Open access • Journal Article • DOI:10.1002/IBD.20539

Intestinal barrier dysfunction in inflammatory bowel diseases — Source link

Michael A. McGuckin, Rajaraman Eri, Lisa A. Simms, Lisa A. Simms ...+3 more authors

Institutions: University of Queensland, Royal Brisbane and Women's Hospital, QIMR Berghofer Medical Research Institute

Published on: 01 Jan 2009 - Inflammatory Bowel Diseases (Inflamm Bowel Dis)

Topics: Inflammatory bowel disease

Intestinal Barrier Dysfunction in Inflammatory Bowel Diseases

Michael A. McGuckin, PhD, Rajaraman Eri, PhD,*† Lisa A. Simms, BSc,‡ Timothy H.J. Florin, MD,† and Graham Radford-Smith, DPhil‡*

Abstract: The etiology of human inflammatory bowel diseases (IBDs) is believed to involve inappropriate host responses to the complex commensal microbial flora in the gut, although an altered commensal flora is not completely excluded. A multifunctional cellular and secreted barrier separates the microbial flora from host tissues. Altered function of this barrier remains a major largely unexplored pathway to IBD. Although there is evidence of barrier dysfunction in IBD, it remains unclear whether this is a primary contributor to disease or a consequence of mucosal inflammation. Recent evidence from animal models demonstrating that genetic defects restricted to the epithelium can initiate intestinal inflammation in the presence of normal underlying immunity has refocused attention on epithelial dysfunction in IBD. We review the components of the secreted and cellular barrier, their regulation, including interactions with underlying innate and adaptive immunity, evidence from animal models of the barrier's role in preventing intestinal inflammation, and evidence of barrier dysfunction in both Crohn's disease and ulcerative colitis.

(*Inflamm Bowel Dis 2009;15:100–113)*

Key Words: mucosal barrier dysfunction, intestinal inflammation, epithelial dysfunction, mucus, inflammatory bowel disease

In all mucosal tissues a complex cellular and secreted barrier separates the external environment from host tissues. The physical, chemical, and microbial challenges to this barrier are intensified in the intestinal tract due to the presence of food and the symbiotic microbial community. Inflammatory bowel diseases (IBDs) are complex polygenetic diseases characterized by an unnecessary or exaggerated inflammatory immune response to the microbial flora inhabiting the lumen of the gut. Environmental factors are important in all human IBD as the penetrance in monozygotic twins is less than 50% in Crohn's disease (CD) and less than 20% in ulcerative colitis (UC)[1] and these diseases require substantial life history before they emerge. Much of IBD research has focused on the nature of the inflammatory response and sought to identify defects in leukocytes involved in innate and adaptive immunity. However, recent data from animal models shows that intestinal inflammation can be initiated by molecular defects restricted to the epithelium in the presence of a normal microbial flora and normal underlying innate and adaptive immunity.[2–4] Given the central role of the epithelium in regulating inflammatory responses, the importance of the intestinal barrier in limiting access of toxins and microbes to underlying tissues, and the antimicrobial nature of the immune responses in IBD, intestinal barrier dysfunction has strong potential as a pathway to at least some subsets of IBD.

Unlike peptic ulcer disease and *Helicobacter pylori*, the best evidence from human IBD does not support a specific IBD-inducing bacterial infection.[5] However, data do suggest a role for chronic or recurrent microbial infection in the ongoing pathogenesis of IBD. First, antibiotics are a useful treatment in pouchitis (reviewed in Refs. 6,7). Second, there is increased translocated bacteria in CD[8–10] with a consequent augmented humoral immune response.[11] Third, in some murine models a colitogenic microbial flora has been described that can induce colitis in mice with normal epithelium and underlying immunity,[12] and inflammation itself can favor colonization of pathogenic aerotolerant bacteria.[13]

While altered barrier function occurs in IBD,[14–16] a key unanswered question confronting this aspect of IBD research is whether dysfunction of the intestinal barrier in human IBD is a primary contributor to inflammation or a consequence of the action of inflammatory mediators. Nevertheless, regardless of whether altered barrier function makes a primary or secondary contribution to pathology, restoring appropriate barrier function remains a worthwhile therapeutic objective in IBD. In this review we will describe the components of the intestinal barrier, examine in vivo evidence that barrier dysfunction leads to intestinal inflammation, explore evidence of

Received for publication May 13, 2008; Accepted May 15, 2008.

From the *Mucin Research Team, Mucosal Diseases Program, Mater Medical Research Institute, and the University of Queensland, Aubigny Place, Mater Health Services, South Brisbane, Queensland, Australia, †IBD Research Team, Mucosal Diseases Program, Mater Medical Research Institute, and the University of Queensland, Aubigny Place, Mater Health Services, South Brisbane, Queensland, Australia, ‡Inflammatory Bowel Disease Laboratory, Royal Brisbane and Women's Research Foundation, Brisbane, Australia; Queensland Institute of Medical Research, Brisbane, Australia; and Department of Gastroenterology, Royal Brisbane and Women's Hospital, Brisbane, Australia.

Reprints: M.A. McGuckin, University of Queensland, Aubigny Place, Mater Health Services, South Brisbane, Qld 4101, Australia (e-mail: mmcguckin@mmri.mater.org.au).

DOI 10.1002/ibd.20539

Published online 11 July 2008 in Wiley InterScience (www.interscience.wiley.com).

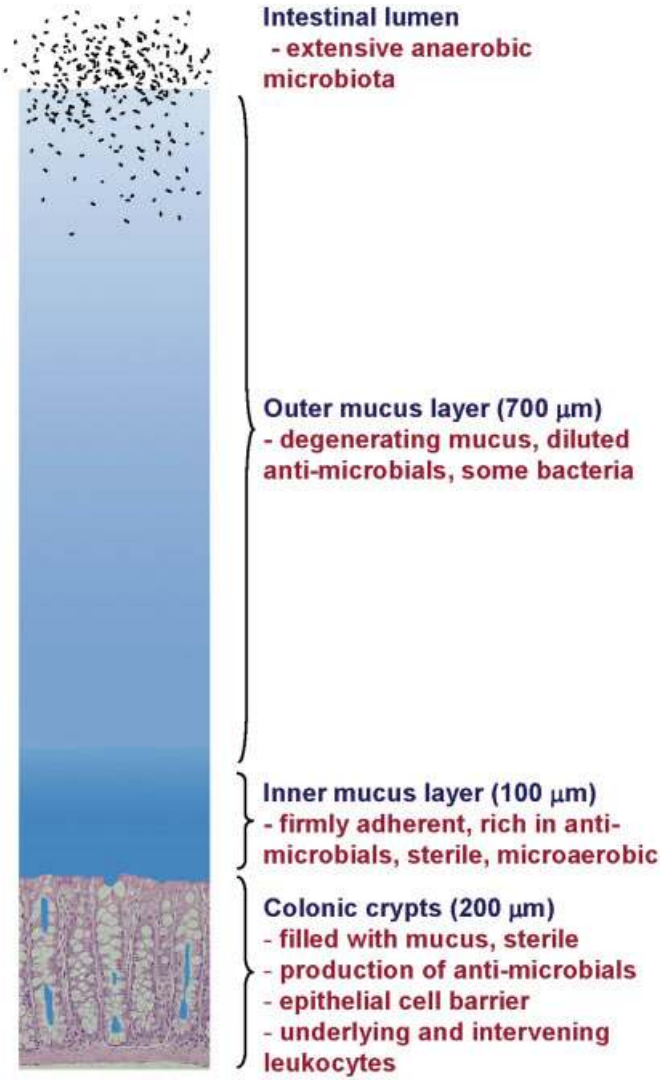

FIGURE 1. Diagrammatic representation of the mucosal barrier in the colon of the mouse showing the thickness of the secreted mucus barrier and the relationship between the luminal microbiota and the epithelium.

barrier dysfunction in human IBD, assess the potential of therapeutic strategies to restore barrier function, and identify the key research objectives for this aspect of IBD research.

COMPONENTS AND ROLES OF THE INTESTINAL BARRIER

The intestinal barrier is composed of a thick secreted mucus layer, a layer of epithelial cells and the underlying nonepithelial mucosal cells, chiefly leukocytes with a range of regulatory and effector functions (see Fig. 1). The elements of the barrier change in different regions of the intestine and can dynamically respond to environmental challenges. Too often elements of this barrier are considered in isolation and one of the major themes of this review will be that these components are intrinsically linked in a complex physiology and therefore need to be considered together in health and disease. The secreted mucus barrier provides both a physical and chemical barrier to microbes, as well as keeping the mucosal surface well hydrated and providing lubrication allowing the continuous flow of luminal contents. The epithelial cells provide a structural barrier, manufacture most, but not all, components of the secreted barrier, are sensors of the external environment, and emit signals regulating underlying innate and adaptive immunity. Nonepithelial mucosal cells, although "underlying" the epithelium, can actually traverse the epithelial barrier, manufacture some components of the secreted barrier, and modulate epithelial cell function, including modulation of the secreted barrier.

The Secreted Barrier

Misconception of the nature of the mucus barrier is probably best represented by the drawings of researchers in the field showing microbes in direct contact with the mucosal epithelial cells. This misconception is fed by the artifactual collapse and loss of the mucus layer during tissue handling, particularly in preparation for histological examination (Matsuo et al[17] took great care to preserve human intestinal mucus and present striking images of intact, although collapsed, mucus). Thus, many of the attempts to measure the mucus layer underestimate mucus thickness. Imaging in live animals and the use of electrodes demonstrate that the mucus layer in the rodent colon is around 800 μm thick, consisting of an inner firmly adherent layer of around 100 μm and an outer more loosely adherent layer of around 700 μm, demonstrated diagrammatically in Figure 1.[18] Thus, a bacterium residing in the colonic lumen needs to traverse around 1000 bacterial cell lengths of thick viscous mucus packed with antimicrobial molecules to make contact with the epithelial cell surface.

The actual location of microbes in the intestine remains controversial. In situ hybridization studies, which are the best available methods of localizing microbes in tissue sections, are dogged by the technical problems referred to above. Using scanning electron microscopy Bollard et al[19] showed that bacteria were positioned exclusively outside the mucus layer in the rat intestine when antibodies were used to stabilize mucus. However, without stabilization prior to processing, even with great care to preserve mucus, bacteria were seen within the mucus, and even within crypts and in contact with epithelial cells.[19] Most in situ studies have failed to try and preserve mucus or relied on Carnoy's fixative to maintain mucus, and consequently the conclusions of these studies are likely to be erroneous. On best evidence at least the inner mucus layer appears largely sterile,[20,21] but whether bacteria reside in large numbers within the outer mucus layer remains unclear, as it is very easily dislodged on handling and lost during tissue processing.

The major macromolecular component of intestinal

TABLE 1. Molecular Elements of the Intestinal Mucus Barrier Relevant to Barrier Function

Component	Source	Regulation	Function
Mucin (MUC2)	Goblet cells, Paneth cells	Constitutive. ↑ TLR ligands, inflammatory cytokines, growth factors, lipid mediators, hormones, neural stimulus	Major macromolecular component of hydrophilic hydrated mucus: physical barrier, hydration, lubrication, retention of antimicrobial molecules
Immunoglobulins (sIgA, IgG, IgM) Secretory component (SC) and FcRn	B lymphocytes, epithelial cells (SC and FcRn)	Constitutive. Regulated by antigen presenting cells, helper T cells and epithelial cells.	Antimicrobial: opsonization of microbes, blocking microbial penetration of mucus SC and FcRn facilitate transport across epithelium. Glycosylated SC protects Fc region of IgA dimer in external environment.
Trefoil peptides (ITF/TFF3)	Goblet cells	Constitutive. Regulation not well understood.	Co-secreted with MUC2, possible modulator of mucin polymerization, stimulator of wound repair.
Antimicrobial peptides (defensins, cathelicidins, lysozyme, PLAP2)	Paneth cells, enterocytes	Constitutive. TLR and NOD ligands, cholinergic stimuli.	Peptides with direct antimicrobial activity.
Phospholipids	Enterocytes	Not known	Hydrophobic element of mucus probably interdispersed in striated layers with hydrated mucus: lubrication and barrier function
Lectins (RegIIIγ, collectins)	Paneth cells, enterocytes	Constitutive and regulated by TLR ligands (e.g,. RegIII expression is lost in MyD88$^{-/-}$ mice)	Direct antimicrobial activity.
Antimicrobial protease inhibitors (SLPI, elafin)	Epithelial cells, leukocytes	Constitutive and increased by inflammation.	Some direct antimicrobial activity.

mucus is the mucin glycoprotein synthesized by goblet cells encoded by the *MUC2* gene, which resides in a cluster of 4 mucin genes on chromosome 11p15.5.[22,23] Each MUC2 subunit protein is over 5000 amino acids in length and over 70% carbohydrate by weight. These subunits dimerize in the endoplasmic reticulum and then following glycosylation in the Golgi undergo further homo-oligomerization to form complex multimers that are primarily responsible for the viscous properties of mucus.[24–26] While mucins provide the matrix, it is vitally important to recognize that mucus is a complex mixture of mucins and other compounds secreted by the epithelium, including phospholipids. Although some specific domains and specific carbohydrates on other mucins have been shown to have direct antimicrobial activity[27,28] or to bind antimicrobial molecules such as histatins and statherin,[28,29] this has not yet been demonstrated for MUC2. However, the intestine secretes many other compounds into mucus that are involved in barrier function, and some of these molecules and their functions are listed in Table 1.

A key function of mucus is to retain a high concentration of antimicrobial molecules in the environment close to the epithelium. It is often not appreciated that the efficacy of these compounds is dependent on their retention in mucus. For example, consider how ineffective defensins in the small intestine would be if they were merely secreted into the large volume and continuous flow of material within the lumen. The defensins are secreted in granules produced by Paneth cells into small intestinal crypts that are completely filled with mucus. In fact, while most of this mucus is derived from goblet cells, Paneth cells also synthesize MUC2, which is packaged into the granules with the defensins and other antimicrobial peptides ensuring that they are co-secreted (McGuckin, unpubl.). It is tempting to speculate that retention of the polycationic defensins in mucus is enhanced by electrostatic interaction with the polyanionic mucin molecules (sulfated and sialylated mucin oligosaccharides are negatively charged). There is much to learn of the complex interactions between components of mucus and how they operate in barrier function in health and disease.

The Cellular Barrier

In addition to producing the major elements of the secreted barrier, underlying epithelial cells form a physical barrier limiting access of microbes and toxins to host tissues. Intestinal epithelial cells form a polarized layer of cells tightly linked via intercellular tight junctions and are covered with a complex apical glycocalyx that includes a family of large cell surface mucin glycoproteins. The epithelial cells

are equipped to phagocytose bacteria, sequester and neutralize toxins, detect prokaryocytic-associated molecular patterns (PAMPs), and respond by bolstering secreted defense, initiating wound repair and activating underlying innate and adaptive immunity.[30] Epithelial crosstalk with underlying immunity is a 2-way communication that is critical in the regulation of the nature of the response to microbial and toxic stimuli.[31] For example, it has recently emerged that epithelial molecules regulate class-switching by B cells in the lamina propria.[32] Some leukocytes are physically integrated within the epithelium as dendritic cells extend processes between adjacent epithelial cells,[31,33] and some specialized T cells and NK cells reside between epithelial cells.[34]

Interaction of the Intestinal Barrier with Commensal and Pathogenic Microbes

The intestinal lumen contains a complex community of microbes[35] generally living in a commensal symbiotic relationship with the host, providing nutrients and maturing the immune system (see reviews 36,37). The intestinal secreted and cellular barrier limits contact between these microbes and underlying host tissues while supporting the growth of the commensal flora. As discussed above, in situ morphological localization studies that preserve the mucus layer show that commensal microbes largely reside on and outside the surface of mucus, positioning them well away from the surface of mucosal epithelial cells.[20] A critical exception to this physical separation occurs in the dome epithelium above Peyer's patches in the small intestine. This epithelium lacks goblet cells and therefore lacks a secreted mucus barrier and has altered expression of cell surface glycoproteins.[38,39] This allows microbes to come in contact with M-cells that phagocytose and transport the microbes to underlying dendritic cells for presentation of antigen in mesenteric lymph nodes.[40] The seminal work of Andrew Macpherson demonstrates that the nature of the ensuing immune response to the commensal microbial flora sampled in this way is noninflammatory and dominated by production of sIgA.[41–43] sIgA is secreted into mucus and helps prevent penetration of the mucus barrier by commensal organisms expressing the target surface antigens.

The strategies used by intestinal pathogens to infect intestinal epithelium perhaps provide the best evidence to date of how the functions of the secreted mucus barrier prevent commensal organisms from penetrating epithelia. Intestinal pathogens, almost by definition, have evolved mechanisms to subvert or avoid the secreted mucus barrier. They are virtually all motile, allowing them to move in mucus, and many bind mucin carbohydrates and produce enzymes that break down the mucin polymer network to facilitate penetration of mucus.[44–48] The protozoan parasite *Entamoeba histolytica* cleaves MUC2 in a manner likely to depolymerize the MUC2 complex, leading to loss of viscosity and disintegration of mucus.[49] In addition, many intestinal pathogens have adhesins that allow them to dock onto the epithelial surface. In contrast, there are other intestinal pathogens that avoid the barrier by exploiting the hole in mucus over the dome epithelium and infect via M-cells.[50–52] While the dome epithelium seems a deficit in host defense, especially in the context of the life-threatening nature of gastrointestinal infections, one can conclude in an evolutionary sense that it has proved more important for mammals to maintain controlled immunity to the normal flora than to completely block access to potential pathogens. In contrast to the noninflammatory sIgA response mounted against commensal flora, when pathogens cross the barrier and disrupt the epithelium, an inflammatory response is generated more akin to the chronic inflammatory response in IBD.[41]

Thus it is conceivable that IBD could ensue due to either the presence of persistent "pathogenic" microbes, defects in the intestinal barrier, inappropriate underlying immune responses to "normal" sampling and presentation of commensal antigens, or to failure of the inflammatory immune response to downregulate once a pathogen is cleared. None of these elements are mutually exclusive and a combination of these factors, both genetic and environmental, could be involved in the development of IBD. The increased incidence of IBD in the developed world in the early 20th Century followed by the more recent increased incidence in Asia coincides with fewer gastrointestinal infections and the advent of treatment of those infections (e.g., with antibiotics) implicating the exposure to these infections inversely with the risk of developing IBD.[53,54]

Regulation of the Intestinal Barrier

Although most of the key elements of the mucus barrier are secreted constitutively, both rates of production and secretion are regulated in an autocrine fashion by epithelial cells responding to PAMPs and other environmental stimuli, and in response to paracrine stimulation by other epithelial cells and lamina propria lymphocytes, and endocrine and nervous stimuli (see Table 1). As an example, MUC2 transcription is increased in response to inflammatory cytokines,[55–59] PAMPs,[60–62] growth factors,[63] lipid mediators[64] and hormones,[65,66] and secretion of the mucin by goblet cells is increased by PAMPs, toxins, and nervous stimuli.[56,58,67–69] Furthermore, it is emerging that the glycosylation of MUC2 can be modulated by inflammatory stimuli.[70,71] Similarly, production and release of defensins, lectins, and other Paneth cell products are upregulated by bacterial products and inflammatory cytokines.[72–77] Thus, the secreted barrier should not be viewed as static or nonresponsive, but rather as an integrated component of innate and adaptive immunity that can be altered in terms of overall quantity and nature, and the relative abundance of the key individual components. Psychological stress can modulate barrier function and this may at least partially explain the links between stress and IBD

(reviewed in 78). In animal models experimental psychological stress increases intestinal permeability, depletes goblet cells and the mucus barrier and can lead to reactivation of colitis.[79,80]

EVIDENCE THAT THE INTESTINAL BARRIER PREVENTS INFLAMMATION

Secreted Barrier Defects

There are no intestinal in vitro culture systems that replicate the complexity of the secreted mucus barrier (for a discussion of this with respect to mucins and mucus, see Ref. 81). Furthermore, no in vitro systems can duplicate the complexity of regulation of the cellular and secreted barrier by underlying innate and adaptive immunity. Indeed, the lack of secreted barriers needs to be carefully considered when interpreting studies of barrier function conducted in vitro. Consequently, the most physiologically relevant data regarding the importance of the intestinal barrier comes from animal model systems of infection and inflammation (some of the most informative models are listed in Table 2).

Somewhat surprisingly, mice with reduced numbers of intestinal secretory lineage cells (both goblet cells and Paneth cells) have not been reported to develop spontaneous inflammation.[82,83] However, both Gfi$^{-/-}$ mice, which have a partial depletion, and particularly Math1$^{-/-}$ mice, which have a much greater depletion of secretory cells, die fairly early in life due to other complications. Intestinal specific knockout of Math1$^{-/-}$ has been achieved with complete loss of secretory lineage cells in 75%–90% of crypt/villus axes in the small intestine and crypts in the large intestine,[84] and these mice survive and do not develop spontaneous colitis. Furthermore, when ≈60% of goblet cells were depleted by expression of the diphtheria toxin under the ITF promoter, mice were unexpectedly more resistant to dextran sodium sulfate (DSS)-induced colitis.[85] These data indicate not only that simple reduction of the total number of goblet cells is insufficient to predispose to colitis, but also suggest that the predisposition to DSS-colitis resides in the goblet cells themselves because reducing the number of goblet cells reduced the predisposition. However, demonstrating that intestinal mucus prevents colitis, knockout of the intestinal mucin gene, *Muc2*, leads to spontaneous colitis at least on some mouse backgrounds.[3] (Although the original report of these mice claimed that intestinal tumors evolved in the absence of inflammation, the mice were reported to have increased crypt length and proliferation, which are well accepted signs of colitis in mice.[86]) Even though the classical goblet cell thecae are lost in *Muc2*$^{-/-}$ mice, the goblet cells are present and produce other secretions and partially compensate by neo-expression of the gastric mucin, Muc6, in the intestine.[86] The development of colitis is not surprising given that Muc2 is the molecule responsible for the viscous properties of mucus and its loss is likely to lead to dispersion of all other epithelial secretions, so bringing the luminal flora in contact with the epithelium and diluting the efficacy of antimicrobial molecules such as defensins and immunoglobulins.

Although the *Muc2*$^{-/-}$ demonstrates the importance of the mucin, and goblet cells are reduced in number and size in UC, there are no humans with total loss of MUC2/goblet cell thecae (although small intestinal goblet cells are sometimes near absent in rare individuals with autoimmune enteritis due to antigoblet-cell antibodies[87]). Using random mutagenesis we have generated and characterized 2 strains of mice with single missense mutations in *Muc2* that develop a UC-like colitis phenotype with mild distal colonic inflammation, increased intestinal permeability, and increased production of IL-1β, TNF-α, IFN-γ, and IL-13.[4] Both of these single nucleotide polymorphism (SNP) mutations cause aberrant assembly of the mucin complex leading to endoplasmic reticulum (ER) stress in goblet cells and Paneth cells, premature apoptosis and reduced mucin secretion, all of which are found in UC (see below). We believe that both ER stress and depletion of the mucus barrier contribute to inflammation in these models, which are the only animal models of spontaneous colitis due to single point mutations in any gene. Environmental ER stressors could duplicate this phenotype by increasing mucin misfolding and reducing mucin production. They may also give rise to inflammatory activation via initiation of the unfolded protein response, which can directly promote inflammation,[88,89] including via activating NF-κB in ER-stressed cells.[90–93] These models resemble UC and demonstrate that underlying defects in MUC2, the molecular machinery of ER stress, or the unfolded protein response could collectively or individually lead to prolonged inflammation once an infection has been cleared.

Changes in mucin glycosylation can also alter barrier function and contribute to colitis susceptibility. Mice lacking a Golgi resident enzyme (core 3 beta1,3-N-acetylglucosaminyltransferase) responsible for production of mucin Core 3 O-glycans show consequent changes in glycosylation of intestinal Muc2 with increased expression of the Tn oligosaccharide.[94] In addition to the glycosylation changes, these mice show decreased intestinal production of Muc2, increased intestinal permeability, and increased susceptibility to induced colitis. Furthermore, we have recently demonstrated increased susceptibility to infection and DSS-induced colitis in mice lacking a sulfate transporter which causes hyposulfatemia and reduced sulfation of intestinal mucins[95] (P. Dawson, unpubl.).

Trefoil peptides (TFFs) are small protease resistant proteins that are co-secreted with mucins into mucus.[96] Intestinal trefoil peptide (ITF, TFF3) is co-produced and secreted with MUC2 by intestinal goblet cells,[97] whereas in the respiratory tract TFF3 is co-secreted with both MUC5B and MUC5AC.[97,98] The viscosity of mucin solutions increases following addition of recombinant TFFs, suggesting that

TABLE 2. Animal Models Demonstrating the Importance of Intestinal Barrier Function

Animal Model	Description	Susceptibility to Intestinal Infection	Severity of Induced Colitis	Spontaneous Colitis	References
Depletion of Secretory Lineage Cells					
Math1$^{-/-}$	Math1 is a transcription factor involved in differentiation of the intestinal secretory lineage. Knockouts and intestinal specific knockouts under a villin promoter produce no secretory lineage cells but die very early in life, apparently without colitis. Intestinal specific knockout under the Fabpl promoter results in a mosaic loss in secretory cells in 75-90% of crypts in the distal small intestine and the colon, and mice survive without colitis.	NT	NT	No	(82, 84)
Gfi1$^{-/-}$	Gfi1 is a transcription factor involved in differentiation of the intestinal secretory lineage. Knockouts produce few secretory lineage cells (more than Math1) and survive longer but still die early, apparently without colitis.	NT	NT	No	(83)
mITF/DT-A	60% depletion of colonic goblet cells by expression of diphtheria toxin under control of the ITF (TFF3) promoter. Actually more resistant to DSS-induced colitis.	NT	Decreased	No	(85)
Alterations in Intestinal Secreted Mucins					
Muc2$^{-/-}$	Muc2 is the main secreted intestinal mucin and knockouts lack goblet cell thecae but continue to make other goblet cell secretions like ITF. Knockouts develop spontaneous colitis (at least on some mouse backgrounds) and show increased susceptibility to DSS-induced colitis. Some compensatory production of gastric mucin Muc6.	NT	Increased	Yes	(3, 86)
Winnie, Eeyore	Mice with single missense mutations in Muc2 leading to aberrant mucin assembly, ER stress and reduced mucin secretion. Develop spontaneous distal colitis with increased intestinal permeability and increased production of both Th1 and Th2 cytokines. Extremely sensitive to low doses of DSS.	Increased	Increased	Yes	(4)
C3GnT transferase$^{-/-}$	Knockout of transferase results in loss of core 3-derived O-glycans on Muc2. Decreased mucin synthesis, increased intestinal permeability and increased susceptibility to DSS-induced colitis.	NT	Yes	No	(94)
Alterations in Intestinal Cell Surface Mucins					
Muc1$^{-/-}$	Cell surface mucin upregulated during intestinal infection. Knockout mice are more susceptible to infection with gastrointestinal pathogens.	Increased	NT	No	(113, 114)
Muc13$^{-/-}$	Cell surface mucin highly expressed in large and small intestine. Knockout mice are more susceptible to DSS-induced colitis and develop more rapid *C. rodentium* infection.	Increased	Increased	No	(McGuckin unpubl. obs.)
Alterations in Intestinal Trefoil Factor (TFF3)					
TFF3$^{-/-}$	ITF is the major intestinal trefoil. Knockout mice do not develop spontaneous colitis but are more susceptible to DSS colitis and radio/chemotherapy induced mucositis.	NT	Increased	No	(105, 169)

TABLE 2. (*Continued*)

Animal Model	Description	Susceptibility to Intestinal Infection	Severity of Induced Colitis	Spontaneous Colitis	References
Alterations in Antimicrobial Peptides and Lectins					
RegIII$\gamma^{-/-}$	Mice deficient in MyD88 do not express RegIIIγ in the ileum and are more susceptible to ileal infection with Listeria. This susceptibility is also seen in mice treated with anti-RegIIIγ antibodies.	Increased	NT	No	(170)
Defensin transgenic	Mice overexpressing human defensin-5 show increased resistance to Salmonella infection.	Decreased	NT	No	(106)
MMP7$^{-/-}$	Defective cryptidin (murine defensin) processing due to loss of metalloproteinase MMP7; other features of any phenotype could be attributable to non-defensin related function of MMP7.	Increased	NT	No	(107)
SLPI$^{-/-}$	Mice lack the secretory leucocyte protease inhibitor (SLPI). More susceptible to respiratory infection but not tested specifically in intestinal infection or colitis but no reported spontaneous colitis.	NT	NT	No	(171)
Alterations in Mucosal Immunoglobulins					
Rag1/2$^{-/-}$	Mice cannot recombine the B-cell receptor and also the T-cell receptor – combined B- and T-cell deficiency.	Increased	Decreased	No	(109, 172)
microMT	Mice have specific B-cell deficiency	Increased	NT	No	(108, 109)
pIgR$^{-/-}$	Mice lack the receptor that transports IgA across the epithelium forming secretory IgA (sIgA). Compensation by increased IgG is possible.	Increased	No	No	(111)
FcRn$^{-/-}$	Mice lack the receptor that transports IgG across the epithelium. Compensation by increased sIgA is possible.	Increased	No	No	(112)
Alterations in Epithelial Cell Barrier Function / Undetermined					
SAMP1/YitFc	Undefined spontaneously generated genetic model that develops spontaneous ileitis. Bone marrow transplantation experiments show that the genetic deficit resides in the epithelial compartment and that secretory cell hyperplasia, production of relm-β and increased intestinal permeability precede inflammation.	NT	NT	Yes	(122, 125, 126, 173)

TFF-mucin interactions could modulate the rheological properties of the mucus gel.[99] Quite separate from this putative role as structural components of the gel, recombinant TFFs at high concentrations have motogenic, proliferative, and anti-apoptotic effects on cells cultured in vitro and are therefore thought to be important in mucosal wound repair.[100–104] TFF3 knockout mice do not develop spontaneous colitis but show enhanced susceptibility to DSS-induced colitis,[105] which could be mediated through poorer mucus quality and/or inadequate wound repair.

A relatively large array of antimicrobial molecules are produced by intestinal epithelia, including defensins, lectins, histatins, statherins, enzymes, and protease inhibitors. The antimicrobial activity of these molecules has been established in vitro, and for some molecules there are in vivo data supporting functional importance in limiting penetration of the mucus barrier by the normal flora and limiting infection by pathogens. However, there are no data demonstrating that loss of individual antimicrobial components leads to spontaneous intestinal inflammation. Because there are no defensin (cryptidin)-deficient mice strains described it is difficult to ascertain the relative importance of these molecules. However, mice transgenic for human defensin-5 show greater resistance to *Salmonella* infection.[106] Conversely, MMP7 knockout mice that have aberrant cryptidin processing are more susceptible to infection,[107] showing that these molecules can limit pathogens in vivo, but they do not develop spontaneous inflammation.[107] Furthermore, *CARD15*/Nod2 knockout mice have decreased cryptidin production, with increased susceptibility to intracellular infection by oral but

not intraperitoneal *Listeria monocytogenes*, but again do not develop spontaneous inflammation.[76] Given the substantial number of different antimicrobial molecules in mucus it is unlikely that loss of, or aberrant function of, any individual molecule would impair innate defense sufficiently to lead to spontaneous inflammation from the commensal microbes. However, defects in molecules regulating their overall production, processing, or secretion may be more likely to compromise innate defense and lead to inflammation.

The adaptive immune system makes a substantial investment in producing mucosal immunoglobulin, with sIgA constituting the greatest proportion of immunoglobulin synthesis. Despite the importance of secreted immunoglobulins in eliminating pathogens and keeping commensal flora away from the epithelial cells, there are no data that demonstrate that deficiencies in immunoglobulin production lead to spontaneous intestinal inflammation. Mice that cannot recombine the B-cell receptor and mice specifically lacking B-cells are more susceptible to infection with intestinal pathogens but do not develop spontaneous colitis.[108,109] Similarly, mice lacking secretory component (pIgR) or FcRn that are responsible for transporting IgA and IgG, respectively, across the mucosa are more susceptible to infection but do not develop spontaneous intestinal inflammation.[108,110–112]

Cellular Barrier Defects

Emerging evidence suggests that the cell surface mucins present in the glycocalyx of all mucosal epithelial cells may be important determinants of infection. We have recently demonstrated that deficiency of the Muc1 mucin renders mice more susceptible to infection by some gastrointestinal pathogens.[113,114] In the case of *Campylobacter jejuni* infection, Muc1 appears to not only impede penetration of the mucosal barrier by the pathogen but to modulate the epithelial cell response to a bacterial genotoxin.[113] In the case of *H. pylori* infection Muc1 knockout mice develop more severe inflammation than wildtype mice, demonstrating that cell surface mucins can modulate the inflammatory response to chronic infection.[114] We have recently generated mice lacking Muc13, which is highly and constitutively expressed in the small and large intestine. Although these mice do not develop overt spontaneous inflammation they appear more susceptible to DSS-induced colitis and *Citrobacter rodentium* infection (McGuckin, unpubl.).

Although increased permeability is a feature of IBD, where it has been demonstrated especially in active ileal CD, and appears in many animal models of intestinal inflammation, there are few compelling data that increased permeability alone results in chronic intestinal inflammation. Mice transgenic for a dominant negative N-cadherin develop spontaneous ileitis but have additional major disturbances in cell proliferation, migration, and apoptosis that are likely to contribute to their phenotype.[115] The inflammatory cytokines IL-1β, TNF-α, IFN-γ, and IL-13 increase intestinal permeability largely by inducing the myosin light chain kinase (MLCK), which in turn destabilizes tight junctions.[116–121] Thus, once inflammation is established altered epithelial permeability ensues and contributes to the severity of pathology and the ability to resolve inflammation and repair wounds.

SAMP1/YitFc mice develop spontaneous ileitis that shows many features similar to Crohn's ileitis and are likely to have multi-allelic contributions to phenotype.[122–124] Despite the observation that T cells from these mice can induce ileitis in lymphopenic recipients,[122] it has recently emerged that the genetic defects in these mice act via the epithelial cells rather than leukocytes.[125] In these mice, hypertrophy of secretory lineage cells and increased intestinal permeability predate inflammation,[125,126] which suggests that a barrier function defect may underlie this phenotype. Further dissection of the mechanism of pathology in mice like SAMP1/YitFc with multi-allelic contributions is likely to increase insights as to the importance of barrier function in preventing intestinal inflammation.

BARRIER FUNCTION IN IBD

Perturbations in barrier function in human IBD include reduction in barrier and antimicrobial secretions, reduced numbers of secretory cells, increased permeability, disabled tight junctions, through to substantial reduction, and even complete loss of the epithelium where ulceration occurs. While many may argue that most of these are consequences rather than causes of inflammation, the data from animal models as described above suggest that barrier dysfunction could plausibly be a primary defect in some subsets of IBD. Even if this is not the case, these features will exacerbate inflammation and complicate resolution of lesions. The mucus layer in IBD appears more highly populated with bacteria, particularly in areas where there is minimal or less severe inflammation[127] (Florin, unpubl.). These studies are beset by technical problems discussed earlier and uncontrolled environmental effects that make interpretation difficult.[128] In the following sections we discuss altered barrier function in CD and UC separately, but it is likely that there are both substantial overlaps between these diseases and substantial variation within each of these broad disease classifications in barrier function phenotypes.

Crohn's Disease

In classical ileal CD the conventional dogma is that there is goblet cell hypertrophy and increased, rather than decreased, mucus formation,[129,130] which suggests that a deficiency in secreted mucus does not underlie this disorder. However, in recent years attention has been focused on Paneth cells in CD because of data indicating decreased defensin production,[131–134] expression of NOD2 within Paneth cells,[135,136] and demonstration that the NOD2 microbial

ligand, MDP, increases transcription of defensin genes.[77] Most of the data on defensins are derived from polymerase chain reaction (PCR) amplification of RNA from tissue biopsies from patients and controls. The relative abundance of epithelium decreases during intestinal inflammation. Therefore, studies based on whole tissue RNA are fraught with the potential to wrongly attribute a decrease in the relative abundance of epithelial specific RNA to decreased production of molecules per remaining epithelial cell rather than to decreases in the relative abundance of epithelial cells.

In this regard, we have recently conducted a study in which we demonstrate increased rather than decreased defensin expression in noninflamed ileal CD.[137] In inflamed tissue the decreased defensin mRNA expression was paralleled by decreased expression of another epithelial-specific gene, villin.[137] Furthermore, by immunohistochemistry we showed that the residual Paneth cells in inflamed ileal CD tissue express protein levels of human defensin-5 that appear similar to Paneth cells from noninflamed CD tissue, regardless of *CARD15* (NOD2) genotype. Interestingly, despite the role of MDP in stimulating defensin production via NOD2,[77] defensin expression was not related to NOD2 mutation status in our study.[137] Our conclusion is that these data do not yet support a fundamental decrease in defensin production underlying ileal CD. In colonic CD there is some evidence of decreased antimicrobial activity in crude cationic protein preparations extracted from biopsies when compared with UC and healthy controls.[138] The same group also reported that there was decreased antimicrobial protease inhibitors SLPI and elafin in inflamed CD compared with inflamed UC.[139] Production of all antimicrobial molecules in inflamed and noninflamed intestine from patients with accurately phenotyped ileal and colonic CD needs to be assessed in thorough analyses that are well controlled for numbers of epithelial and inflammatory cells in the individual biopsies. These studies need to be sufficiently powered to permit subanalysis for the major CD subsets.

Using noninvasive techniques many, but not all,[123] studies have demonstrated increased intestinal permeability in CD.[14,15,140-142] While the mechanism and cellular site of the increased permeability measured in humans are not precisely known, this is likely to be attributable to the actions of the Th1 cytokines TNF-α and IFN-γ that are characteristic of this disease and known to increase permeability.[143] Underlying this increased permeability are reduced numbers of tight junctions and reduced expression and relocation of claudin isoforms involved in tight junctions in active CD.[144] Bacterial translocation is frequently reported in CD,[8–10] suggesting that the altered permeability to small molecules and disrupted tight junctions is functionally significant. However, increased bacterial translocation could be due to multiple factors including impaired bacterial processing possibly associated with *CARD15* and *ATG16L1* mutations,[145–147] a more virulent bacterial flora with increased epithelial adhesins,[148] or increased ligands for bacterial adhesins in CD.[149] Evidence that clinically unaffected family members of CD sufferers can have increased intestinal permeability suggests that this could be an early predisposing factor.[150]

Ulcerative Colitis

Unlike CD, UC is usually characterized by a reduction in goblet cells, reduced size of goblet cell thecae, decreased MUC2 production,[151,152] decreased mucin sulfation,[152–154] and a diminished mucus barrier. The mucus diarrhea often seen clinically in UC may be indicative of poor mucus quality leading to reduced retention at the mucosal surface. These characteristics are usually dismissed as being a response to inflammation, as similar changes in goblet cells can occur in infectious colitis. However, these features are also observed, albeit to a lesser degree, in unaffected proximal intestine of patients with distal UC, suggesting that it could be an early predisposing factor predating histological colitis. Not so well known but equally relevant, vacuolization of the ER and Golgi is observed in both inflamed and noninflamed secretory cells in the intestines of UC but not CD,[155–160] suggesting that ER stress is occurring. We and others have reported accumulation of the nonglycosylated MUC2 precursor in UC,[4,154,161,162] and biochemical evidence of ER stress and activation of the unfolded protein response.[4,92] This has led us to propose a model whereby genetic predisposition to misfolding of proteins, or inappropriately responding to protein misfolding and/or environmental ER stressors, could initiate a chronic, or resolving and relapsing cycle of inflammation producing an UC phenotype.[4]

In contrast to CD, increased intestinal permeability is not easily demonstrated in UC.[15,142] This possibly relates to the sensitivity of the noninvasive permeability techniques, which are best at quantifying permeability in the small bowel.[163] There is ultrastructural evidence of inadequate tight junctions between epithelial cells in UC which could be attributed to the cytokines produced in UC including IL-1β, TNF-α, and IL-13, which are all known to alter tight junctions and intestinal permeability in cell cultures.[143,164,165] However, these abnormalities in the UC colon do not appear to be functionally significant in terms of bacterial translocation, which, in contrast to CD, is not a feature of UC.

THERAPEUTIC RESTORATION OF BARRIER FUNCTION

Therapeutic restoration of barrier function could improve pathophysiology and clinical outcomes in both CD and UC. Unfortunately, there are few data regarding the direct effects of current IBD therapeutics on intestinal barrier function. Immunomodulating drugs and biologicals are likely to act in part directly or indirectly via restoration of some aspects of barrier function and further research is required to

dissect out the importance of these aspects of their activity. For example, anti-TNF-α antibodies could directly interfere with the effect of this inflammatory cytokine on intestinal permeability[166] and ER stress in secretory cells,[167] while drugs that suppress T cells more generally (e.g., steroids) will indirectly influence these pathways. A range of different agents under study in preclinical models could affect epithelial cells, for example, growth factors, trefoil peptides, antioxidants, differentiation agents, iNOS inhibitors, and NF-κB inhibitors. New drugs that directly stimulate production of constituents of the secreted barrier may not resolve advanced lesions but have promise as agents to maintain patients in remission without the side effects of the classical immunomodulating drugs. If ER stress in secretory cells proves to be an important component of disease etiology in some patients with IBD, then drugs that ameliorate ER stress or UPR signaling[168] could be similarly utilized to diminish the initial triggers for inflammation. Manipulation of the luminal environment (e.g., modifying exposure to dietary toxins and modifying the microbial microflora with anti-, pro-, or prebiotics) may bolster the efficacy of the mucosal barrier in patients with IBD.

SUMMARY AND KEY FUTURE RESEARCH OBJECTIVES

In summary, emerging experimental evidence from animal models suggests that altered barrier function is a potential pathway to intestinal inflammation in IBD that has received far less attention than pathways involving underlying immunity. Increased exploration of the complexity of barrier function and its connections with the microbial community and the underlying innate and adaptive immunity will enhance understanding of the etiology of human IBD and could lead to more effective therapeutic strategies for these diseases. Key future research objectives include:

- Identification of changes in the constituency and/or function of the microbial flora in IBD and their relationship with the mucosal barrier.
- More detailed characterization of all elements of the mucosal barrier and their interactions in health and IBD.
- Further understanding of the regulation of the mucosal barrier by epithelial cells and underlying elements of innate and adaptive immunity.
- Development of animal model systems encompassing both biologically relevant genetic predisposition and appropriate environmental challenges affecting the mucosal barrier.

REFERENCES

1. Halfvarson J, Bodin L, Tysk C, et al. Inflammatory bowel disease in a Swedish twin cohort: a long-term follow-up of concordance and clinical characteristics. *Gastroenterology.* 2003;124:1767–1773.
2. Nenci A, Becker C, Wullaert A, et al. Epithelial NEMO links innate immunity to chronic intestinal inflammation. *Nature.* 2007;446:557–561.
3. Van der Sluis M, De Koning BA, De Bruijn AC, et al. Muc2-deficient mice spontaneously develop colitis, indicating that MUC2 is critical for colonic protection. *Gastroenterology.* 2006;131:117–129.
4. Heazlewood CK, Cook MC, Eri R, et al. Aberrant mucin assembly in mice causes endoplasmic reticulum stress and spontaneous inflammation resembling ulcerative colitis. *PLoS Med.* 2008;5:e54.–
5. Selby W, Pavli P, Crotty B, et al. Two-year combination antibiotic therapy with clarithromycin, rifabutin, and clofazimine for Crohn's disease. *Gastroenterology.* 2007;132:2313–2319.
6. Sandborn WJ, McLeod R, Jewell DP. Medical therapy for induction and maintenance of remission in pouchitis: a systematic review. *Inflamm Bowel Dis.* 1999;5:33–39.
7. Gionchetti P, Amadini C, Rizzello F, et al. Diagnosis and treatment of pouchitis. *Best Pract Res Clin Gastroenterol.* 2003;17:75–87.
8. Ryan P, Kelly RG, Lee G, et al. Bacterial DNA within granulomas of patients with Crohn's disease—detection by laser capture microdissection and PCR. *Am J Gastroenterol.* 2004;99:1539–1543.
9. Darfeuille-Michaud A, Boudeau J, Bulois P, et al. High prevalence of adherent-invasive Escherichia coli associated with ileal mucosa in Crohn's disease. *Gastroenterology.* 2004;127:412–421.
10. Martin HM, Campbell BJ, Hart CA, et al. Enhanced Escherichia coli adherence and invasion in Crohn's disease and colon cancer. *Gastroenterology.* 2004;127:80–93.
11. Adams RJ, Heazlewood SP, Gilshenan KS, et al. IgG antibodies against common gut bacteria are more diagnostic for Crohn's disease than IgG against mannan or flagellin. *Am J Gastroenterol.* 2008;103:386–396.
12. Garrett WS, Lord GM, Punit S, et al. Communicable ulcerative colitis induced by T-bet deficiency in the innate immune system. *Cell.* 2007; 131:33–45.
13. Stecher B, Robbiani R, Walker AW, et al. Salmonella enterica serovar typhimurium exploits inflammation to compete with the intestinal microbiota. *PLoS Biol.* 2007;5:2177–2189.
14. Issenman RM, Jenkins RT, Radoja C. Intestinal permeability compared in pediatric and adult patients with inflammatory bowel disease. *Clin Invest Med.* 1993;16:187–196.
15. Jenkins RT, Ramage JK, Jones DB, et al. Small bowel and colonic permeability to 51Cr-EDTA in patients with active inflammatory bowel disease. *Clin Invest Med.* 1988;11:151–155.
16. Welcker K, Martin A, Kolle P, et al. Increased intestinal permeability in patients with inflammatory bowel disease. *Eur J Med Res.* 2004;9: 456–460.
17. Matsuo K, Ota H, Akamatsu T, et al. Histochemistry of the surface mucous gel layer of the human colon. *Gut.* 1997;40:782–789.
18. Atuma C, Strugala V, Allen A, et al. The adherent gastrointestinal mucus gel layer: thickness and physical state in vivo. *Am J Physiol Gastrointest Liver Physiol.* 2001;280:G922–929.
19. Bollard JE, Vanderwee MA, Smith GW, et al. Location of bacteria in the mid-colon of the rat. *Appl Environ Microbiol.* 1986;51:604–608.
20. van der Waaij LA, Harmsen HJ, Madjipour M, et al. Bacterial population analysis of human colon and terminal ileum biopsies with 16S rRNA-based fluorescent probes: commensal bacteria live in suspension and have no direct contact with epithelial cells. *Inflamm Bowel Dis.* 2005;11:865–871.
21. Swidsinski A, Sydora BC, Doerffel Y, et al. Viscosity gradient within the mucus layer determines the mucosal barrier function and the spatial organization of the intestinal microbiota. *Inflamm Bowel Dis.* 2007;13: 963–970.
22. Rousseau K, Byrne C, Kim YS, et al. The complete genomic organization of the human MUC6 and MUC2 mucin genes. *Genomics.* 2004;83:936–939.
23. Gum JR, Byrd JC, Hicks JW, et al. Molecular cloning of human intestinal mucin cDNAs. Sequence analysis and evidence for genetic polymorphisms. *J Biol Chem.* 1989;264:6480–6487.
24. Asker N, Axelsson MAB, Olofsson SO, et al. Dimerization of the human MUC2 mucin in the endoplasmic reticulum is followed by a N-glycosylation-dependent transfer of the mono- and dimers to the Golgi apparatus. *J Biol Chem.* 1998;273:18857–18863.
25. Asker N, Baeckstrom D, Axelsson MAB, et al. The human MUC2 mucin apoprotein appears to dimerize before O-glycosylation and shares epitopes with the insoluble mucin of rat small intestine. *Biochem J.* 1995;308:873–880.

26. Godl K, Johansson ME, Lidell ME, et al. The N terminus of the MUC2 mucin forms trimers that are held together within a trypsin-resistant core fragment. *J Biol Chem.* 2002;277:47248–47256.
27. Kawakubo M, Ito Y, Okimura Y, et al. Natural antibiotic function of a human gastric mucin against Helicobacter pylori infection. *Science.* 2004;305:1003–1006.
28. Bruno LS, Li X, Wang L, et al. Two-hybrid analysis of human salivary mucin MUC7 interactions. *Biochim Biophys Acta.* 2005;1746:65–72.
29. Iontcheva I, Oppenheim FG, Offner GD, et al. Molecular mapping of statherin- and histatin-binding domains in human salivary mucin MG1 (MUC5B) by the yeast two-hybrid system. *J Dent Res.* 2000;79:732–739.
30. Neal MD, Leaphart C, Levy R, et al. Enterocyte TLR4 mediates phagocytosis and translocation of bacteria across the intestinal barrier. *J Immunol.* 2006;176:3070–3079.
31. Rimoldi M, Chieppa M, Salucci V, et al. Intestinal immune homeostasis is regulated by the crosstalk between epithelial cells and dendritic cells. *Nat Immunol.* 2005;6:507–514.
32. He B, Xu W, Santini PA, et al. Intestinal bacteria trigger T cell-independent immunoglobulin A(2) class switching by inducing epithelial-cell secretion of the cytokine APRIL. *Immunity.* 2007;26:812–826.
33. Rescigno M, Urbano M, Valzasina B, et al. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nat Immunol.* 2001;2:361–367.
34. Cheroutre H. IELs: enforcing law and order in the court of the intestinal epithelium. *Immunol Rev.* 2005;206:114–131.
35. Wang X, Heazlewood SP, Krause DO, et al. Molecular characterization of the microbial species that colonize human ileal and colonic mucosa by using 16S rDNA sequence analysis. *J Appl Microbiol.* 2003;95:508–520.
36. Hooper LV, Midtvedt T, Gordon JI. How host-microbial interactions shape the nutrient environment of the mammalian intestine. *Annu Rev Nutr.* 2002;22:283–307.
37. Backhed F, Ley RE, Sonnenburg JL, et al. Host-bacterial mutualism in the human intestine. *Science.* 2005;307:1915–1920.
38. Sharma R, Schumacher U. Carbohydrate expression in the intestinal mucosa. *Adv Anat Embryol Cell Biol.* 2001;160:III–IX, 1–91.
39. Hathaway LJ, Kraehenbuhl JP. The role of M cells in mucosal immunity. *Cell Mol Life Sci.* 2000;57:323–332.
40. Kraehenbuhl JP, Neutra MR. Epithelial M cells: differentiation and function. *Annu Rev Cell Dev Biol.* 2000;16:301–332.
41. Macpherson AJ, Uhr T. Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria. *Science.* 2004;303:1662–1665.
42. Macpherson AJ, Gatto D, Sainsbury E, et al. A primitive T cell-independent mechanism of intestinal mucosal IgA responses to commensal bacteria. *Science.* 2000;288:2222–2226.
43. Macpherson AJ. IgA adaptation to the presence of commensal bacteria in the intestine. *Curr Top Microbiol Immunol.* 2006;308:117–136.
44. Corfield AP, Wagner SA, Clamp JR, et al. Mucin degradation in the human colon: production of sialidase, sialate O-acetylesterase, N-acetylneuraminate lyase, arylesterase, and glycosulfatase activities by strains of fecal bacteria. *Infect Immun.* 1992;60:3971–3978.
45. Haider K, Hossain A, Wanke C, et al. Production of mucinase and neuraminidase and binding of Shigella to intestinal mucin. *J Diarrhoeal Dis Res.* 1993;11:88–92.
46. Corfield AP, Wagner SA, O'Donnell LJ, et al. The roles of enteric bacterial sialidase, sialate O-acetyl esterase and glycosulfatase in the degradation of human colonic mucin. *Glycoconj J.* 1993;10:72–81.
47. Homer KA, Whiley RA, Beighton D. Production of specific glycosidase activities by Streptococcus intermedius strain UNS35 grown in the presence of mucin. *J Med Microbiol.* 1994;41:184–190.
48. Roberton AM, Wright DP. Bacterial glycosulphatases and sulphomucin degradation. *Can J Gastroenterol.* 1997;11:361–366.
49. Lidell ME, Moncada DM, Chadee K, et al. Entamoeba histolytica cysteine proteases cleave the MUC2 mucin in its C-terminal domain and dissolve the protective colonic mucus gel. *Proc Natl Acad Sci U S A.* 2006;103:9298–9303.
50. Vazquez-Torres A, Fang FC. Cellular routes of invasion by enteropathogens. *Curr Opin Microbiol.* 2000;3:54–59.
51. Siebers A, Finlay BB. M cells and the pathogenesis of mucosal and systemic infections. *Trends Microbiol.* 1996;4:22–29.
52. Jones B, Pascopella L, Falkow S. Entry of microbes into the host: using M cells to break the mucosal barrier. *Curr Opin Immunol.* 1995;7:474–478.
53. Danese S, Fiocchi C. Etiopathogenesis of inflammatory bowel diseases. *World J Gastroenterol.* 2006;12:4807–4812.
54. Green C, Elliott L, Beaudoin C, et al. A population-based ecologic study of inflammatory bowel disease: searching for etiologic clues. *Am J Epidemiol.* 2006;164:615–623; discussion 624–618.
55. Levine SJ, Larivee P, Logun C, et al. Tumor necrosis factor-alpha induces mucin hypersecretion and MUC-2 gene expression by human airway epithelial cells. *Am J Respir Cell Mol Biol.* 1995;12:196–204.
56. Enss ML, Cornberg M, Wagner S, et al. Proinflammatory cytokines trigger MUC gene expression and mucin release in the intestinal cancer cell line LS180. *Inflamm Res.* 2000;49:162–169.
57. Louahed J, Toda M, Jen J, et al. Interleukin-9 upregulates mucus expression in the airways. *Am J Respir Cell Mol Biol.* 2000;22:649–656.
58. Kim YD, Jeon JY, Woo HJ, et al. Interleukin-1beta induces MUC2 gene expression and mucin secretion via activation of PKC-MEK/ERK, and PI3K in human airway epithelial cells. *J Korean Med Sci.* 2002;17:765–771.
59. Iwashita J, Sato Y, Sugaya H, et al. mRNA of MUC2 is stimulated by IL-4, IL-13 or TNF-alpha through a mitogen-activated protein kinase pathway in human colon cancer cells. *Immunol Cell Biol.* 2003;81:275–282.
60. Dohrman A, Miyata S, Gallup M, et al. Mucin gene (MUC2 and MUC5AC) upregulation by Gram-positive and Gram-negative bacteria. *Biochim Biophys Acta.* 1998;1406:251–259.
61. Li JD, Dohrman AF, Gallup M, et al. Transcriptional activation of mucin by Pseudomonas aeruginosa lipopolysaccharide in the pathogenesis of cystic fibrosis lung disease. *Proc Natl Acad Sci U S A.* 1997;94:967–972.
62. Lemjabbar H, Basbaum C. Platelet-activating factor receptor and ADAM10 mediate responses to Staphylococcus aureus in epithelial cells. *Nat Med.* 2002;8:41–46.
63. Perrais M, Pigny P, Copin MC, et al. Induction of MUC2 and MUC5AC mucin by factors of the epidermal growth factor family is mediated by EGF-R/Ras/Raf/MAPK signaling cascade and Sp1. *J Biol Chem.* 2002;19:19.
64. Gaudier E, Jarry A, Blottiere HM, et al. Butyrate specifically modulates MUC gene expression in intestinal epithelial goblet cells deprived of glucose. *Am J Physiol Gastrointest Liver Physiol.* 2004;287:G1168–1174.
65. El Homsi M, Ducroc R, Claustre J, et al. Leptin modulates the expression of secreted and membrane-associated mucins in colonic epithelial cells by targeting PKC, PI3K, and MAPK pathways. *Am J Physiol Gastrointest Liver Physiol.* 2007;293:G365–373.
66. Hokari R, Lee H, Crawley SC, et al. Vasoactive intestinal peptide upregulates MUC2 intestinal mucin via CREB/ATF1. *Am J Physiol Gastrointest Liver Physiol.* 2005;289:G949–959.
67. Florey HW. The secretion and function of intestinal mucus. *Gastroenterology.* 1962;43:326–329.
68. McCool DJ, Forstner JF, Forstner GG. Regulated and unregulated pathways for MUC2 mucin secretion in human colonic LS180 adenocarcinoma cells are distinct. *Biochem J.* 1995;312:125–133.
69. Shekels LL, Lyftogt CT, Ho SB. Bile acid-induced alterations of mucin production in differentiated human colon cancer cell lines. *Int J Biochem Cell Biol.* 1996;28:193–201.
70. Lamblin G, Degroote S, Perini JM, et al. Human airway mucin glycosylation: a combinatory of carbohydrate determinants which vary in cystic fibrosis. *Glycoconj J.* 2001;18:661–684.
71. Kanoh A, Takeuchi H, Kato K, et al. Interleukin-4 induces specific pp-GalNAc-T expression and alterations in mucin O-glycosylation in colonic epithelial cells. *Biochim Biophys Acta.* 2008;1780:577–584.
72. O'Neil DA, Porter EM, Elewaut D, et al. Expression and regulation of the human beta-defensins hBD-1 and hBD-2 in intestinal epithelium. *J Immunol.* 1999;163:6718–6724.
73. Ayabe T, Satchell DP, Wilson CL, et al. Secretion of microbicidal

alpha-defensins by intestinal Paneth cells in response to bacteria. *Nat Immunol.* 2000;1:113–118.
74. Ogushi K, Wada A, Niidome T, et al. Salmonella enteritidis FliC (flagella filament protein) induces human beta-defensin-2 mRNA production by Caco-2 cells. *J Biol Chem.* 2001;276:30521–30526.
75. Bajaj-Elliott M, Fedeli P, Smith GV, et al. Modulation of host antimicrobial peptide (beta-defensins 1 and 2) expression during gastritis. *Gut.* 2002;51:356–361.
76. Kobayashi KS, Chamaillard M, Ogura Y, et al. Nod2-dependent regulation of innate and adaptive immunity in the intestinal tract. *Science.* 2005;307:731–734.
77. Voss E, Wehkamp J, Wehkamp K, et al. NOD2/CARD15 mediates induction of the antimicrobial peptide human beta-defensin-2. *J Biol Chem.* 2006;281:2005–2011.
78. Hart A, Kamm MA. Review article: mechanisms of initiation and perpetuation of gut inflammation by stress. *Aliment Pharmacol Ther.* 2002;16:2017–2028.
79. Qiu BS, Vallance BA, Blennerhassett PA, et al. The role of CD4+ lymphocytes in the susceptibility of mice to stress-induced reactivation of experimental colitis. *Nat Med.* 1999;5:1178–1182.
80. Castagliuolo I, Lamont JT, Qiu B, et al. Acute stress causes mucin release from rat colon: role of corticotropin releasing factor and mast cells. *Am J Physiol.* 1996;271:G884–892.
81. Linden SK, Driessen KM, McGuckin MA. Improved in vitro model systems for gastrointestinal infection by choice of cell line, pH, microaerobic conditions and optimization of culture conditions. *Helicobacter.* 2007;12:341–353.
82. Yang Q, Bermingham NA, Finegold MJ, et al. Requirement of Math1 for secretory cell lineage commitment in the mouse intestine. *Science.* 2001;294:2155–2158.
83. Shroyer NF, Wallis D, Venken KJ, et al. Gfi1 functions downstream of Math1 to control intestinal secretory cell subtype allocation and differentiation. *Genes Dev.* 2005;19:2412–2417.
84. Shroyer NF, Helmrath MA, Wang VY, et al. Intestine-specific ablation of mouse atonal homolog 1 (Math1) reveals a role in cellular homeostasis. *Gastroenterology.* 2007;132:2478–2488.
85. Itoh H, Beck PL, Inoue N, et al. A paradoxical reduction in susceptibility to colonic injury upon targeted transgenic ablation of goblet cells. *J Clin Invest.* 1999;104:1539–1547.
86. Velcich A, Yang W, Heyer J, et al. Colorectal cancer in mice genetically deficient in the mucin Muc2. *Science.* 2002;295:1726–1729.
87. Akram S, Murray JA, Pardi DS, et al. Adult autoimmune enteropathy: Mayo Clinic Rochester experience. *Clin Gastroenterol Hepatol.* 2007;5:1282–1290.
88. Majors AK, Austin RC, de la Motte CA, et al. Endoplasmic reticulum stress induces hyaluronan deposition and leukocyte adhesion. *J Biol Chem.* 2003;278:47223–47231.
89. Zhang K, Shen X, Wu J, et al. Endoplasmic reticulum stress activates cleavage of CREBH to induce a systemic inflammatory response. *Cell.* 2006;124:587–599.
90. Hu P, Han Z, Couvillon AD, et al. Autocrine tumor necrosis factor alpha links endoplasmic reticulum stress to the membrane death receptor pathway through IRE1alpha-mediated NF-kappaB activation and down-regulation of TRAF2 expression. *Mol Cell Biol.* 2006;26:3071–3084.
91. Deng J, Lu PD, Zhang Y, et al. Translational repression mediates activation of nuclear factor kappa B by phosphorylated translation initiation factor 2. *Mol Cell Biol.* 2004;24:10161–10168.
92. Shkoda A, Ruiz P, Daniel H, et al. Interleukin 10 blocked endoplasmic reticulum stress in intestinal epithelial cells: impact on chronic inflammation. *Gastroenterology.* 2007;132:190–207.
93. Pahl HL, Baeuerle PA. A novel signal transduction pathway from the endoplasmic reticulum to the nucleus is mediated by transcription factor NF-kappa B. *EMBO J.* 1995;14:2580–2588.
94. An G, Wei B, Xia B, et al. Increased susceptibility to colitis and colorectal tumors in mice lacking core 3-derived O-glycans. *J Exp Med.* 2007;204:1417–1429.
95. Dawson PA, Beck L, Markovich D. Hyposulfatemia, growth retardation, reduced fertility, and seizures in mice lacking a functional NaSi-1 gene. *Proc Natl Acad Sci U S A.* 2003;100:13704–13709.
96. Thim L. Trefoil peptides: from structure to function. *Cell Mol Life Sci.* 1997;53:888–903.
97. Madsen J, Nielsen O, Tornoe I, et al. Tissue localization of human trefoil factors 1, 2, and 3. *J Histochem Cytochem.* 2007;55:505–513.
98. Wiede A, Jagla W, Welte T, et al. Localization of TFF3, a new mucus-associated peptide of the human respiratory tract. *Am J Respir Crit Care Med.* 1999;159:1330–1335.
99. Wright NA, Hoffmann W, Otto WR, et al. Rolling in the clover: trefoil factor family (TFF)-domain peptides, cell migration and cancer. *FEBS Lett.* 1997;408:121–123.
100. Taupin DR, Kinoshita K, Podolsky DK. Intestinal trefoil factor confers colonic epithelial resistance to apoptosis. *Proc Natl Acad Sci U S A.* 2000;97:799–804.
101. Kalabis J, Rosenberg I, Podolsky DK. Vangl1 protein acts as a downstream effector of intestinal trefoil factor (ITF)/TFF3 signaling and regulates wound healing of intestinal epithelium. *J Biol Chem.* 2006;281:6434–6441.
102. Goke MN, Cook JR, Kunert KS, et al. Trefoil peptides promote restitution of wounded corneal epithelial cells. *Exp Cell Res.* 2001;264:337–344.
103. Kanai M, Mullen C, Podolsky DK. Intestinal trefoil factor induces inactivation of extracellular signal-regulated protein kinase in intestinal epithelial cells. *Proc Natl Acad Sci U S A.* 1998;95:178–182.
104. Tan XD, Liu QP, Hsueh W, et al. Intestinal trefoil factor binds to intestinal epithelial cells and induces nitric oxide production: priming and enhancing effects of mucin. *Biochem J.* 1999;338:745–751.
105. Mashimo H, Wu DC, Podolsky DK, et al. Impaired defense of intestinal mucosa in mice lacking intestinal trefoil factor. *Science.* 1996;274:262–265.
106. Salzman NH, Ghosh D, Huttner KM, et al. Protection against enteric salmonellosis in transgenic mice expressing a human intestinal defensin. *Nature.* 2003;422:522–526.
107. Wilson CL, Ouellette AJ, Satchell DP, et al. Regulation of intestinal alpha-defensin activation by the metalloproteinase matrilysin in innate host defense. *Science.* 1999;286:113–117.
108. Maaser C, Housley MP, Iimura M, et al. Clearance of Citrobacter rodentium requires B cells but not secretory immunoglobulin A (IgA) or IgM antibodies. *Infect Immun.* 2004;72:3315–3324.
109. Simmons CP, Clare S, Ghaem-Maghami M, et al. Central role for B lymphocytes and CD4+ T cells in immunity to infection by the attaching and effacing pathogen Citrobacter rodentium. *Infect Immun.* 2003;71:5077–5086.
110. Yoshida M, Claypool SM, Wagner JS, et al. Human neonatal Fc receptor mediates transport of IgG into luminal secretions for delivery of antigens to mucosal dendritic cells. *Immunity.* 2004;20:769–783.
111. Wijburg OL, Uren TK, Simpfendorfer K, et al. Innate secretory antibodies protect against natural Salmonella typhimurium infection. *J Exp Med.* 2006;203:21–26.
112. Yoshida M, Kobayashi K, Kuo TT, et al. Neonatal Fc receptor for IgG regulates mucosal immune responses to luminal bacteria. *J Clin Invest.* 2006;116:2142–2151.
113. McAuley JL, Linden SK, Png CW, et al. MUC1 cell surface mucin is a critical element of the mucosal barrier to infection. *J Clin Invest.* 2007;117:2313–2324.
114. McGuckin MA, Every A, Skene CD, et al. Muc1 mucin limits both Helicobacter pylori colonization of the murine gastric mucosa and associated gastritis. *Gastroenterology.* 2007;133:1210–1218.
115. Hermiston ML, Gordon JI. Inflammatory bowel disease and adenomas in mice expressing a dominant negative N-cadherin. *Science.* 1995;270:1203–1207.
116. McDermott JR, Humphreys NE, Forman SP, et al. Intraepithelial NK cell-derived IL-13 induces intestinal pathology associated with nematode infection. *J Immunol.* 2005;175:3207–3213.
117. Wang F, Graham WV, Wang Y, et al. Interferon-gamma and tumor necrosis factor-alpha synergize to induce intestinal epithelial barrier dysfunction by up-regulating myosin light chain kinase expression. *Am J Pathol.* 2005;166:409–419.
118. Utech M, Ivanov AI, Samarin SN, et al. Mechanism of IFN-gamma-induced endocytosis of tight junction proteins: myosin II-dependent vacuolarization of the apical plasma membrane. *Mol Biol Cell.* 2005;16:5040–5052.

119. Shen L, Black ED, Witkowski ED, et al. Myosin light chain phosphorylation regulates barrier function by remodeling tight junction structure. *J Cell Sci.* 2006;119:2095–2106.
120. Wang F, Schwarz BT, Graham WV, et al. IFN-gamma-induced TNFR2 expression is required for TNF-dependent intestinal epithelial barrier dysfunction. *Gastroenterology.* 2006;131:1153–1163.
121. Al-Sadi R, Ye D, Dokladny K, et al. Mechanism of IL-1β-induced increase in intestinal epithelial tight junction permeability. *J Immunol.* 2008;180:5653–5661.
122. Kosiewicz MM, Nast CC, Krishnan A, et al. Th1-type responses mediate spontaneous ileitis in a novel murine model of Crohn's disease. *J Clin Invest.* 2001;107:695–702.
123. Rivera-Nieves J, Bamias G, Vidrich A, et al. Emergence of perianal fistulizing disease in the SAMP1/YitFc mouse, a spontaneous model of chronic ileitis. *Gastroenterology.* 2003;124:972–982.
124. Bamias G, Okazawa A, Rivera-Nieves J, et al. Commensal bacteria exacerbate intestinal inflammation but are not essential for the development of murine ileitis. *J Immunol.* 2007;178:1809–1818.
125. Olson TS, Reuter BK, Scott KG, et al. The primary defect in experimental ileitis originates from a nonhematopoietic source. *J Exp Med.* 2006;203:541–552.
126. Vidrich A, Buzan JM, Barnes S, et al. Altered epithelial cell lineage allocation and global expansion of the crypt epithelial stem cell population are associated with ileitis in SAMP1/YitFc mice. *Am J Pathol.* 2005;166:1055–1067.
127. Schultsz C, van den Berg FM, ten Kate FW, et al. The intestinal mucus layer from patients with inflammatory bowel disease harbors high numbers of bacteria compared with controls. *Gastroenterology.* 1999; 117:1089–1097.
128. Swidsinski A, Ladhoff A, Pernthaler A, et al. Mucosal flora in inflammatory bowel disease. *Gastroenterology.* 2002;122:44–54.
129. Dvorak AM, Osage JE, Monahan RA, et al. Crohn's disease: transmission electron microscopic studies. III. Target tissues. Proliferation of and injury to smooth muscle and the autonomic nervous system. *Hum Pathol.* 1980;11:620–634.
130. Trabucchi E, Mukenge S, Baratti C, et al. Differential diagnosis of Crohn's disease of the colon from ulcerative colitis: ultrastructure study with the scanning electron microscope. *Int J Tissue React.* 1986;8:79–84.
131. Wehkamp J, Schmid M, Stange EF. Defensins and other antimicrobial peptides in inflammatory bowel disease. *Curr Opin Gastroenterol.* 2007;23:370–378.
132. Wehkamp J, Schmid M, Fellermann K, et al. Defensin deficiency, intestinal microbes, and the clinical phenotypes of Crohn's disease. *J Leukoc Biol.* 2005;77:460–465.
133. Wehkamp J, Salzman NH, Porter E, et al. Reduced Paneth cell alpha-defensins in ileal Crohn's disease. *Proc Natl Acad Sci U S A.* 2005; 102:18129–18134.
134. Wehkamp J, Harder J, Weichenthal M, et al. NOD2 (CARD15) mutations in Crohn's disease are associated with diminished mucosal alpha-defensin expression. *Gut.* 2004;53:1658–1664.
135. Ogura Y, Lala S, Xin W, et al. Expression of NOD2 in Paneth cells: a possible link to Crohn's ileitis. *Gut.* 2003;52:1591–1597.
136. Lala S, Ogura Y, Osborne C, et al. Crohn's disease and the NOD2 gene: a role for Paneth cells. *Gastroenterology.* 2003;125:47–57.
137. Simms LA, Doecke JD, Walsh MD, et al. Reduced alpha-defensin expression is associated with inflammation and not NOD2 mutation status in ileal Crohn's disease. *Gut.* 2008 (in press).
138. Nuding S, Fellermann K, Wehkamp J, et al. Reduced mucosal antimicrobial activity in Crohn's disease of the colon. *Gut.* 2007;56:1240–1247.
139. Schmid M, Fellermann K, Fritz P, et al. Attenuated induction of epithelial and leukocyte serine antiproteases elafin and secretory leukocyte protease inhibitor in Crohn's disease. *J Leukoc Biol.* 2007;81: 907–915.
140. Munkholm P, Langholz E, Hollander D, et al. Intestinal permeability in patients with Crohn's disease and ulcerative colitis and their first degree relatives. *Gut.* 1994;35:68–72.
141. Peeters M, Ghoos Y, Maes B, et al. Increased permeability of macroscopically normal small bowel in Crohn's disease. *Dig Dis Sci.* 1994; 39:2170–2176.
142. Miki K, Moore DJ, Butler RN, et al. The sugar permeability test reflects disease activity in children and adolescents with inflammatory bowel disease. *J Pediatr.* 1998;133:750–754.
143. Prasad S, Mingrino R, Kaukinen K, et al. Inflammatory processes have differential effects on claudins 2, 3 and 4 in colonic epithelial cells. *Lab Invest.* 2005;85:1139–1162.
144. Zeissig S, Burgel N, Gunzel D, et al. Changes in expression and distribution of claudin 2, 5 and 8 lead to discontinuous tight junctions and barrier dysfunction in active Crohn's disease. *Gut.* 2007;56:61–72.
145. Hugot JP, Chamaillard M, Zouali H, et al. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature.* 2001;411:599–603.
146. Ogura Y, Bonen DK, Inohara N, et al. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature.* 2001;411: 603–606.
147. Hisamatsu T, Suzuki M, Reinecker HC, et al. CARD15/NOD2 functions as an antibacterial factor in human intestinal epithelial cells. *Gastroenterology.* 2003;124:993–1000.
148. Barnich N, Carvalho FA, Glasser AL, et al. CEACAM6 acts as a receptor for adherent-invasive E. coli, supporting ileal mucosa colonization in Crohn disease. *J Clin Invest.* 2007;117:1566–1574.
149. Keita A, Salim S, Jiang T, et al. Increased uptake of non-pathogenic E. coli via the follicle-associated epithelium in longstanding ileal Crohn's disease. *J Pathol.* 2008;215:135–144.
150. Peeters M, Geypens B, Claus D, et al. Clustering of increased small intestinal permeability in families with Crohn's disease. *Gastroenterology.* 1997;113:802–807.
151. Tytgat KMAJ, van der Wal JWG, Einerhand AWC, et al. Quantitative analysis of MUC2 synthesis in ulcerative colitis. *Biochem Biophys Res Commun.* 1996;224:397–405.
152. Van Klinken BJW, van der Wal JWG, Einerhand AWC, et al. Sulphation and secretion of the predominant secretory human colonic mucin MUC2 in ulcerative colitis. *Gut.* 1999;44:387–393.
153. Corfield AP, Myerscough N, Bradfield N, et al. Colonic mucins in ulcerative colitis: evidence for loss of sulfation. *Glycoconj J.* 1996;13: 809–822.
154. Hanski C, Born M, Foss HD, et al. Defective post-transcriptional processing of MUC2 mucin in ulcerative colitis and in Crohn's disease increases detectability of the MUC2 protein core. *J Pathol.* 1999;188: 304–311.
155. Gonzalez-Licea A, Yardley JH. Nature of the tissue reaction in ulcerative colitis. Light and electron microscopic findings. *Gastroenterology.* 1966;51:825–840.
156. Donnellan WL. Early histological changes in ulcerative colitis. A light and electron microscopic study. *Gastroenterology.* 1966;50:519–540.
157. Nagle GJ, Kurtz SM. Electron microscopy of the human rectal mucosa. A comparison of idiopathic ulcerative colitis with inflammation of known etiologies. *Am J Dig Dis.* 1967;12:541–567.
158. O'Connor JJ. An electron microscopic study of inflammatory colonic disease. *Dis Colon Rectum.* 1972;15:265–277.
159. Dvorak AM, Dickersin GR. Crohn's disease: transmission electron microscopic studies. I. Barrier function. Possible changes related to alterations of cell coat, mucous coat, epithelial cells, and Paneth cells. *Hum Pathol.* 1980;11:561–571.
160. Delpre G, Avidor I, Steinherz R, et al. Ultrastructural abnormalities in endoscopically and histologically normal and involved colon in ulcerative colitis. *Am J Gastroenterol.* 1989;84:1038–1046.
161. Hinoda Y, Akashi H, Suwa T, et al. Immunohistochemical detection of MUC2 mucin core protein in ulcerative colitis. *J Clin Lab Anal.* 1998;12:150–153.
162. Shaoul R, Okada Y, Cutz E, et al. Colonic expression of MUC2, MUC5AC, and TFF1 in inflammatory bowel disease in children. *J Pediatr Gastroenterol Nutr.* 2004;38:488–493.
163. Tibble JA, Sigthorsson G, Bridger S, et al. Surrogate markers of intestinal inflammation are predictive of relapse in patients with inflammatory bowel disease. *Gastroenterology.* 2000;119:15–22.
164. Heller F, Florian P, Bojarski C, et al. Interleukin-13 is the key effector Th2 cytokine in ulcerative colitis that affects epithelial tight junctions, apoptosis, and cell restitution. *Gastroenterology.* 2005;129:550–564.
165. Al-Sadi RM, Ma TY. IL-1beta causes an increase in intestinal epithelial tight junction permeability. *J Immunol.* 2007;178:4641–4649.

166. Zeissig S, Bojarski C, Buergel N, et al. Downregulation of epithelial apoptosis and barrier repair in active Crohn's disease by tumour necrosis factor alpha antibody treatment. *Gut.* 2004;53:1295–1302.
167. Xue X, Piao JH, Nakajima A, et al. Tumor necrosis factor alpha (TNFalpha) induces the unfolded protein response (UPR) in a reactive oxygen species (ROS)-dependent fashion, and the UPR counteracts ROS accumulation by TNFalpha. *J Biol Chem.* 2005;280:33917–33925.
168. Boyce M, Bryant KF, Jousse C, et al. A selective inhibitor of eIF2alpha dephosphorylation protects cells from ER stress. *Science.* 2005;307: 935–939.
169. Beck PL, Wong JF, Li Y, et al. Chemotherapy- and radiotherapy-induced intestinal damage is regulated by intestinal trefoil factor. *Gastroenterology.* 2004;126:796–808.
170. Brandl K, Plitas G, Schnabl B, et al. MyD88-mediated signals induce the bactericidal lectin RegIII gamma and protect mice against intestinal Listeria monocytogenes infection. *J Exp Med.* 2007;204:1891–1900.
171. Nishimura J, Saiga H, Sato S, et al. Potent antimycobacterial activity of mouse secretory leukocyte protease inhibitor. *J Immunol.* 2008;180: 4032–4039.
172. Kim TW, Seo JN, Suh YH, et al. Involvement of lymphocytes in dextran sulfate sodium-induced experimental colitis. *World J Gastroenterol.* 2006;12:302–305.
173. Barnes SL, Vidrich A, Wang ML, et al. Resistin-like molecule beta (RELMbeta/FIZZ2) is highly expressed in the ileum of SAMP1/YitFc mice and is associated with initiation of ileitis. *J Immunol.* 2007;179: 7012–7020.