Central role of the gut epithelial barrier in the pathogenesis of chronic intestinal inflammation: lessons learned from animal models and human genetics

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The gut mucosa is constantly challenged by a bombardment of foreign antigens and environmental microorganisms. As such, the precise regulation of the intestinal barrier allows the maintenance of mucosal immune homeostasis and prevents the onset of uncontrolled inflammation. In support of this concept, emerging evidence points to defects in components of the epithelial barrier as etiologic factors in the pathogenesis of inflammatory bowel diseases (IBDs). In fact, the integrity of the intestinal barrier relies on different elements, including robust innate immune responses, epithelial paracellular permeability, epithelial cell integrity, as well as the production of mucus. The purpose of this review is to systematically evaluate how alterations in the aforementioned epithelial components can lead to the disruption of intestinal immune homeostasis, and subsequent inflammation. In this regard, the wealth of data from mouse models of intestinal inflammation and human genetics are pivotal in understanding pathogenic pathways, for example, that are initiated from the specific loss of function of a single protein leading to the onset of intestinal disease. On the other hand, several recently proposed therapeutic approaches to treat human IBD are targeted at enhancing different elements of gut barrier function, further supporting a primary role of the epithelium in the pathogenesis of chronic intestinal inflammation and emphasizing the importance of maintaining a healthy and effective intestinal barrier.

Keywords: intestinal epithelial cells, intestinal barrier function, gut immune homeostasis, innate immunity, Crohn’s disease, ulcerative colitis, inflammatory bowel disease genetics, animal models of intestinal inflammation

INTRODUCTION
The gastrointestinal tract, from the beginning of extrauterine life, is chronically exposed to a huge burden of foreign antigens, various microorganisms, and toxic molecules. Therefore, its ability to act as a barrier against potentially harmful molecules and to defend against pathogenic bacteria is pivotal in maintaining gut immune homeostasis. In fact, evolution has selected different mechanisms by which the gut serves as an effective protective barrier. Of paramount importance is the intestinal epithelium of which intestinal epithelial cells (IECs) are the primary cell type coming into contact with the external environment and act as the host’s first line of the defense against potential harmful stimuli. Despite their non-hematopoietic derivation, IECs also represent a core element of innate immunity within the gut mucosa, displaying a wide array of immune functions. In fact, IECs are able to recognize pathogens through the expression of innate immune receptors, to release anti-microbial molecules, and to secrete cytokines and chemokines that link innate and adaptive immune responses. Moreover, IECs also represent the main structural component of the physical barrier between the luminal microenvironment and host, allowing selective absorption of nutrients and denying entry of noxious molecules and antigens. The intestinal epithelium constitutes the largest exposed surface area of the human body and its permeability is finely regulated by the presence of tight junctions (TJs), large molecular complexes which, together with adherens junctions (AJs), link IECs to each other, and seal the intercellular spaces on the luminal surface, regulating molecule passage through the paracellular spaces. Finally, IECs produce the mucus layer covering the entire length of the gastrointestinal tract, whose role is to further protect the mucosal surface from harmful molecules and bacteria, and reinforce the overall intestinal barrier. As such, any defect in these IEC-specific processes can cause a breakdown in gut barrier and consequently, a disruption of normal mucosal immune homeostasis that can potentially lead to uncontrolled chronic inflammation, such as that observed in inflammatory bowel disease (IBD).

DEFECTS IN EPITHELIAL-SPECIFIC INNATE IMMUNE FUNCTIONS LEAD TO INTESTINAL INFLAMMATION
Intestinal epithelial cells, located at the interface between the external environment and the internal mucosal immune system, must be able to mount early and appropriate defense responses against various pathogens in order to maintain homeostasis. Central to this process are innate immune receptor molecules, referred to as pattern-recognition receptors (PRRs), whose function is to sense highly conserved structures or pathogen-associated molecular patterns (PAMPs) present among several different pathogens (1). Archetypal molecules belonging to PRRs are the toll-like receptors.
(TLRs), which are type I integral transmembrane glycoproteins expressed by several types of cells, including IECs. The TLR family consists of at least 13 members with slightly different structures that recognize, through an extracellular domain containing large leucine-rich repeats, different PAMPs, such as lipopolysaccharide (LPS), peptidoglycan (PGN), muramyl dipeptide (MDP), lipoteichoic acids (LTAs), and bacterial DNA (2). Recognition of each TLR-specific PAMP initiates downstream signaling through two different pathways: via the myeloid differentiating factor 88 (MyD88) pathway and via an alternative “MyD88-independent” pathway, both of which lead to activation of NF-κB, triggering of other innate immune responses, production of cytokines and chemokines, and finally, recruitment of the adaptive immune system (2). Interestingly, the MyD88 pathway is activated by a cytoplasmic domain similar to the interleukin-1 receptor (IL-1R) (3), and both TLR and IL-1R stimulation leads to NF-κB activation (4).

**GENETICALLY ENGINEERED MODELS AFFECTING EPITHELIAL INNATE RESPONSES**

Several lines of evidence using genetically manipulated mouse models suggest that deletion/dysregulation of genes and specific chromosomal loci associated with epithelial barrier function can lead to chronic intestinal inflammation (Figure 1). In fact, epithelial barrier defects are clearly present in most animal models of IBD (summarized in Table 1), which have become seminal tools in understanding normal epithelial physiology as well as the role of IECs in the development of gut inflammation.

Toll-like receptor-bearing IECs are of critical importance for organizing the first line defense against pathogenic microorganisms and in maintaining normal barrier function. For example, the development of spontaneous intestinal inflammation has been reported in TLR5 knockout (KO) mice, with around 35–40% of these mice presenting with colitis and exhibiting areas of extensive mononuclear infiltration, epithelial hyperplasia, and focal epithelial crypt destruction (5). An increase in intestinal permeability was also noted in this model, even though it appeared to be secondary to the inflammatory process and not the triggering event. Instead, the primary defect leading to colitis in these mice is speculated to be the waning ability to clear bacteria due to an inherent defect in innate immune responses. Thus, the lack of TLR5 promotes an increase in colonic bacterial burden, and this process may enhance the activation of other proinflammatory pathways. In fact, the absence of colonic inflammation in TLR4/5 and IL-1R/TLR5 double KO mouse strains (5, 6) strongly suggests that activation of other Toll/IL-1 receptor pathways, such as TLR4 and IL-1R, is essential for the onset of disease.

The importance of TLR5 signaling in the development of spontaneous gut inflammation has also been brought to light using the spontaneous C3H/HeBir model of colitis as well as studies in IBD patients, which suggest a central role for TLR5 and bacterial flagellin, its natural ligand, in the pathogenesis of Crohn’s disease (CD), one of the major forms of IBD. The colitis characteristic of C3H/HeBir mice is primarily localized to the cecum and resolves by 3 months of age (7). Interaction with the commensal bacterial flora is important in this model, as innate responses to TLR ligands are impaired compared to the colitis-resistant C57BL/6 strain (8), with the major class of antigens identified as commensal bacterial flagellins, recognized by TLR5 (9). In fact, serum IgG anti-flagellin antibodies have been identified in three different mouse models and in approximately 50% of CD patients evaluated, but not in either UC patients or controls (9). In addition, flagellin-reactive Th1 cells isolated from C3H/HeBir mice have the ability...
Table 1 | Inflammatory bowel disease animal models with primary defects of intestinal epithelial origin.

| Animal model | Disease location/phenotype of inflammation | Histologic features | Identified gene(s) involved | Epithelial-specific dysfunction |
|--------------|------------------------------------------|---------------------|-----------------------------|-------------------------------|
| CHEMICALLY INDUCED MODELS | | | | |
| DSS-induced colitis (57) | Superficial colitis | Superficial ulcerations, Infiltration of acute inflammatory cells, Crypt distortion, Loss of goblet cells | Loci increasing susceptibility to the induced model found on Chr5 and Chr2 | Chemical destruction of mucosal barrier with consequent increase in luminal bacterial translocation |
| TNBS-induced colitis (61) | Transmural colitis | Ulceration, Infiltration of acute/chronic inflammatory cells, Crypt distortion, Loss of goblet cells | N/A | Ethanol-induced destruction of mucosal barrier facilitating hapten penetration and contact with underlying mucosal immune system |
| GENETICALLY ENGINEERED MODELS AFFECTING EPITHELIAL BARRIER INTEGRITY | | | | |
| Mdr1a knockout (103) | Transmural colitis | Colonic thickening with crypt hyperplasia, Focal ulcerations, Crypt abscesses, Leukocyte infiltration, Increased number of granulocytes | Mdr1a deletion | Increased basal colonic ion transport, Dysregulated epithelial cell growth, Increased permeability (dependent on bacterial colonization), Decreased phosphorylation of TJ proteins (ZO-1 and occludin) |
| Dominant negative N-cadherin transgenic (62) | Patchy foci of ileal inflammation | Cryptitis and crypt abscesses, Epithelial hyperplasia, Presence of lymphoid aggregates | Dominant negative N-cadherin expression in small intestinal IEC | Breakdown of intestinal epithelial apical junctional complexes, Aberrant cell migration, proliferation, and apoptosis in small intestinal crypts |
| Ga2 knockout (64) | Superficial pancolitis; increased severity in distal colon | Cryptitis and crypt abscesses with crypt distortion, Mucosal PMN infiltrates | Ga2 deletion | Possible impairment of epithelial TJ assembly |
| JAM-A knockout (66) | Colitis | Normal epithelial architecture, Increased PMN infiltration, Formation of large lymphoid aggregates | JAM-A deletion | Impaired TJ structure and consequent increase in epithelial permeability, Increase in the TJ proteins, claudin-10 and -15 |
| GENETICALLY ENGINEERED MODELS AFFECTING EPITHELIAL INNATE RESPONSES | | | | |
| TLR5 knockout (5) | Colitis; 10% incidence of rectal prolapse | Mononuclear infiltrates, Epithelial hyperplasia, Focal crypt loss, Goblet cell depletion | TLR5 deletion | Increased epithelial permeability (secondary to inflammation), Ineffective bacterial clearance |
| NEMOIEC-KO (23) | Pancolitis | Mucosal thickening, Enlarged crypts, Loss of goblet cells, Extensive epithelial destruction, Marked infiltration of mononuclear cells in mucosa/submucosa | IEC-specific inhibition of NFκB via conditional ablation of NEMO (IKKg) | Colonic epithelial cell apoptosis (via increased sensitivity to TNF), Impaired expression of anti-microbial peptides, Dysregulated epithelial barrier integrity |
| TAK1IEC-KO (24) | Enterocolitis | Complete disruption of small intestine structure, Less severe alterations of colonic tissue | IEC specific conditional ablation of TAK1 | Colonic epithelial cell apoptosis (via increased sensitivity to TNF), Impaired innate immune response |

(Continued)
Table 1 | Continued

| Animal model     | Disease location/phenotype of inflammation | Histologic features                                                                 | Identified gene(s) involved                                                                 | Epithelial-specific dysfunction                                      |
|------------------|--------------------------------------------|--------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|----------------------------------------------------------------------|
| **GENETICALLY ENGINEERED MODELS AFFECTING EPITHELIAL CELL INTEGRITY AND MUCUS PRODUCTION** |
| XBP1 IEC-KO      | Focal non-granulomatous enteritis          | Absence of Paneth cells                                                              | IEC specific conditional ablation of XBP1                                                 | impaired innate immune response due to Paneth cell loss by apoptosis |
|                  |                                             | Loss of goblet cells                                                                 |                                             | Endoplasmic reticulum (ER) stress secondary to the lack of XBP1       |
|                  |                                             | Lamina propria mononuclear infiltrate                                               |                                             | increased proinflammatory signaling due to increased                |
|                  |                                             | Crypt abscesses                                                                     |                                             | JNK/SAPK activation secondary to the lack of XBP1                    |
|                  |                                             | Mucosal ulcerations                                                                 |                                             |                                                                      |
|                  |                                             | Villus shortening with a reduction of villus:crypt ratio                            |                                             |                                                                      |
| AP1M2 knockout   | Transmural colitis                         | Epithelial hyperplasia                                                              | Epithelia-specific membrane trafficking factor AR-1B deficiency induced via AP1M2         | Loss of IEC polarity                                                 |
|                  |                                             | Crypt distorsion                                                                    | deletion                                    | impaired epithelial production of anti-microbial peptides           |
|                  |                                             | Loss of goblet cells                                                                |                                             | Defective luminal transport of secretory IgA                       |
|                  |                                             | Mucosal and submucosal inflammatory infiltrate                                      |                                             |                                                                      |
| RBP-J IEC-KO     | Colitis; rectal prolapse                   | Goblet cell hyperplasia                                                             | IEC-specific impairment of Notch signaling via conditional ablation of RBP-J              | Retarded IEC turnover                                               |
|                  |                                             | Aberrant accumulation of mucus under the tunica serosa                               |                                             | Increased epithelial permeability                                   |
|                  |                                             | Neutrophilic infiltrate                                                             |                                             | Impaired epithelial defense against bacteria                        |
| MUC2 knockout    | Superficial colitis; more severe in the distal colon | Complete lack of goblet cells                                                        | MUC2 deletion                                | altered bacterial stimulation of IECs due to a diminished mucus layer |
|                  |                                             | Crypt hyperplasia                                                                   |                                             |                                                                      |
|                  |                                             | Flattening of the epithelial layer and superficial erosions                         |                                             |                                                                      |
|                  |                                             | Mild inflammatory infiltration                                                       |                                             |                                                                      |
|                  |                                             | Lamina propria distorsion                                                           |                                             |                                                                      |
| MUC2 mutant      | Superficial colitis; more severe in the distal colon | Focal epithelial erosions                                                           | MUC2 missense mutations                     | altered bacterial stimulation of IECs due to a diminished mucus layer |
| Winnie and       |                                             | Crypt elongation                                                                    |                                             | increased endoplasmic reticulum (ER) stress due to mutated MUC2     |
| Eeyore strains   |                                             | Neutrophilic infiltrate                                                             |                                             | protein misfolding and accumulation in ER                            |
|                  |                                             | Crypt abscesses                                                                     |                                             |                                                                      |
|                  |                                             | Goblet cell loss                                                                     |                                             |                                                                      |
| POFUT1 IEC-KO    | Enterocolitis                              | Crypt hyperplasia                                                                   | IEC specific conditional ablation of POFUT1                                               | Notch signaling impairment with consequent goblet cell hyperplasia   |
|                  |                                             | Dilated and mucus filled crypts                                                     |                                             | and mucus hypersecretion, leading to associated gut microbiota     |
|                  |                                             | Hyperplasia of Paneth cells and enteroendocrine cells                               |                                             | alterations                                                        |
|                  |                                             | Inflammatory infiltrate of the lamina propria                                        |                                             |                                                                      |
|                  |                                             | Crypt abscesses                                                                     |                                             |                                                                      |
|                  |                                             | Crypt abscesses                                                                     |                                             |                                                                      |
|                  |                                             | Transmural inflammation                                                             |                                             |                                                                      |
| **SPONTANEOUS MODELS** |
| SAMP1/YitFc      | Segmental, discontinuous, transmural ileitis; increased severity in the terminal ileum with 2–3% incidence of perianal disease | Villous blunting/crypt hypertrophy Paneth cell/goblet cell hyperplasia PMN mononuclear cell infiltration in lamina propria and submucosa Aphthous inflammatory lesions Granuloma formation Cryptitis/crypt microabscesses Basal plasmacytosis | Multigenic etiology; susceptibility found on Chr6, Chr8, Chr9, and ChrX | Primary non-hematopoietic (i.e., epithelial) dysfunction Increased epithelial permeability independent of commensal bacterial colonization Altered T4 protein expression (increase in claudin-2, decrease in occludin) Dysregulated epithelial innate responses |

(Continued)
to induce colitis upon transfer to naïve SCID recipients. Analysis of
quantitative trait locus mapping of C3H/HeBJbir mice backcrossed
with IL-10 KO mice identified several potential colitogenic loci on
chromosome 3, 1, 2, 8, 17, and 18, named, respectively, cytokine
deficiency-induced colitis susceptibility 1–6 (Cdc31–6) (10). The
strongest association with the colitic phenotype was seen for
Cdc31, which includes two attractive candidate genes: encoding nuclear
factor κB subunit 1 (Nfkb1) (8). Quite remarkably, both of these genes
encode pivotal proteins in TLR downstream signaling, corrobora-
ting data on the impairment of TLR5. Although a direct link to
epithelial dysfunction has not been made to the colitis phenotype,
these data suggest that C3H/HeBJbir mice exhibit defects in TLR5-
dependent host-microflora interactions, resulting in increased T
cell responses to bacterial antigens (i.e., flagellin).

Activation of the TLR5 pathway also appears to be the mech-
anism by which adherent-invasive Escherichia coli (AIEC) excacer-
brates inflammation in dextran sulfate sodium (DSS)-induced
coliitis (11). In these studies, BALB/cJ mice treated with DSS and
orally challenged with LF82, the reference strain for AIEC that has
the ability to adhere to and invade IECs (12) and notably colo-
nizes the inflamed mucosa of ileal CD patients (13), worsened the
severity of colitis and induced a sevenfold increase in colonic tissue
levels of TLR5 compared to mice infected with a mutated strain
of LF82 that lacks the flic gene encoding flagellin (11). These data
further support a central role of TLR5 activation in bacterial-host
interactions that drive chronic intestinal inflammation. Moreover,
a dominant-negative TLR5 polymorphism, which has been shown
to dampen adaptive immune responses to flagellin, appears to
reduce the production of IgG against flagellin and to be protective
against the development of CD in a Jewish population, suggesting
that mucosal immune responses to flagellin promote pathogenic
responses in CD (14).

Aside from TLR5, other TLRs, such as TLR4, also appear to
play a role in gut mucosal immune homeostasis and in regu-
lating epithelial barrier function against invasive bacteria. TLR4
and MyD88 KO mice both develop more severe colitis induced by
DSS compared to wildtype (WT) controls, with increased bac-
terial translocation, shown by the greater positivity of mesenteric
lymph node cultures for E. coli and Pseudomonas fluorescens (15).
Remarkably, the analysis of intestinal mucosa from IBD patients
has shown a strong upregulation of TLR4 that is normally not
expressed in healthy individuals (16), while genetic association
studies have linked carriage of the TLR4 Asp299Gly polymor-
phism, which has been reported to impair LPS sensing, with IBD
susceptibility in different patient populations (17). The preva-
ience of other TLR genetic polymorphisms has been reported in
IBD. TLR1, -2, and -6 non-synonymous polymorphisms have also
been shown to be associated with UC and CD colonic disease
extension (18). While mechanistic studies have not been reported
for TLR1 and -6, TLR2 KO mice have been shown to be more
susceptible to DSS colitis than WT controls (19). These mice dis-
play an increase in IEC apoptosis that is secondary to defective
goblet cell production of trefoil factor 3 (TFF3), a peptide with
anti-apoptotic functions that also enhances mucosal healing (20).
Similarly, impairment of TLR9 may also promote the develop-
ment of intestinal inflammation. Indeed, IECs constitutively expressing
TLR9 release potent amounts of the proinflammatory cytokines,
TNF, and IL-8, in response to CpG DNA, TLR9’s natural ligand
(21). Nonetheless, while a TLR9 polymorphism has been reported
to be associated with CD (22), more experimental data are war-
tanted to clarify the role of TLR9- and other TLR-dependent
pathways in the pathogenesis of IBD.

Downstream of TLR signaling, NF-κB activation occurs that
results in the initiation of a proinflammatory cascade. Increasing
evidence suggests that the NF-κB pathway plays a critical role in
regulating epithelial innate responses and maintaining gut home-
ostasis. This is best illustrated by the NEMO KO model in which
IEC-specific inhibition of NF-κB, through conditional ablation of
NEMO, results in the generation of spontaneous pancolitis (23). In
these mutant mice, specific IEC deletion of NF-κB specifically
resulted in apoptosis of colonic epithelial cells, impaired expres-
sion of anti-microbial peptides, and increased translocation of
bacteria into the gut mucosa. In addition, deficiency of the gene
encoding MyD88, positioned upstream of NEMO in the NF-κB
signaling cascade, prevented colitis and demonstrates that TLR
activation by the gut microbiota is essential for disease patho-
genesis in this model (23). Similarly, TGF-β-activated kinase 1
(TAK1) is an essential intermediate of innate signaling pathways,
and its expression also leads to downstream NF-κB activation. Spe-
cific deletion of TAK1 in IECs results in death on postnatal Day
1 in mutant mice, due to severe intestinal bleeding, while TAK1
knockdown in 4-week-old mice leads to the onset of intestinal
inflammation, characterized by a complete loss of small intestinal
architecture and a marked increase in IEC apoptosis within the
crypts of both the ileum and colon (24). In this model however,
impairment of innate immunity due to ineffective downstream
TLR signaling is not the only mechanism proposed to induce the
aforementioned gut pathologies. In fact, double mutant mice,
bearing both the intestinal epithelium-specific TAK1 deletion and

| Animal model          | Disease location/phenotype of inflammation | Histologic features                     | Identified gene(s) involved                                                                 | Epithelial-specific dysfunction                          |
|-----------------------|------------------------------------------|----------------------------------------|------------------------------------------------------------------------------------------|--------------------------------------------------------|
| C3H/HeBJbir (7)       | Colitis, primary localization in the cecum| Acute and chronic inflammatory infiltrate | Multigenic etiology; susceptibility found on Chr3, Chr1, Chr2, Chr8, Chr17, and Chr18 | Dysregulated epithelial innate responses                  |
|                       |                                          | Crypt abscesses                        |                                                                                          | Ineffective bacterial clearance                         |
|                       |                                          | Ulcerations                            |                                                                                          | Hyper-responsiveness to flagellin stimulation           |
|                       |                                          | Regenerative hyperplasia               |                                                                                          |                                                        |

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the tumor necrosis factor receptor 1 (TNFRI) deletion develop less severe gut inflammation and IEC apoptosis, suggesting that TAK1 confers IEC resistance toward TNF-mediated apoptosis during the inflammatory process (24). Taken together, these data indicate that NF-κB not only serves as a master regulator of proinflammatory cytokines, but also functions to control epithelial barrier integrity and interactions between the mucosal immune system and the gut microflora. The role, however, of the TLR/NF-κB pathway in the development of IBD is complex and may be cell-specific in its overall contribution to disease pathogenesis. In fact, whereas TLR/NF-κB engagement on IECs appears to be mostly protective, the activation of the same pathway in cells participating to adaptive immunity is more likely to contribute to intestinal inflammation.

**IBD SUSCEPTIBILITY GENES ASSOCIATED WITH EPITHELIAL INNATE IMMUNE FUNCTIONS**

Emerging evidence suggests that human genetic studies investigating the pathogenesis of IBD strongly corroborate the hypothesis of a fundamental influence of innate immunity in maintaining gut mucosal immune homeostasis. In fact, apart from genes associated with the TLR pathway, the most convincing genetic data linking dysregulated innate immune responses with IBD centers around genes of the caspase recruitment domain/nucleotide-binding oligomerization domain (CARD/NOD) family, as well as autophagy-related genes.

**CARD15/NOD2** was the first CD susceptibility gene identified in 2001 (25, 26); the discovery of its association to CD ignited interest in the potential mechanistic defects of innate immunity in the pathogenesis of IBD. **CARD15/NOD2** is located on chromosome 16 and encodes a cytoplasmic protein constitutively expressed in myeloid cells (27), IECs, and Paneth cells of the small bowel (28). The CARD15/NOD2 protein is a PRR involved in recognition of the bacterial cell wall component, MDP (29, 30). Similar to other PRRs, sensing of MDP by CARD15/NOD2 triggers NF-κB activation and subsequent expression of proinflammatory cytokines, including TNF, IL-1, IL-6, IL-8, and IL-18 (27, 30, 31). **CARD15/NOD2** is also critical for the release of inflammatory cytokines, including TNF, IL-1, IL-6, IL-8, and IL-18 associated with the TLR pathway, the most convincing genetic data linking dysregulated innate immune responses with IBD centers around genes of the caspase recruitment domain/nucleotide-binding oligomerization domain (CARD/NOD) family, as well as autophagy-related genes.

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Although less developed than the evidence supporting a role for **CARD15/NOD2** in the pathogenesis of IBD, recent genetic findings have focused on the importance of another branch of innate immunity, that is, autophagy, in the regulation of intestinal inflammation. Indeed, large genome-wide association studies have identified two autophagy-related genes, autophagy-related 16-like 1 (**ATG16L1**) (49) and immunity-related GTPase family M (**IRGM**) (50) on chromosomes 2 and 5, respectively, as CD susceptibility genes. Autophagy is an intracellular process through which cells rearrange their cytoplasm and organelles by means of lysosomal digestion (51), and is considered a response of innate immunity as it represents a major mechanism of defense against intracellular pathogens, such as Salmonella or Mycobacterium species (49, 52, 53). In fact, functional studies have shown that **ATG16L1** knockdown in IEC lines impairs the clearance *S. typhimurium* infection (49). In addition, since cells undergo a structural de-arrangement during the autophagic process, autophagy has the potential to alter overall epithelial integrity (51). Interestingly, mice that are genetically engineered to under-express the **ATG16L1** protein display profound alterations in Paneth cell morphology and function (54). Although these mice do not develop spontaneous gut inflammation, they show a lack of lysozymes in the intestinal mucus, hyperactivation of proinflammatory pathways, and the production of adipokines and acute phase reactants (54).

Taken together, epithelial innate immune function, including appropriate activation of PRR pathways and of autophagy processes, plays a central role in the overall maintenance of intestinal immune homeostasis. Defects in epithelial innate function can result in dysregulated mucosal immune responses that lead to chronic intestinal inflammation and IBD (Figure 2). As such, IECs embody much more than the mere lining of the gut lumen, but represent the first line of host defense, controlling penetration, and invasion of pathogens, which is critical in limiting adaptive immune activation.

**DEFECTS IN EPITHELIAL BARRIER FUNCTION LEAD TO INTESTINAL INFLAMMATION**

In addition to the central role of IECs in maintaining mucosal barrier function through early activation of innate immune responses, the intestinal epithelium also constitutes an impermeable layer that has the ability to selectively absorb what is necessary to sustain the organism, while denying passage of other pathogenic and
ory, as in the case of IBD (55), a growing body of evidence has supported this theory. Central to this hypothesis, several studies have reported ultrastructural changes in IEC junctional complexes, spontaneously and without further manipulation. For example, mice genetically engineered to express a dominant negative N-cadherin specifically in small intestinal IECs develop a spontaneous IBD phenotype resembling CD (62). Cadherins, together with catenins, are the main constituent of the AJs, cell-to-cell

**ROLE OF TIGHT JUNCTIONS IN INTESTINAL INFLAMMATION**

 Tight junctions are pivotal in regulating intestinal permeability and the diffusion of ions and molecules across the epithelial luminal surface. TJAs consist of at least 50 different membrane-associated proteins located between apical and lateral regions of polarized epithelial cells that link neighboring cells and regulate molecular flow through intercellular spaces (56). TJ proteins include: (1) integral membrane proteins, such as junctional adhesion molecules, claudins, and occludins, which extend into the intercellular spaces and function as a gate, (2) cytoplasmic cytoskeletal linker proteins, such as cingulin, zona occludens-1, -2, -3 (ZO-1, -2, -3), which anchor membrane proteins to the cytoskeleton, and (3) a number of signaling proteins that can activate various downstream cascades, act as transcription factors, and serve as cell cycle regulators (56). Several lines of evidence support the concept that a direct link exists between TJ protein impairment and intestinal inflammation, with the large majority of data generated from animal models of intestinal inflammation.

**IMPAIRED GUT PERMEABILITY IN ANIMAL MODELS OF INTESTINAL INFLAMMATION**

 Chemically induced colitis likely represents the most highly utilized animal models to induce intestinal inflammation. A prototypic example is colitis induced by DSS, which is fed to directly damage the colonic epithelium, resulting in disruption of barrier integrity and a subsequent increase in luminal antigen/bacterial translocation to the underlying components of the gut mucosal immune system (57). In fact, mice exposed to DSS develop an increase in intestinal permeability before the onset of colonic inflammation. Moreover, several changes in TJ assembly occur during the pre-inflammatory stage of this model, such as complete loss of ZO-1 expression and a doubling of occludin-1 expression in colonic epithelia (58). The ensuing inflammation is acute in nature, primarily consisting of neutrophil and macrophage infiltration and expression of associated cytokines. The sensitivity to DSS challenge differs in various mouse strains (e.g., C3H/HeJ mice display increased susceptibility compared to C57BL/6J mice), while genetic studies have identified quantitative trait loci conferring susceptibility to the development of DSS-induced colonic inflammation. These loci, named DSS colitis 1 and 2 (Dssc1 and 2), are located on chromosomes 5 and 2, respectively, but further investigation is needed to evaluate the actual susceptibility genes within these loci (59). As such, although a viable model to investigate the process of epithelial damage/repair and the subsequent acute inflammatory events, it should also be noted that acute DSS colitis does not require the presence of T and B cells (60), and therefore, does not represent an appropriate model when investigating more chronic and adaptive immune responses related to IBD pathogenesis.

 Similarly, trinitrobenzene sulfonic acid (TNBS)-induced colitis is another alternative chemically induced model in which an ethanol solvent is first administered that permeabilizes the epithelium, followed by TNBS that functions as a sensitizing hapten driving cellular immune responses toward a Th1 polarized phenotype (61). Despite the artificial nature for initiating gut inflammation, chemically induced models of colitis highlight the importance of epithelial barrier disruption, which is likely the primary event leading to the development of colonic inflammation characteristic of these models.

 Interestingly, while several genetically engineered mouse models have been commonly challenged with either DSS and/or TNBS to assess their susceptibility toward a colitic phenotype, only a few of these mutant mice with deletion or transgenic expression of genes related to specific junctional complexes develop colitis spontaneously and without further manipulation. For example, mice genetically engineered to express a dominant negative N-cadherin specifically in small intestinal IECs develop a spontaneous IBD phenotype resembling CD (62). Cadherins, together with catenins, are the main constituent of the AJs, cell-to-cell
adhesion structures, and are essential for normal gut development. N-cadherin is a transmembrane molecule that regulates calcium-dependent intercellular adhesion and relies on its association with the actin cytoskeleton. In these mice, altered expression of N-cadherin interferes with E-cadherin and leads to ruptures in the epithelial monolayer and to the generation of patchy inflammatory lesions. N-cadherin mutant mice also demonstrate aberrant epithelial proliferation, migration, and programmed cell death in small intestinal crypts that eventually lead to adenoma formation. In support of these data, mice conditionally knocked-out for epithelial p120-catenin, a direct cytoplasmic regulator of E-cadherin expression and function, show a phenotype similar to dominant negative N-cadherin mice (65).

Another IBD model that results from a primary epithelial permeability defect is the Gαi2 KO mouse strain that develops a pancolitis at 8–12 weeks of age, and for which early indications point to a defective epithelial barrier that occurs prior to histologic inflammation (64). Gαi2 is an inhibitory isoform of G protein subunit α found in IEC as well as lymphocytes that plays an important role in regulating signal transduction through adenylylate cyclase; this subunit is also critical in regulating epithelial permeability. In fact, it has been shown that Gαi2 overexpression in IEC monolayers induces TJ assembly, increasing transepithelial electrical resistance (TER) (65). Therefore, a possible impairment of TJ assembly may be responsible for decreased barrier integrity leading to superficial colitis, which is most severe in the distal colon of this mouse strain (64).

Deletion of the JAM-A gene, encoding a transmembrane TJ protein, has generated a unique model of intestinal inflammation. JAM-A deficient mice display increased colonic epithelial permeability in homozygous mutants that corresponds to an increase in claudin-10 and -15 (66). While the colonic mucosa has normal epithelial architecture, increased polymorphonuclear infiltration, and formation of large lymphoid aggregates are observed in JAM-A KO mice that are absent in WT controls (66). JAM-A is also localized to TJs of endothelial cells as well as on the surface of leukocytes, serving as a critical protein in mediating leukocyte migration (67). However, when mice with specific inactivation of JAM-A in endothelial and hematopoietic cells (Tie-2-Cre-JAM-A−/− mice) (68) were treated with DSS, resulting colonic inflammation was comparable to controls and much less severe than in JAM-A KO mice, strengthening the hypothesis of a primary defect of epithelial origin that leads to colitis (69). Interestingly, in the same study, reduced levels of JAM-A expression were detected in inflamed tissues from IBD patients compared to controls (69), suggesting that JAM-A may also play an important role in human disease. However, recent studies in JAM-A KO mice have also shown that dysregulation in adaptive immunity also plays a role in the development of the colitis phenotype since the lack of T and B cells and, more prominently the absence of CD4+ T cells, increases the severity of intestinal inflammation in these mice (70).

Indeed, the phenotype displayed by the three aforementioned strains of genetically modified mice suggests a primary involvement of epithelial barrier dysfunction in the pathogenesis of intestinal inflammation. However, it should be pointed out that, at present, no other mouse strain in which assembly of the intestinal epithelial junctional structure has been altered, develops any significant signs of intestinal inflammation. As an example, even though occludin KO mice exhibit pronounced morphologic alterations within the gastric mucosa, such as mucus cell hyperplasia and complete loss of parietal cells, no evidence of inflammation has been detected in both the stomach as well as along the entire length of the gut (71). Quite surprisingly, the absence of occludin in these mice does not seem to affect intestinal permeability, which appears to be comparable to that observed in WT littermates (71). Similarly, claudin-15 deficient mice display an enhanced proliferation of intestinal crypt cells, resulting in an overt megaintestine phenotype in the upper small bowel, but neither gut inflammation nor increased epithelial paracellular permeability are observed in these mice, despite a decrease in the number of TJ strands within the distal jejunum and without a compensatory increase in the synthesis of other claudins (72). These observations suggest that both occludin and claudin-15 may be involved in epithelial differentiation/growth regulation, but likely do not play a critical role in the regulation of intestinal epithelial permeability. Similarly, transgenic mice expressing constantly active myosin light chain kinase (CA-MLCK) show a marked increase in intestinal permeability and overexpression of IFNγ, TNF, and IL-4, but no overt histologic signs of intestinal inflammation. MLCK is a kinase that, upon TNF stimulation, phosphorylates myosin II regulatory light chain, leading to TJ rearrangement, and reduction of intestinal barrier function. Interestingly, CD4+CD45RBhi adaptive transfer into CA-MLCKRAG1−/− mice causes a much more severe colitis, with an earlier onset, compared to transfer into RAG−/− mice (73).

Taken together, these data suggest that, perhaps, deletion, or deficiency of a single TJ protein may not be sufficient to disrupt intestinal barrier function alone, and may require a particular combination or a greater number of TJs to be altered and/or dysregulated before overt gut inflammation is observed. Alternatively, the possibility exists that dysfunction of TJ assembly or TJs themselves may not be causal for the generation of chronic intestinal inflammation.

The aforementioned animal models, although extremely useful in investigating specific molecular mechanisms in the pathogenesis of IBD, do not fully recapitulate disease observed in patients since IBD is clearly multifactorial and not caused by a single mutation or defect in cellular and molecular immune pathways. As such, animal models that occur spontaneously in the absence of chemical, genetic, or immunologic manipulation are likely more representative of the human disease condition. Two animal models that spontaneously develop chronic intestinal inflammation similar to human IBD are the C3H/HeJ and SAMP1/YitFc (SAMP) mouse strains, and their phenotypes are likely due to multiple defects in both innate and adaptive immune responses (7, 74). While gut inflammation in both models appears to be due to multiple defects, epithelial innate dysfunction also plays a central role in disease pathogenesis of these two mouse strains. As previously described, C3H/HeJ show an impairment of epithelial innate immune responses, especially against flagellin (8, 9), leading to overaggressive adaptive immune responses. The SAMP mouse strain is another spontaneous model of IBD that most closely resembles CD for its histologic features and localization to the terminal ileum (74, 75). The ileitis characteristic of these mice is discontinuous in nature, with inflammatory lesions occurring sporadically along...
length of the ileum, alternating with areas of relative normalcy, and with a small percent of mice (2–3%) developing perianal fistulas (76). Alterations in epithelial morphology and architecture occur early in the disease process with expansion of IECs of primarily secretory cells lineage, including Paneth cells and goblet cells, and a decrease of mature absorptive enterocytes (77). Remarkably, in vivo and ex vivo experiments using SAMP mice have shown significantly increased epithelial paracellular permeability in the ilea in comparison to control mice; increased gut permeability was observed in both older mice with established inflammation, and in young mice (3 weeks of age), before the onset of the disease (78). This epithelial barrier defect appears to be independent of commensal bacterial colonization as increased permeability is also observed in SAMP mice raised under germ-free conditions. The increase in gut permeability is likely related to altered TJ protein expression and localization in that epithelial expression of claudin-2 is eightfold greater than in controls, while occludin is markedly suppressed, prior to the onset of intestinal inflammation. As such, the balance between different TJ proteins appears to play a crucial role in regulating TJs assembly/stabilization, and therefore, paracellular permeability. High expression of occludin and of most claudin isoforms usually reinforces the intestinal barrier; on the contrary, claudin-2 expression appears to establish lower affinity interactions with other claudin isoforms on neighboring IEC, leading to a leakier epithelial layer (79). The study of the SAMP genome revealed several quantitative trait loci with linkage to ileal inflammation, located on chromosomes 6, 8, 9, 17, and X (80). Within these loci, several potential candidate susceptibility genes have been identified, and most are involved in either immune regulation and/or intestinal epithelial functions. Among these, several were related to the structural formation of the apical junctional complex, including E-cadherin (Cdh1) on chromosome 8, JAM-C (Jam3), cingulin-like 1 (Cgnl1), nectin-1 (Pvrll1) and β-catenin (Ctnmbi) on chromosome 9, afadin (Mllt4) on chromosome 17, and interestingly, claudin-2 (Cldn2) on chromosome X (81). In addition to these data, further observations suggest a primary involvement of IEC in the pathogenesis of the ileitis characterizing SAMP mice; in fact, the primary defect appears to originate from a non-hematopoietic source since bone marrow (BM) chimeras consisting of irradiated non-inflamed control AKR recipients receiving donor pathogenic SAMP BM did not confer disease, while recipient SAMP mice receiving donor AKR BM resulted in severe ileitis (78). Taken together, these data suggest that epithelial barrier dysfunction is likely the primary, initiating trigger that leads to gut inflammation in the SAMP model of CD-like ileitis as well as other experimental models of chronic intestinal inflammation, and that is phenomenon may share similarities to patients with IBD.

It should be pointed out, however, that SAMP mice (similar to the C3H/HeJ/Bir strain), demonstrate defects in more than one component of normal mucosal immune function. In fact, mice generated by crossing the RAG-2 KO mutation onto the SAMP background, resulting in SAMP mice that lack mature T and B lymphocytes, do not develop ileitis (unpublished results), indicating that despite the presence of the epithelial barrier defect, the adaptive arm of the immune system is still required for the disease phenotype to occur. Therefore, the concept of “multiple hits” or defects in interacting components of host mucosal immune responses (i.e., of both innate and adaptive origin) is likely the cause of chronic intestinal inflammation in the spontaneous murine models of IBD, and is likely also necessary for the initiation and perpetuation of disease observed in patients with IBD.

**TJ PROTEIN IMPAIRMENT IN HUMAN IBD**

In IBD patients, several lines of evidence suggest that gut permeability changes could play a pivotal role in disease development; in fact, a decrease in intestinal epithelial barrier function and altered expression of TJ proteins have been observed in patients affected by CD and UC and their relatives (82, 83). Moreover, it has also been shown that in CD patients, an increase in epithelial permeability precedes episodes of disease relapse and the onset of symptoms by up to 1 year (84, 85). Interestingly, as observed in SAMP mice, claudin-2, and occludin appear to be involved in these permeability changes. IEC from IBD patients express much more claudin-2 and less occludin, claudin-3 and -4 in comparison with gut epithelial from healthy controls, particularly in active UC patients (86, 87). These findings are further corroborated by the observation that claudin-2 is markedly upregulated in the epithelium of dogs affected by idiopathic lymphocytic-plasmacytic colitis, another model of spontaneous intestinal inflammation (88).

In addition to expression data, genetic studies have also revealed a potential link between genetic polymorphisms/mutations in TJ-associated genes and the development of IBD. A recent large GWA study, including more than 2300 cases and 5400 control subjects, identified novel epithelial-related susceptibility genes for UC (89); this study found the greatest association with HNF4A, a gene encoding hepatocyte nuclear factor 4α, a transcription factor that regulates the synthesis of several TJ, AJ, and desmosome proteins (90). In fact, IEC-targeted deletion of HNF4A causes the perinatal death of experimental mice, due to severe defects in embryonic development of the gastrointestinal tract, characterized by the absence of crypt formation, reduced epithelial cell proliferation, and defective goblet cell maturation (91). In addition, conditional deletion of IEC HNF4A showed increased intestinal permeability and greater susceptibility to chemically induced colonic injury, suggesting the importance of this gene in intestinal inflammation (92). In the same GWA study, authors identified laminin β1 subunit (LAMB1) and E-cadherin (CDH1) as possible susceptibility genes for UC (89), corroborating the data on the dominant negative N-cadherin (62) and conditional IEC p120-catenin KO mouse models (63).

Other IBD susceptibility genes identified thus far appear to be primarily involved in TJ assembly. In fact, variants of myosin IXB (MYO9B), partitioning defective protein 3 (PARD3) gene, and membrane-associated guanylate kinase, WW, and PDZ domain-containing protein 2 gene (MAGI2), were found to be associated with UC, with a weaker association with CD for MYO9B and MAGI2 (93–95). MYO9B encodes an unconventional myosin molecule involved in actin remodeling of epithelial enterocytes, and in TJ assembly (96), while PARD3 and MAGI2 encode for adaptor proteins also participating to this process (95). Interestingly, CD patients, carrying MAGI2 variants associated with IBD, display higher serum levels of antibodies against antigens from intestinal microbiorganisms, such as anti-Saccharomyces cerevisiae (ASCA),
anti-CBir1 flagellin (CBir1), and anti-outer membrane porin C (OmpC) (94), further confirming the central role of intestinal barrier function in the pathogenesis of IBD. It is worth noting that MYO9B, PARD3, and MAGI2 were also reported to be celiac disease susceptibility genes (95, 97). If that is the case, a common, primary causal mechanism involving epithelial permeability defects may be hypothesized as a potential trigger for the development of chronic intestinal inflammation, as recapitulated in Figure 3. Again, it would be fair to say that in human disease, as postulated by the “multiple hit” theory, impairment of intestinal permeability may represent only one of the aberrancies that, if combined with others, can lead to the development of chronic intestinal inflammation, such as that observed in IBD.

**EPITHELIAL CELL INTEGRITY**

The integrity and function of IECs appear to be additional determinants in intestinal barrier function. As such, alterations in structural proteins or in proteins that are pivotal in maintaining cell homeostasis may lead to a breakdown of the epithelial “wall,” as summarized in Figure 4. The profound dysfunction in IECs caused by the loss of cell polarity has the ability to trigger potent inflammatory responses within the gut. In fact, the onset of spontaneous colitis has been described in mice deficient in Ap1m2, a master regulator of IEC polarization; these mice display impaired epithelial innate immune functions, as a consequence of a reduction in β-defensin release, followed by a pathogenic Th17 immune response (98). Inflammation also develops when normal IEC turnover is disrupted, as in the case of recombination signal protein for Ig κ J region (RPB-J); this protein is involved in the regulation of the Notch signaling pathway, which plays a major role in the regulation of intestinal epithelium differentiation and proliferation. The conditional KO of RBP-J in IECs results in the development of a spontaneous Th17 dominant colitis, characterized by impaired epithelial defense against bacteria, goblet cell hyperplasia, retarded IEC turnover, and altered TJ assembly (99). In addition, the onset of intestinal inflammation can by initiated by targeting the endoplasmic reticulum (ER) stress response, which is pivotal for the development and survival of secretory cells. Mice deficient in the transcription factor, X-box-binding protein 1 (XBP1), a key component in the activation of the ER stress response, spontaneously develop small intestine inflammation, which displays a patchy pattern, is not granulomatous, and has severity varying from the presence of mild polymorphonuclear infiltrates in lamina propria to the presence of crypt abscesses and ulcerations (100). Striking features of these mice are the complete ablation of functional Paneth cells, a marked reduction in number and size of small intestine goblet cells, and villus shortening with a reduced villus:crypt ratio, which are a sign of the regenerative response. In the absence of XBP1, Paneth cells are unable to process and secrete the anti-bacterial peptides and undergo early apoptosis, while small intestinal, but not colonic, goblet cells present with a reduced number of secretory granules and low levels of MUC2 expression (100). Notably, these mice do not exhibit alteration in intestinal permeability, but are much more susceptible to *Listeria monocytogenes* infection compared to WT littermates, showing a 10-fold higher burden of *L. monocytogenes* translocating into liver.
FIGURE 4 | Role of epithelial cell integrity and mucus production in gut health and disease. Proteins regulating cell structure (e.g., DLG5) or metabolic functions (e.g., XBP1) maintain IEC integrity. IECs in constant contact with luminal toxins and xenobiotics dispose of these harmful molecules by means of several transporter proteins, such as MDR1, OCTN1, and 2. IECs secrete a thick layer of mucus, whose production is finely regulated by different proteins, including MUC family members and POFTUT1. Loss of control over ER stress, resulting from XBP1 dysfunction and accumulation of toxic molecules inside IECs, secondary to transporter molecule loss of function, cause IEC damage, defective defensin secretion from Paneth cells, and release of proinflammatory mediators leading to immune activation. Direct exposure of IEC to luminal toxins/antigens is increased by deletion of MUC2, 3, and 4, which leads to dramatic reduction of mucus production, and eventually to intestinal inflammation. Conversely, overproduction of mucus is also harmful, leading to bacterial overgrowth in intestinal crypts, as seen in POFTUT1 deficiency, causing a dysregulation of the epithelial transcription factor, NOTCH that controls IEC proliferation and differentiation.

and spleen 72 h after oral infection (100). Therefore, in XBP1 KO mice, the impairment of Paneth cell function, and the consequent defect in bacterial clearance, appears to be the prominent trigger for intestinal inflammation, rather than epithelial leakiness due to suffering IECs. However, the lack of inflammation and crypt colonization by intestinal microbes in Paneth cell or cryptdin deficient mice (101) suggests that other defects are required in order to initiate intestinal inflammation. In fact, silencing of XBP1 in the murine IEC line, MODE-K, leads to the activation of Jun N-terminal kinase (JNK)/Stress-activated protein kinase (SAPK) signaling, enhancing IEC inflammatory responses to proinflammatory stimulation, such as flagellin or TNF (100). Thus, ER stress due to knocking down XBP1 directly puts IECs into a proinflammatory state. Even though the XBP1 KO phenotype consists of ileitis, colonic IECs are also prone to ER stress and are, in fact, more sensitive to harmful events. As such, these mice are much more susceptible to DSS colitis than WT controls, exhibiting more severe clinical and histological signs of disease activity after the DSS challenge (100). Finally, XBP1 may also play a major role in human IBD as inflamed and non-inflamed tissue biopsies from CD and UC patients show an increased expression of this transcription factor (100). In addition, several SNPs in XBP1 have been shown to be associated with both CD and UC (100), strongly indicating the involvement of XBP1 in human IBD pathogenesis.

The murine multiple drug resistance 1 a (mdr1a) gene, corresponding to human MDR1, encodes P-glycoprotein 170, which is an efflux pump for amphipathic and hydrophobic molecules, mainly xenobiotics, and is expressed in many cell lineages, including IECs (102). This molecule participates in the transmembrane transport of macromolecules, thus regulating epithelial transcellular permeability, but also in cellular detoxification processes. The importance of P-glycoprotein 170 in preserving intestinal
homeostasis is depicted in mdrla KO mice, wherein the lack of this protein causes development of spontaneous and severe colonic inflammation. The colitis phenotype, which closely resembles human UC, is characterized by massive thickening of the mucosa, leukocyte infiltration in the lamina propria, occasional crypt abscesses and ulcerations, crypt elongation, and dysregulated IEC growth (103). Not surprisingly, these mice display signs of intestinal barrier dysfunction. In fact, increased bacterial translocation that correlates to disease severity, greater basal colonic ion transport, decreased TER, are typical features of these mice. Indeed, the increase in epithelial permeability does not appear to be a consequence of the inflammation since it is observed as early as 4 weeks of age, prior to the onset of colitis (104). Moreover, the development of the disease requires the presence of the normal gut flora, as antibiotic treatment virtually prevents intestinal inflammation (103). Indeed, the genetic background greatly influences the development of the inflammatory phenotype; in fact, whereas mdrla deficiency in FVB mice causes spontaneous colitis, the same genetic defect triggers colonic inflammation only in C57/BL6 mice when they are exposed to DSS (105). Gender also plays an important role in this model, as male mdrla deficient mice develop severe colitis earlier and show increased epithelial permeability compared to females. In addition, barrier dysfunction is accompanied by decreased phosphorylation of the TJ proteins, occluding, and ZO-1. Interestingly, a recent study showed that colons from young, 4- to 5-week-old mdrla deficient mice are disease-free and display no evidence of increased permeability compared to controls (106). In these mice, a distinct pattern of upregulated genes was observed in local tissues that are associated with bacterial recognition and the ubiquitin-proteasome system, suggesting that P-glycoprotein may be critical in regulating interactions with the enteric microflora leading to colitis, albeit prior to epithelial barrier disruption. Similarly to SAMP mice, the generation of BM chimeras in this model also strongly indicates an inherent epithelial defect as a primary mechanism for the development of colitis. In fact, irradiated non-infused control FVB recipients receiving donor pathogenic BM from mdrla KO mice did not show signs of disease, while recipient mdrla KO mice receiving donor BM from FVB mice developed overt colitis. Taken together, these data suggest that the initiating factor for the development of colitis in mdrla KO mice is likely the result of an epithelial-derived dysfunction; however, controversy remains as to whether the primary event is solely due to a permeability defect, given the expression of P-glycoprotein 170 in hematopoietic cells as well.

In support of the relevance of this transporter in gut inflammation, a polymorphism (Ala893) of MDR1 has been reported to be associated to IBD (107). Likewise, IBD genetic studies identified two genes, encoding epithelial transporter proteins, as IBD susceptibility genes. Solute carrier 22A4/organic cation transporter 1 (SLC22A4/OCTN1) and SLC22A5/OCTN2, localized on chromosome 5, encode transporter proteins involved in xenobiotic and toxin removal, physiological substrate uptake, and carnitine metabolism (108). Functional nucleotide polymorphisms (SNPs) of these genes, leading to impairment of OCTN promoter activity and consequently to severe alterations in transporter functions, were found to confer susceptibility to the development of CD (109). Therefore, it could be hypothesized that the carriage of MDR1, OCTN1, and OCTN2 variants might interfere with IEC homeostasis at different levels: variants of all three of these genes could alter transepithelial permeability to large molecules, carriage of the MDR1 polymorphism could dampen the ability of IEC to eliminate potentially toxic molecules, while OCTN1 and OCTN2 SNPs could damage mucosal energetic metabolism through a defective carnitine intake.

Other genetic variants that may affect the integrity of IEC structure and polarity could represent alternative predisposing factors for the onset of IBD. In fact, variants of the gene encoding the structural protein Disk large homolog 5 (DLG5) appear to predispose individuals to CD (110). DLG5 is located on chromosome 10 and encodes a scaffolding protein expressed in IEC from both the colon and small bowel, and is involved in the maintenance of IEC integrity, regulation of cell growth, as well as epithelial polarization (111). Haplotype D, or R30Q variant (SNP 113G → A), influences CD susceptibility as it encodes an amino acid substitution that results in a mutation that likely interferes with DLG5 scaffolding (110). Similar to MYOIX, PARD3, and MAGI2 variants, the DLG5 R30Q polymorphism has also been reported to be associated with celiac disease (112), corroborating the concept that a common mechanism, that is breakdown of the intestinal barrier, may exist between celiac disease and IBD. In addition to the scaffolding function, which also involves the constitution of AJs, DLG5 is also important in innate immune responses. Recent studies have identified an exon, which encodes for a CARD domain, that has been identified within the DLG5 gene and its expression in colonic tissues has been confirmed (113). As such, DLG5 may belong, or be closely related to, the CARD/NOD family, and therefore, may also participate directly in bacteria-host interactions within the gut. Interestingly, data generated in pediatric and adult IBD populations showed a gender effect in analyzing DLG5 R30Q carriage and CD susceptibility. While this haplotype represents a susceptibility factor for CD in males, it confers protection against the development of disease in females (114, 115). Further studies, however, are warranted to investigate whether gender differences exist and to mechanically determine how DLG5 is involved in IBD pathogenesis.

GENETIC REGULATION OF EPITHELIAL MUCUS PRODUCTION

Aside from their absorptive, immunological, and barrier functions, IECs are also specialized to produce a large amount of mucus. As such, the epithelial mucosal surface is covered by a more than 100 μm thick layer of mucus, secreted by goblet cells (116). The purpose of this mucus layer, aside from the lubrication between the luminal contents of the gut and the epithelial surface, is to add further protection to the intestinal barrier. The main constituents of mucus are phospholipids and mucins, both of which are highly negatively charged; therefore, the mucus layer represents both a mechanical and chemical barricade overlying the IEC lining. In addition, the mucus layer also supports the presence of proteins in close proximity to the intestinal wall that are pivotal in controlling luminal bacterial burden, such as secretory IgA (117) and lactoferrin (118).

Goblet cells may also play a primary role in the activation of the intestinal inflammatory process. Goblet cells contain potent regulators of the inflammatory cascade, such as components of
the kallikrein-kinin system, within their cytoplasmic secretory granules. Kallikrein is an enzyme present in different isoforms, in plasma, and in tissues that cleaves high molecular weight kininogen to release bradykinin and activates both coagulation and inflammatory events (119). Conversely, kallistatin, a member of the serine proteinase inhibitor family, is its specific antagonist (120). Tissue expression of kallikrein and kallistatin significantly varies in active IBD; in fact, localization in normal intestinal tissues, in non-involved area of IBD patients, and in specimens from diverticulitis patients, is confined to the cytoplasm of intestinal goblet cells. During the specific inflammatory process characterizing active IBD, goblet cells are depleted of both kallikrein and kallistatin, which are, instead, massively present in the interstitium (121, 122). Thus, goblet cells appear to actively secrete kallikrein and kallistatin in the interstitium, directly regulating local gut inflammatory responses.

Similar to that observed with other components that are important in maintaining intestinal barrier function, dysfunction of mucus production may also lead to intestinal inflammation. For example, while MUC2 KO mice lack the complete gene for MUC2 mucin (123), different strains of MUC2 mutant mice, Winnie and Eeyore, are characterized by two distinct non-complementing missense mutations in MUC2 (124). The different mouse strains spontaneously develop colitis, exhibiting watery diarrhea, rectal bleeding, and prolapse. Histological features include mucosal thickening, superficial erosions, crypt elongation, goblet cell loss, neutrophilic infiltration, and crypt abscesses. Surprisingly, the colitic phenotype of Winnie and Eeyore mice appears to be worse and more penetrant than MUC2 KO mice, the latter being present only under certain genetic backgrounds (124). This phenomenon could be explained considering that the mutations in Winnie and Eeyore mice cause MUC2 protein misfolding and a consequential accumulation in the cytoplasm leading to ER stress (124). This observation, again, highlights the importance of the “multiple hit” concept, since the complete lack of MUC2, predisposing to bowel inflammation, is not sufficient to initiate the cascade of molecular events leading to intestinal inflammation. If the impairment of the mucus barrier is associated with other pathogenic noxae, for example, the disease manifests itself in full.

In fact, reduced production of MUC2 has been reported in human IBD, particularly UC, although it is not well understood whether this decrease represents a primary defect or if it is secondary to the epithelial damage induced by inflammation (125). However, genetic polymorphisms involved in the regulation of mucus production have also been associated with human IBD. In particular, it has been suggested that a few, rare variable number of tandem repeat (VNTR) alleles of the human intestinal mucin gene, MUC3, and non-synonymous SNPs of MUC3A, which is part of the MUC3 gene and encodes a membrane-bound mucin with epi- dermal growth factor (EGF)-like motifs that alter IEC signaling, may confer a genetic predisposition to UC (126) and CD (127), respectively. Thus, impairment of the bowel mucosal barrier, due to different MUC3A variants, may be involved in the pathogenesis of both UC and CD. On the other hand, an hyperproduction of mucus, leading to an alteration of mucus-associated flora, has been implicated as the basis for the enterocolitic phenotype presented by mice with selective deletion of protein O-fucosyltransferase 1 (Pofut1) in IECs (128). POFUT1 is an enzyme required for correct signaling of the Notch pathway. Mice defective in IEC-specific POFUT1 display marked hyperplasia and hypertrophy of goblet cells in both the small intestine and colon, leading to an over-secretion of mucus. Other features of these mice are Paneth and enteroendocrine cell hyperplasia (128). At 4 weeks of age, these mice start to develop ileal and colonic inflammation, evident by thickening of the intestinal wall and by an increase in intestinal permeability. Inflammation increases until 36 weeks of age, with 100% penetration at this age. The enterocolitis is histologically characterized by crypt hyperplasia, inflammatory infiltrates within the lamina propria, crypt abscesses, and transmural inflammation. Moreover, significantly high levels of Th1 and Th17 cytokines are detectable in the inflamed tissues, while a shift toward Gram-negative bacteria is evident in the gut microflora, with spiral-shaped organisms accumulating in dilated and mucus filled crypts (128).

In further support of intestinal mucus production playing a pivotal role in maintaining gut homeostasis, is the observation that thickening of mucus secretions, secondary to the common cystic fibrosis transmembrane conductance regulator (CFTR) ΔF508 mutation, appears to confer protection against the development of CD. In fact, heterozygosity for this mutation has recently been shown to be negatively associated with CD in two independent European cohorts, but had no impact on the risk of developing UC (129). CFTR encodes a transmembrane transporter that pumps chloride anions out of the cell, regulating both secretory and absorptive functions, and the production of mucus from IECs; however, it can also bind the TLR4 ligand, LPS, impacting on the interactions between IECs and bacteria. The ΔF508 mutation causes loss of one phenylalanine from the CFTR amino acid sequence and consequently, the misfolding of this protein. This mutation completely eradicates the functional activity of CFTR, and therefore TER is increased, while PRR signaling remains intact (129). The fact that carriage of one allele of this mutation confers protection against CD further underscores the importance of genetic contributions in regulating epithelial barrier function, as well as intestinal permeability and epithelial innate responses, in maintaining normal mucosal immune homeostasis.

**THERAPIES THAT ENHANCE INTESTINAL BARRIER FUNCTION IN IBD**

Since emerging evidence in recent years has implicated the importance of intestinal barrier function in maintaining gut immune homeostasis, increasing efforts and investment in developing tools by which to manipulate epithelial innate immunity and permeability have been made in order to obtain therapeutic effects in human disease. At present, several approaches have been made and/or proposed to enhance intestinal barrier function using several different strategies. Such drug development could boost epithelial innate immunity, decrease the permeability of the epithelial barrier, or improve the quality and quantity of mucus production (Figure 5).

**ENHANCERS OF EPITHELIAL INNATE IMMUNITY**

The use of granulocyte-monocyte colony stimulating factor (GM-CSF), or Sargramostim, has been proposed for the treatment of
FIGURE 5 | Therapeutic agents that enhance epithelial barrier function. Several drugs can potentially improve different components of intestinal barrier function by (from left to right): (1) enhancing mucosal innate immunity through increased expression of TLRs and production of anti-microbial peptides, (2) decreasing epithelial permeability through the expression and assembly of TJ and AJ proteins, and (3) restoring epithelial cell and mucus layer integrity by reducing IEC apoptosis and inducing mucus production.

patients suffering from CD. A randomized placebo controlled clinical trial demonstrated a significant improvement in disease activity in patients treated with GM-CSF compared to those treated with placebo (130), even though the clinical remission rates did not differ in the two groups. As such, instead of serving as an immunosuppressant or anti-inflammatory agent, GM-CSF has been proposed to serve an enhancer of innate immune responses and has been used for the treatment of inflammatory diseases. This paradox may be explained by considering that GM-CSF is capable of boosting innate immune functions, and therefore, may reinforce the intestinal mucosal barrier (131). In fact, GM-CSF stimulation increases neutrophil expression of PRRs (132), and neutrophil, monocyte, and macrophage bactericidal activity (133). At present, most of the data regarding GM-CSF’s effects on innate immunity come from experiments involving hematopoietic cells since it has been shown that IECs, including Paneth cells, display receptors for GM-CSF, and proliferate after GM-CSF stimulation (134). It is therefore plausible that GM-CSF may have a direct influence on IEC barrier function.

Several lines of evidence also suggest that probiotics can modulate host epithelial innate immune responses. Indeed, it is believed that probiotics have the ability to stimulate mucosal defenses through TLRs and upregulation of innate-type cytokines (135, 136). For example, experimental data has shown that administration of Lactobacillus casei has the ability to potently increase cellular expression of TLR2 (137), and the E. coli strain, Nissle 1917, can induce the expression of β-defensins (138), as well as both TLR2 and TLR4 (139). Moreover, E. coli strain Nissle 1917 has been shown to ameliorate DSS colitis in C57BL/6 WT mice, but not in mice deficient in TLR2 or TLR4, suggesting that activation of both TLR2 and TLR4 signaling is pivotal for this microorganism in order to exert its beneficial effects (139). Enhancement of innate immune function is not the only way by which probiotics may aid in the control of intestinal pathogens; probiotic supplementation can alter the microbiota of IBD patients (140) through competition with enterotoxigenic and enteropathogenic bacteria for energy sources and for IEC surface receptors. In this way, probiotics may block binding of pathogens to the intestinal epithelial surface, inhibiting their invasivity, and thereby reducing potential bacterial translocation (141, 142).

DRUGS THAT DECREASE EPITHELIAL PERMEABILITY AND LEAKINESS

The modulation of Tα protein expression may also represent a promising target for novel therapies for the treatment of inflammatory gut disorders. At present, the use of compounds with the ability to enhance Tα expression and function has already been proposed for celiac disease. Indeed, celiac disease shares several features with IBD, including an increase in intestinal permeability, and as mentioned earlier, a few susceptibility genes involved in Tα assembly. An octapeptide, called AT-1001, derived from Vibrio cholera’s zonula occludens toxin (ZOT), has been proposed for the treatment of celiac disease. This peptide has the ability to interfere with IEC cytoskeleton rearrangement and Tα disassembly, secondary to gliadin exposure (143). Thus, AT-1001 antagonizes the increase in paracellular permeability induced by ZOT analogs, and the effect of gliadin on epithelial permeability in the duodenal mucosa of celiac patients (143). In addition, IL-10 KO mice that develop spontaneous colitis displayed less severe intestinal disease when treated with AT-1001 (144). Therefore, the rationale for the use of AT-1001 in gut inflammatory disorders has become quite apparent. Currently, only two randomized placebo-controlled safety and dose-ranging studies, involving 20 and 86
It has been demonstrated that activation of ERβ and androgen receptors (154). Thus, alterations in TJs triggered by selective ERβ activation may be protective against the development of intestinal inflammation, suggesting a possible role of ERβ in modulating immune responses and intestinal permeability. It is well established that the prevalence of inflammatory and autoimmune disorders is higher in female than in male subjects (150), and this gender bias is present, albeit to a much lesser extent, in IBD patients. In fact, CD is slightly more common and aggressive in female compared to male patients (151). However, how estrogens and androgens can modify mucosal immune responses still remains somewhat obscure. The administration of dehydroepiandrosterone and 7-alpha-hydroxydehydroepiandrosterone in rats with TNBS colitis attenuated the degree of mucosal damage and inflammation (152); surprisingly, despite the potential role of estrogens in triggering autoimmunity, 17alpha-ethynyl-17beta-estradiol also ameliorated the severity of disease in HLA-B27 transgenic rats, which spontaneously develop colitis (153). One possible explanation is differential signaling through the estrogen receptors (ER), ERα, and ER-β. Interestingly, it has been demonstrated that activation of ERβ on IECs, through estradiol or specific agonists, increases epithelial barrier function by upregulating occludin and JAM-A, both in vitro and in vivo systems (154). Thus, alterations in TJs triggered by selective ERβ activation may be protective against the development of intestinal inflammation, suggesting a possible role of ERβ agonists in the treatment of IBD.

Parenteral heparin and low molecular weight heparins (LMWHs), such as tinzaparin, deligoparin, enoxaparin, reviparin, and dalteparin, have been proposed as a therapeutic option in IBD, primarily due to their anti-inflammatory properties. Their actual efficacy, however, is debatable, at least considering the parenteral administration route (155). Data recently obtained using chemically induced colitis models suggest a potential role of two different LMWHs in ameliorating gut inflammation if administered directly on the epithelial surface. In one study, parnaparin, a LMWH of 5000 kD, was administered to rats intracolonically, and improved disease severity (156). In another study, colitic mice treated with microspheres loaded with enoxaparin (4500 kD), specifically designed to release the drug into the colon, resulted in a reduction of inflammatory activity after parenteral administration (157). The same approach was tested in a pilot study in patients with mild to moderate active distal UC; sodium parnaparin, dispersed in a multimatrix formulation (MMX) in order to obtain a controlled colonic-release of the drug, showed no side effects and possible efficacy (158). A later, randomized, placebo-controlled clinical trial validated these results, showing a significant clinical response in parnaparin- vs. placebo-treated patients (159). The potential efficacy of heparins may be explained not only by their primary anti-inflammatory properties, but also their ability to regulate epithelial permeability. In fact, the lack of heparan sulfate and syndecan-1, two proteoglycans belonging to the heparinoid compound family, from the basolateral surface of IECs is closely linked with protein-losing enteropathy (PLE), a syndrome, characterized by the leakage of proteins into the intestinal lumen through the gut wall (160–162). Knockdown of syndecan-1 causes augmented paracellular permeability in in vitro monolayers of the HT29 colon cancer cell line, while heparan sulfate and syndecan-1 deficient mice display increased intestinal protein loss that can be prevented by the parenteral administration of heparins, including 2,3-de-O-sulfated heparin, which does not have any anticoagulant properties (163).

Probiotics, as well, appear influence epithelial paracellular permeability, as indicated by the increased TER obtained following treatment of in vitro intestinal epithelial monolayers (164). The mechanism(s) by which intestinal permeability is decreased following probiotic treatment involves rearrangement of TJ proteins, such as ZO-2, protein kinase C ζ (PKCζ) and occludins, that are pivotal in stabilizing TJ complexes (165, 166). It has also been shown that probiotics can decrease intestinal permeability, in vivo, during chemically induced colitis, restoring gut barrier integrity through the maintenance of TJ protein expression, and the prevention of IEC apoptosis (167). Accordingly, VSL#3, a multiple probiotic formulation, proved to be efficacious in restoring early epithelial permeability defects in young SAMP mice, preventing/delaying the development of intestinal inflammation. This effect appears to be related to the increase of TNF production by IECs (168); in fact, co-administration of VSL#3 and anti-TNF antibodies significantly dampened the efficacy of probiotics (169). Thus, increased TNF production by IECs induced by probiotics, may enhance different components of mucosal innate immunity and/or epithelial barrier function.

RESTORATION OF EPITHELIAL CELL INTEGRITY AND BIOLOGY

The integrity of IECs themselves, their cell membrane, and the mucus layer covering the epithelium of the entire gastrointestinal tract, are of paramount importance in maintaining gut immune homeostasis. Indeed, a defect in mucus production has been suggested as a primary feature of UC since the 1980s (170).

Anti-TNF monoclonal antibody therapies have shown great efficacy in obtaining and/or maintaining clinical and endoscopic remission in patients with IBD (171). Aside from their potent
immunomodulating effects, anti-TNF antibody administration has the ability to restore mucosal barrier integrity, normalizing intestinal permeability, in CD patients (172, 173). According to experimental data from studies on human endoscopic biopsies (174) and on SAMP mice (175), this effect could be related to homeostatic regulation of mucosal cell apoptosis induced by anti-TNF strategies. Similarly, manipulation of the enteric flora may provide an alternative strategy to increase epithelial barrier integrity by inducing anti-apoptotic processes. Administration of specific commensal *E. coli* in a mouse model of intestinal development was shown to prevent staurosporine-induced apoptosis, increasing the tissue expression of IFNα and guanylate binding protein-1 (GBP-1), a recently identified anti-apoptotic protein (176).

Growth factors and mediators that promote mucosal wound healing have also been proposed to enhance epithelial barrier integrity by inducing epithelial repair and restitution processes. EGF, for example, has been proposed and tested as a potential therapeutic agent for the treatment of UC. The addition of EGF enemas to mesalamine treatment in UC patients has been shown to increase the response rate to the therapy, confirming the importance of enhancing mucosal integrity for IBD patients (177). In addition, as mentioned earlier, trefoil factors are proteins synthesized by IECs that initiate and improve wound healing after a mucosal injury. Selective deletion of TFF3, abundantly produced by goblet cells in both the large and small intestine, increases the susceptibility to chemically induced colitis (178). Interestingly, exogenous administration of TFF3 was able to reverse colitis, suggesting a potential role of TFF3 in IBD management (178). As such, a pilot clinical trial using human recombinant TFF3 enemas as a novel therapeutic strategy for the treatment of UC was performed (179); this trial, however, did not show any significant difference between patients treated with topical TFF3 in combination with oral 5-ASA vs. those treated with oral 5-ASA alone. Therefore, further studies, perhaps with different clinical conditions or with different administration routes are needed in order to investigate the true therapeutic value of this molecule.

The intestinal barrier can also be structurally manipulated through modification of the IEC membrane composition. Administration of sphingomyelinase decreases TER in monolayers of Caco-2 cells (180) through the hydrolysis of IEC membrane sphingomyelin into ceramide (181), thus altering the composition of cholesterol and sphingolipids in TJ membrane microdomains (182) and transmembrane signaling (180). The end result of this complex chain of molecular events leads, in the end, to an increase in paracellular and transcellular epithelial permeability. Similarly, intestinal epithelial permeability is directly modified by cell membrane lipid content (183–185). As such, omega-3 polyunsaturated fatty acids have been proposed as an adjuvant therapy in IBD (186, 187), even though their efficacy in inducing and maintaining remission is still debatable (188, 189). Interestingly, recent randomized and controlled clinical trials showed that the administration of phosphatidylcholine by slow release ameliorates the inflammatory activity in UC patients, also in steroid refractory subset of patients (190, 191). The rationale behind this rather new therapeutic approach is that phosphatidylcholine and other phospholipids (192) serve as major components of the intestinal mucus layer, generating a hydrophobic protective layer overlying IECs, and therefore take part in establishing the gut mucosal barrier. Mucus from UC patients has been shown to be defective in phosphatidylcholine compared to controls and CD patients (192); thus, this deficiency could be of pivotal importance in the pathogenesis of disease since it significantly alters colonic barrier functions. In addition, intestinal mucus production can be boosted by the administration of cathelicidin, an anti-microbial peptide secreted by IECs. Cathelicidin has been given intrarectally to mice challenged with DSS; in this experiment, mice treated with cathelicidin displayed increased mucus production, an overexpression of the mucin genes *MUC1*, *MUC2*, *MUC3*, and *MUC4*, and milder colitis than untreated controls (193). The ability of cathelicidin to induce mucus gene expression has been confirmed, in part, in the colonic epithelial cell line, HT-29, which responded by upregulating *MUC1* and *MUC2*, but not *MUC3* and *MUC4* (193). Therefore, aside from its bactericidal activity, cathelicidin may have the potential to treat colitis due to its effect on enhancing gut mucus production.

Similar to cathelicidin, butyrate, a short chain fatty acid produced by intestinal microbial fermentation of dietary fibers, has the ability to reinforce epithelial barrier function through an increase in mucus production. In fact, administration of butyrate to human colon cancer cell lines or to endoscopic colon biopsies clearly upregulates mucin production (194, 195), while intrarectal delivery of butyrate into rats increased colonic mucus secretion (196). As such, several studies suggest a primary role of butyrate in the modulation of epithelial permeability. Butyrate can increase TER in Caco-2 and HT-29 cell monolayers by augmenting TJ protein expression (197, 198); however, this effect appears to be dose and cell type dependent. In fact, a higher concentration of butyrate increased paracellular permeability in Caco-2 monolayers (198) and in rat distal colon specimens (199). Interestingly, butyrate exerts many other effects on IECs, specifically contributing to the energetic balance of cells, controlling oxidative stress, and in regulating the inflammatory status of cells (200). Its role in maintaining the epithelial barrier may be more complex, including several pivotal functions for the preservation of IEC homeostasis and integrity. Currently, it is unclear which of these many actions confers the most potent therapeutic effect induced by butyrate administration (201, 202).

In summary, several therapeutic agents demonstrate the potential of modifying the intestinal barrier and enhancing various IEC functions. Their true clinical efficacy in the management of chronic intestinal inflammatory disorders, however, is still largely unknown. This is primarily due to the lack or scarcity of evidence-based information and data regarding the effectiveness of these compounds for the treatment of intestinal inflammation. Therefore, while a deeper knowledge of the cellular events leading to the impairment of mucosal barrier function and the onset of gut inflammation is needed, more safety and efficacy trials are warranted to assess the feasibility of manipulating the intestinal barrier as a novel therapeutic approach for the treatment of chronic inflammation in the GI tract.
CONCLUSION

The etiology of IBD is complex and as researchers deepen their knowledge regarding the mechanisms underlying the pathogenesis of chronic intestinal inflammation, emerging evidence has revealed that the intestinal epithelium plays a central role. Defects of primary epithelial etiology leading to chronic gut inflammation globally include dysfunction of innate immune responses and of epithelial barrier integrity. However, it is likely that the development of IBD occurs as the result of a concomitant presence of different defects in various compartments, as postulated by the “multiple hit” theory, and as supported by several mouse model of chronic intestinal inflammation. As such, impairment of normal intestinal epithelial function, although likely not sufficient by itself to sustain the inflammatory process, plays a primary role in the onset and maintenance of disease. Further investigation is needed to define its precise role in the pathogenesis of IBD, which will lead to more targeted therapies and strategic approaches to specifically boost intestinal epithelial function to ultimately treat patients with IBD.

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Intestinal epithelium regulates mucosal immunity

INTRODUCTION

The etiology of IBD is complex and as researchers deepen their knowledge regarding the mechanisms underlying the pathogenesis of chronic intestinal inflammation, emerging evidence has revealed that the intestinal epithelium plays a central role. Defects of primary epithelial etiology leading to chronic gut inflammation globally include dysfunction of innate immune responses and of epithelial barrier integrity. However, it is likely that the development of IBD occurs as the result of a concomitant presence of different defects in various compartments, as postulated by the “multiple hit” theory, and as supported by several mouse model of chronic intestinal inflammation. As such, impairment of normal intestinal epithelial function, although likely not sufficient by itself to sustain the inflammatory process, plays a primary role in the onset and maintenance of disease. Further investigation is needed to define its precise role in the pathogenesis of IBD, which will lead to more targeted therapies and strategic approaches to specifically boost intestinal epithelial function to ultimately treat patients with IBD.
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