reduces epithelial permeability in vitro.

GM-CSF administration has been shown to be protective in experimental colitis acting on innate immunity mechanisms. Accordingly, GM-CSF KO mice are more susceptible to inducible colitis. GM-CSF may be beneficial also in humans. It should be noted that GM-CSF has other effects in addition to macrophage stimulation, including epithelial cell proliferation and repair.[87] Other relevant cytokines are IL-22, IL17A, epidermal growth factor, keratinocyte growth factor, IL-18, IL-6, IL-1β, etc, but they are not covered here.

TRANSLOCATION

As mentioned above, one of the basic roles of MBF is to contain the microbiota and luminal macromolecules. Translocation is the passage of molecules or microorganisms through the mucosal barrier and to the bloodstream or lymphatic system. Although there is essentially no translocation in normal conditions, it is being identified as a contributing factor in a growing number of conditions. This is usually a consequence of weakening of MBF by alterations in any of its components. For instance, TREM-1 (triggering receptor expressed on myeloid cells 1), KO mice exhibit enhanced *Klebsiella pneumoniae* translocation after oral infection due to inefficient intestinal clearance.[88] Although the implications exceed the limits of this review, such MBF alterations have been associated with ischemia/reperfusion, pancreatitis, shock, diabetes, metabolic syndrome, chronic kidney disease, burn injury, hepatic disease, sepsis, psychogenic stress, etc. Whatever the initial insult, translocation of LPS resulting in endotoxemia may further weaken MBF, particularly in the ileum,[76] associated typically with a local inflammatory response and enterocyte apoptosis.

INFLAMMATORY BOWEL DISEASE AND MBF

It has been long known that IBD is associated with increased permeability in the gut. However, because actual intestinal inflammation disrupts MBF regardless of the cause, it is difficult to tell apart cause from effect in many cases. Notably, healthy relatives of patients with IBD exhibit augmented permeability values as well, indicating that this MBF defect precedes the onset on inflammation.[89] Because spouses do not share this phenotype, a genetic basis is inferred. Increased permeability has been associated with augmented epithelial shedding and gap formation in the epithelial layer in the duodenum of patients with IBD.[90] Using confocal laser endomicroscopic analysis, it is possible to predict to a certain extent which patients will relapse in the following 6 months.[91] Evidence of permeability defects occurring before inflammation is also available in animal models. Thus, in lymphocyte transfer colitis, in which inflammation develops gradually in contrast with the more widely used chemically induced models such as DSS colitis, decreased epithelial resistance has been shown to precede overt microscopic inflammation.[92] Similarly, in recombination signal-binding protein for Ig κ J region (RBP-J)$^{\Delta IEC}$ KO mice, which have disrupted Notch signaling, bacterial translocation precedes the onset of spontaneous colitis.[93] Another example is commensal *Enterococcus faecalis* metalloprotease GelE–induced colitis in IL-10 KO and TNF (ΔARE/WT) mice, where impairment of MBF precedes colitis.[94]

It is interesting to note that some patients with IBD with quiescent disease display irritable bowel syndrome–like symptoms, which correlate with increased permeability and subclinical inflammation.[95] In fact, in recent years, evidence has accumulated supporting the occurrence of defective MBF and low-grade inflammation in patients with irritable bowel syndrome.[96] These alterations may be the long-term result of intestinal infections, because these have been related to irritable bowel syndrome etiology.

Although altered permeability and translocation may result in intestinal inflammation, it is equally clear this is not an inevitable outcome. Thus, most PRR KO mice do not develop spontaneous colitis, but respond to DSS or other colitogenic stimuli with a more intense inflammatory phenotype, suggesting that stressful conditions uncover subtle defects in the system. In addition, physiological redundancy exists, so that a defect in 1 element of the MBF may be compensated by the activation of other elements.[97] One example is provided by increased IgG and IgM levels in patients with defective sIgA. In junctional adhesion molecule A KO mice, there is increased permeability but unchanged sensitivity to colitis, because of potentiation of adaptive immunity.[98] Similarly, in RegIIIγ KO mice, there is an increase in T cells and sIgA secretion.[99]

OVERVIEW

The concept of MBF has evolved from being initially dismissed as a relevant cause of inflammation in case of failure to its actual consideration as a major contributor to gastrointestinal homeostasis. It is important to recognize that in many instances, the effects of therapeutic interventions on MBF may be very difficult or impossible to differentiate from those on the inflammatory response as such. Thus, a given anti-inflammatory maneuver will typically enhance MBF, whereas colonic inflammation is expected to increase permeability and bacterial/LPS

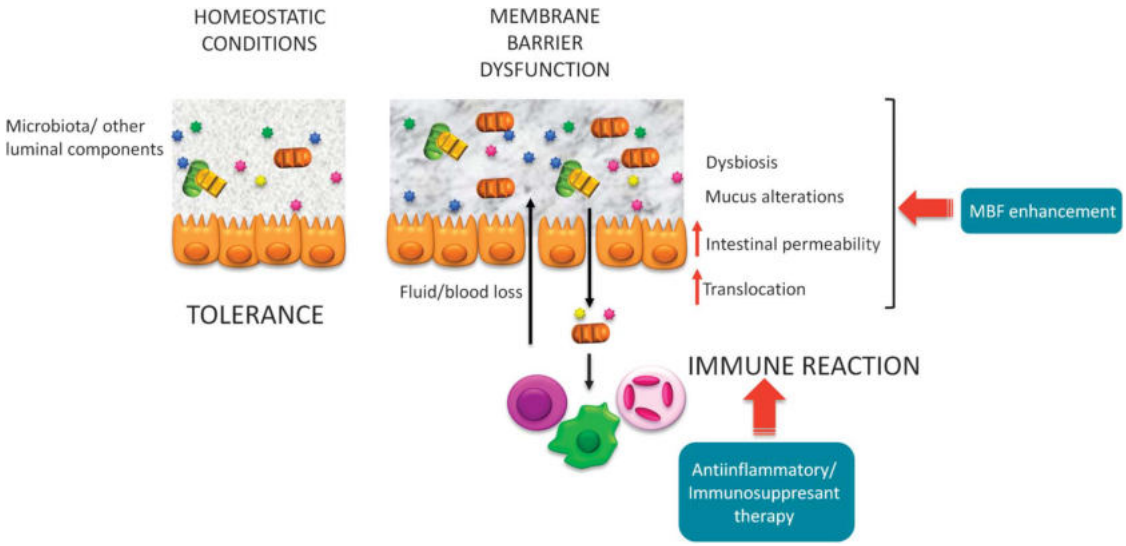

FIGURE 3. Diagram showing the operation of the mucosal barrier in homeostatic conditions (left) and in the context of barrier dysfunction (right). In the normal intestine, there is minimal or no translocation and the intestinal immune system is in a state of tolerance, with no signs of inflammation. Conversely, when the mucosal barrier fails, there is increased translocation of molecules/microorganisms from the lumen, with enhanced stimulation of the resident immune cells, dysbiosis, and alterations in epithelial repair and permeability. This creates an imbalance that may ultimately give rise to an overt inflammatory reaction. The equilibrium may be restored by stimulation of barrier function elements or by inhibition of the secondary inflammatory cascade.

translocation. However, we now have substantial evidence indicating that defects in MBF may act as an etiopathogenic factor in intestinal inflammation and particularly IBD, based on the sequence of events in patients and animal models, and also by observation that inflammation or, more often, increased susceptibility to inflammation, is the frequent consequence of alterations in different elements of the intestinal barrier (Fig. 3). At the same time, a single defect rarely causes an inflammatory reaction, consistent with a multifactorial etiology of IBD. Another argument in favor of the relevance of MBF is that many IBD-related gene loci identified to date correspond to genes involved in MBF.

There is also ample evidence that intestinal inflammation can be ameliorated by a vast number of interventions, which are in many cases of opposite nature, such as enhancing or blocking PRR activation, NFκB function, and so forth. This is perplexing but may be explained by the unique nature of the intestinal barrier (Fig. 3). Because inefficient containment of microbes/molecules may lead to an exacerbated response by the immune system, leading to inflammation (the real life equivalent being bringing in the army when the police fails), this can be prevented/managed by augmenting the basic capacity to control translocation or by dampening immune function. The end result will depend on timing, cell type targeted, and the existence of additional proinflammatory/anti-inflammatory factors.

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