Gastroenterology 2022;162:1602–1616

REVIEWS IN BASIC AND CLINICAL GASTROENTEROLOGY AND HEPATOLOGY

Pattern Recognition Receptor Signaling and Cytokine Networks in Microbial Defenses and Regulation of Intestinal Barriers: Implications for Inflammatory Bowel Disease

Clara Abraham,[1] Maria T. Abreu,[2] and Jerrold R. Turner[3]

[1]Department of Internal Medicine, Yale University, New Haven, Connecticut; [2]Division of Gastroenterology and Hepatology, Department of Medicine, University of Miami Leonard Miller School of Medicine, Miami, Florida; and [3]Laboratory of Mucosal Barrier Pathobiology, Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts

Inflammatory bowel disease is characterized by defects in epithelial function and dysregulated inflammatory signaling by lamina propria mononuclear cells including macrophages and dendritic cells in response to microbiota. In this review, we focus on the role of pattern recognition receptors in the inflammatory response as well as epithelial barrier regulation. We explore cytokine networks that increase inflammation, regulate paracellular permeability, cause epithelial damage, up-regulate epithelial proliferation, and trigger restitutive processes. We focus on studies using patient samples as well as speculate on pathways that can be targeted to more holistically treat patients with inflammatory bowel disease.

Keywords: Toll-Like Receptor; TNF; IL23; Tight Junction; Claudin; MLCK; JAK-STAT; Genetics.

The high density of microbes within the intestinal lumen provides multiple benefits, including nutrient breakdown and immune education. In the absence of appropriate regulation, however, responses to these microbes can cause disease. Such immune dysregulation occurs, for example, in inflammatory bowel disease (IBD). As the key structure that separates luminal materials from the underlying lamina propria, the intestinal epithelium is a critical determinant of the extent to which immune cells and other cells are exposed to microbes and their products. A broad range of microbial products are sensed by pattern recognition receptors (PRRs; which include toll-like receptors [TLRs], NOD-like receptors [NLRs], and RIG-I-like receptors). PRR signaling is, therefore, carefully regulated, particularly with respect to downstream cytokine secretion. Although innate immune cells, eg, macrophages and dendritic cells, are central to these responses, many other cell types, including epithelial, stromal, endothelial, and adaptive immune cells express PRRs. Moreover, PRR-initiated responses in one cell subset can modulate responses in other cell types via PRR-independent processes, many of which are essential for mucosal homeostasis. Regulation of microbial exposure and sensing is, therefore, essential for intestinal immune conditioning and responses that limit bacterial burden and prevent excessive inflammatory responses (Figure 1). Here we review mechanisms of cross-talk between immune and epithelial cells within the intestinal environment, contributions of PRRs and cytokines to these processes, and their contributions to intestinal homeostasis or, when dysregulated, disease.

PRRs and Shaping of Innate Immune Responses in the Intestine

Innate immune cells are key mediators of PRR-initiated responses to microbes. There is a diverse spectrum of innate cells in the intestine; these cells cross-regulate each other and are also modulated by local factors, including the microbiome.[1–5] Intestinal macrophages are one critical innate immune cell subset. Experiments in mice have shown that intestinal macrophages are continuously replenished by peripheral monocytes in a CCR2-dependent manner.[6] Depending on the intestinal environment into which the peripheral monocytes are recruited, they can give rise to various myeloid cell phenotypes, including anti-inflammatory resident intestinal macrophages, and macrophages that mediate microbial clearance, intestinal injury, or resolution of inflammation after injury.[6–11]

Under homeostatic conditions intestinal macrophages are more effective at clearing bacteria but have reduced cytokine secretion relative to peripheral macrophages.[12,13] This allows for microbial clearance with minimal tissue injury. A range of factors within the intestinal environment contributes to these dual outcomes. The prolonged exposure to microbial products occurring in the intestine leads to chronic PRR stimulation that up-regulates antimicrobial processes while down-regulating inflammatory cytokines.[14–16] The importance of PRRs in conditioning

Abbreviations used in this paper: **CD, Crohn's disease; IBD, inflammatory bowel disease; IEC, intestinal epithelial cell; Ig, immunoglobulin; IL, interleukin; ILC, innate lymphoid cell; MLCK, myosin light chain kinase; NLR, NOD-like receptor; PRR, pattern recognition receptor; Reg, Regenerating family member; TGF, transforming growth factor; Th, T helper; TLR, toll-like receptor; TNF, tumor necrosis factor; Treg, regulatory T cell; TSLP, thymic stromal lymphopoietin; UC, ulcerative colitis.**

Most current article

0016-5085/$36.00
https://doi.org/10.1053/j.gastro.2021.12.288

intestinal immune cells is highlighted by the adverse outcomes induced by deletion of specific TLRs, eg, TLR4 or TLR5, or essential components of TLR signaling pathways. TLR signaling defects can result in an increased bacterial burden, altered microbiota, increased expression of inflammatory mediators, dysregulation of immune cells, and intestinal inflammation (Figure 1).[17–26] The Crohn's disease (CD)–associated PRR NOD2 has been well-studied in this regard.[15,27] In the absence of NOD2, antimicrobial pathways are impaired and inflammatory cytokines are not adequately down-regulated. As a result, experimental colitis severity can increase in NOD2-deficient mice,[27,28] whereas mice with NOD2 overexpression have resistance to experimental colitis.[29] Combined deficiency of NOD2 with other IBD-associated pathways and/or additional risk factors can further increase colitis severity.[30,31] For example, combined deficiency of NOD2 and gp91phox (nicotinamide adenine dinucleotide phosphate oxidase member) in mice can lead to early-onset spontaneous intestinal inflammation associated with accumulation of *Mucispirillium schaedleri*.[31]

The conditioning of intestinal macrophages through PRRs leads to the up-regulation of antimicrobial pathways.[14] One such pathway is autophagy,[16] and genetic variants (*ATG16L1*, *IRGM*, *MTMR3*) leading to a loss-of-autophagy increase susceptibility to IBD.[32] PRR stimulation also promotes production of reactive oxygen species and reactive nitrogen species in macrophages, and these pathways cooperate to limit systemic dissemination of intestinal microbiota in mouse models (Figure 1).[33] The importance of PRR signaling and microbial conditioning for mounting defenses to intestinal infectious challenges extends to additional cell subsets, including neutrophils[34] and natural killer cells.[35] Innate lymphoid cells (ILCs) are also important for homeostasis and regulating bacteria. For example, ILCs regulate dissemination of bacteria (eg, Alcaligenes)[36] and commensal bacteria-specific CD4+ T-cell responses;[37] ILCs are expanded in human IBD and can also contribute to the inflammation in experimental colitis.[38,39] Interestingly, although PRR-initiated signaling in myeloid cells promotes antimicrobial pathways, downstream consequences of PRR signaling (eg, endosomal acidification) can also serve as cues for up-regulating microbial virulence factors and intracellular microbial growth (eg, SPI2 genes in *Salmonella* Typhimurium).[40,41] Microbial-dependent dietary products also contribute to beneficial conditioning of intestinal macrophages, as is observed with butyrate-induced autophagy and antimicrobial proteins.[42,43]

Intestinal myeloid cells are continuously exposed to microbial products and down-regulating inflammatory responses to these products is essential. Mechanisms mediating the down-regulation of PRR-initiated inflammatory pathways in myeloid cells include up-regulation of inhibitory cell surface molecules (eg, Tyro, Axl, Mer), inhibitory intracellular molecules (eg, IRAKM, Tollip, IRF4, ZNRF4, A20), transcriptional repressors (eg, Twists, NFκB1), and autocrine/paracrine inhibitory secreted mediators (eg, interleukin [IL]10, transforming growth factor [TGF]β).[11–13,15,28,44–53] Anti-inflammatory mechanisms in myeloid cells can be induced by specific microbes, microbial products, and/or metabolites (eg, *Clostridium* species, polysaccharide).[54,55] Additional mechanisms contributing to cytokine down-regulation in intestinal myeloid cell subsets include the uptake of apoptotic cells (eg, epithelial cells).[56–58]

Because autocrine/paracrine cytokine pathways are important drivers of PRR-initiated responses, mechanisms inhibiting inflammatory cytokine-initiated signaling pathways (eg, suppressor of cytokine signaling family members) or promoting anti-inflammatory cytokine signaling (eg, SMAD family members) also contribute to down-regulatory mechanisms in innate immune cells. As such the ability of innate immune cells to respond to IL10 is critical to anti-inflammatory conditioning in the intestine and to regulating intestinal inflammation.[59,60] Additional anti-inflammatory mediators, such as TGFβ, which can be secreted by intestinal stromal cells, also down-regulate PRR-induced inflammatory cytokine secretion from myeloid cells.[12] Mechanisms allowing for increased TGFβ responsivity can be modulated in the intestine. For example, PRRs up-regulate the integrin αvβ8, which promotes TGFβ activation, and these pathways are up-regulated in intestinal dendritic cells.[61] On the other hand, intestinal myeloid cells from patients with IBD and animal models of colitis can demonstrate reduced responsivity to anti-inflammatory cytokines, including to IL10[62] and to TGFβ due to up-regulation of SMAD7.[63] Consistently, intestinal myeloid cells from patients with IBD demonstrate an inflammatory phenotype with increased production of inflammatory cytokines (eg, IL23, IL12, tumor necrosis factor [TNF], IL1β).[64] Therefore, on the one hand, microbes and PRR-initiated pathways promote inflammatory cytokines (eg, IL23, IL12, TNF, IL1β) and colitis.[65,66] On the other hand, intestinal microbiota and PRRs mediate numerous protective effects in intestinal tissues, such that in the absence of PRR signaling colitis can be more severe and the ability of microbial communities to mediate beneficial effects may be impaired.[21–26]

PRRs and Regulation of Adaptive Immunity

Adaptive immunity plays an important role in both intestinal immune homeostasis and in the inflammation observed in patients with IBD. T helper (Th) 1 (interferonγ, TNF), Th17 (IL17, IL21, IL22), and Th9 (IL9) cells are present in intestinal tissues under homeostatic conditions, but their dysregulation can lead to IBD.[67–72] Regulatory T cells (Tregs) are similarly present in intestinal tissues during homeostasis and are critical for down-regulating inflammatory pathways. The proper recognition and response to microbial products through PRRs is required for optimally regulating T- and B-cell adaptive immune responses; this regulation can occur through pathways both intrinsic and extrinsic to adaptive immune cells.

Direct PRR stimulation of T cells can regulate T-cell outcomes, which can, in turn, modulate colitis outcomes.[21,73,74] Distinct PRRs can also cross-regulate each other (eg, RIG-I–like receptors can suppress activation by TLRs[75]) to modulate T cells. In contrast, some studies have shown that expression of the TLR adaptor protein MyD88

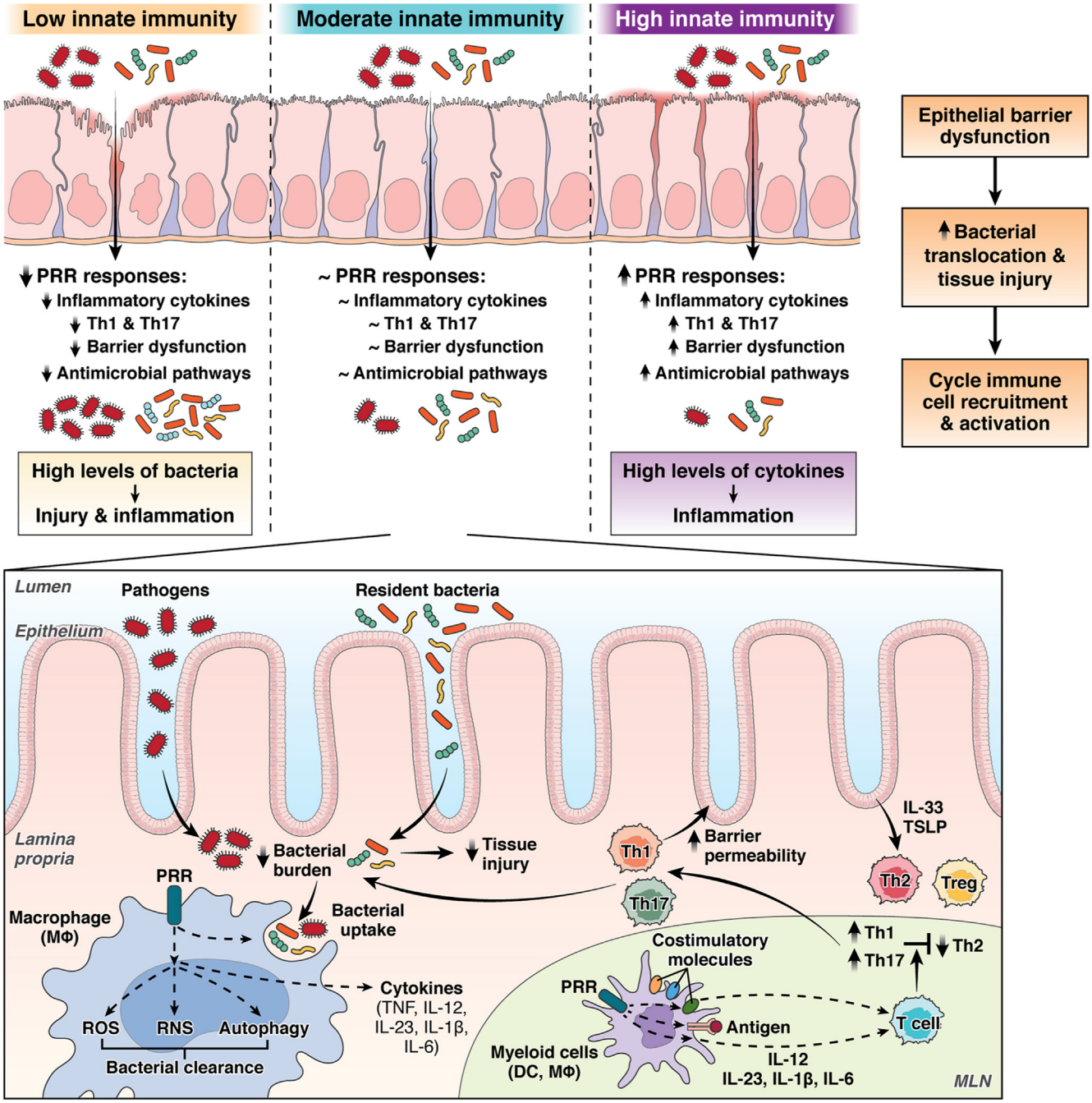

Figure 1. Upon microbial challenges, balancing PRR responses in innate immune cells is critical for regulating interactions between innate and adaptive immunity, cytokines, and epithelial barrier function. Either low or high levels of innate immune responses through PRR-initiated pathways can ultimately lead to intestinal inflammation. With initial inadequately low innate immune responses, the low levels of antimicrobial pathways and Th1- and Th17-conditioned adaptive pathways (which are also required for bacterial responses) can lead to high levels of bacterial burden in intestinal tissues resulting in injury and subsequent inflammation. With excessive PRR responses on innate immune cells the high levels of cytokines can similarly lead to intestinal inflammation, which in turn disrupts the epithelial barrier, thereby further amplifying the inflammation. MLN, mesenteric lymph node.

by T cells is not necessary for either pathogenic or regulatory functions in the intestine.[66] With respect to adaptive immune cell-extrinsic PRR stimulation, PRR stimulation of innate immune cells, which can serve as antigen-presenting cells, enhances antigen uptake and antigen presentation and up-regulates costimulatory molecules and cytokine secretion, thereby modulating T-cell activation and differentiation.[76] When PRR pathways are deficient in myeloid cells (eg, TRAF6, LACC1, IRF5[77–79]), Th1 and Th17 cells can be reduced and Th2 cells, in turn, can increase. Although this T-cell modulation can reduce tissue damage in some situations, it can also reduce efficacy of microbial clearance

and increase susceptibility to intestinal injury and subsequent inflammation (Figure 1). The proper recognition and sensing of microbial products can also regulate myeloid cell–dependent trafficking of microbes to mesenteric lymph nodes, thereby regulating both T- and B-cell responses.[80] Adaptive immune cells can also cross-regulate myeloid cells and intestinal microbiota. For example, adaptive immune-dependent IL17 can recruit neutrophils, which in turn regulate IL22, antimicrobial peptides, and expansion of potentially harmful microbiota in mice (eg, segmented filamentous bacteria).[81]

Consistent with the role of PRRs in regulating T cells, specific microbes can promote distinct T-cell phenotypes. For example, various *Clostridia* strains can promote Tregs in colonic mucosa and thereby protect from colitis.[82] On the other hand, microbial-driven IL23 increases with inflammation and promotes more pathogenic Th17 cells, which can drive disease pathology.[68,83,84] Importantly, transfer of microbiota from patients with IBD into germ-free mice can lead to increased Th1 and Th17 cells and reduced Treg cells, epithelial barrier dysfunction, increased bacterial translocation, and more severe experimental colitis.[85–87] Microbes can also regulate intestinal adaptive immune outcomes through PRR-independent mechanisms. For example, intestinal microbiota regulate bile acids (eg, lithocholic acid derivatives), which in turn can regulate adaptive immune outcomes (eg, Th17, Tregs).[88] Microbial-derived butyrate generated upon fermentation of dietary fiber induces colonic Tregs in mice.[89]

Antibody production to microbial products in the intestine requires PRR signaling.[90] For example, PRR stimulation (eg, TLR5) of lamina propria myeloid cells can result in their production of retinoic acid and differentiation of naïve B cells into immunoglobulin (Ig)A-producing plasma cells[91] and MyD88 on intestinal T cells coordinates germinal center responses and IgA+ B cells;[92] this can in turn modulate the ability of bacteria to breach the intestinal epithelial barrier.[93] TLRs can also promote IgG responses from B cells, which protect from systemic bacteria[94] and IL10-producing intestinal B cells, which are able to reduce colitis severity.[95] Taken together, PRRs and cytokines cooperate to modulate the spectrum and specificity of adaptive immune responses in the intestine. The coordination of PRRs, cytokines, and immune responses in turn cross-regulate intestinal epithelial cells.

Epithelial Barrier Function

The intestinal barrier separates the lamina propria and deeper tissues from the harsh luminal environment. Diminished function of this barrier is commonly cited as both a cause and consequence of disease. Although useful as an overarching term, understanding the mechanisms that lead to altered barrier function in specific settings has profound pathophysiological and therapeutic implications. In general, barrier function is considered to be the opposite of permeability. Although this generally refers to paracellular permeability, there are multiple pathways by which materials can cross the epithelium. These can be divided into transcellular and paracellular routes. Transcellular transport is exemplified by absorption of most nutrients and salts, in which distinct apical and basal transporters ensure vectorial, ie, directionally oriented, solute movement. Transcellular transport can also be mediated by vesicular transport, of which IgA transcytosis into the lumen and antigen presentation by M cells are examples. In contrast to transcellular transport, paracellular transport is passive, with the direction of net transepithelial flux determined by existing gradients. Because these gradients can be established by transcellular transport, paracellular transport can be directed by transcellular transport. For example, diarrhea induced by TNF, a prototypic effector of increased paracellular permeability and net fluid efflux, requires both protein kinase C–mediated inhibition of NHE3-mediated Na^+ absorption, which reduces the osmotic driving force for paracellular water absorption, as well as myosin light chain kinase (MLCK)–induced tight junction permeability increases that mediate paracellular water efflux.[96]

Intestinal permeability is frequently equated with permeability of the tight junction, which seals the space between cells and is the rate-limiting step of paracellular flux. It is, however, important to also consider barrier loss and increased passive flux that occurs at sites of epithelial damage. Flow at these sites does not display the size- and charge-selectivity that characterize trans-tight junction flux and is, therefore, referred to as unrestricted. The unrestricted pathway is the route by which bacterial invasion and massive macromolecular flux occur (Figure 2). This contrasts sharply with the 2 routes across the tight junction that, as discussed next, are highly selective.

Trans-tight junction routes differ in molecular composition, physical characteristics, and mechanisms of regulation. The pore pathway is created by some members of the claudin protein family and represents physical channels that traverse the tight junction.[97–99] These actively gated channels create a high-capacity route that is both charge- and size-selective. In the gut, the channels are generally cation selective and have maximum diameters of 6–8 Å (0.6–0.8 nm) that restrict flux to water and small ions.[99–102] This pathway does not, however, have the structural specificity of the transmembrane transporters that drive transcellular transport and also transports other small cations that transmembrane transporters exclude. The second trans-tight junction flux route, the leak pathway, is regulated by the dense ring of actin and myosin that encircles the tight and adherens junctions. Activation of MLCK, which induces remodeling of the perijunctional ring and triggers endocytic removal of some tight junction proteins, eg, occludin, increases permeability of the low-capacity, charge-nonselective leak pathway, which accommodates molecules up to ~125 Å (12.5 nm) in diameter, including small proteins.[96,103–106] This size-selectivity prevents bacteria from crossing pore or leak pathways. However, the relative absence of specificity allows the leak pathway to mediate flux of some bacterial products, including small metabolites and lipopolysaccharide (Figure 2).

Measuring Barrier Function In Vivo

In vivo, epithelial barrier function has been variably defined and measured using a range of approaches including assessment of mucus thickness, epithelial morphology, tight junction protein expression, recovery of orally delivered inert probes, or presence of endogenous markers thought to correlate with barrier permeability in serum or urine. As might be expected, these all report on different aspects of barrier function that may be disrupted by a wide range of factors. All of the approaches do, therefore, have utility depending on the question being studied (Table 1). However, only recovery of inert probes measures intestinal permeability directly.

In patients, the most commonly used measure of intestinal barrier function measures urinary recovery of lactulose and mannitol, which have diameters of 11.5 Å and 7 Å, respectively. Both of these cross the leak pathway but are too large to be accommodated by the pore pathway in vitro. It remains to be determined, however, if some mannitol crosses the pore pathway in vivo. Because mammals lack transporters and enzymes necessary for absorption and metabolism of lactulose and mannitol, they can only cross the barrier at tight junctions or at sites of epithelial damage. Once absorbed, both probes are small enough to pass through the glomerular filter into the renal tubules and, because they cannot be actively resorbed, recovered in the urine. Of course, factors other than intestinal permeability can affect urinary recovery, including impaired renal perfusion and renal disease. Some investigators have also reported that mannitol in the food chain has become a confounder and have turned to ^{13}C-mannitol instead of unlabeled mannitol.[107] Breakdown of lactulose and mannitol by bacteria can skew results in patients with small bowel bacterial overgrowth and also prevents their use to measure colonic permeability. Sucralose, which is slightly larger than lactulose and resistant to bacterial degradation, can be used to overcome this problem and is often used in single-probe assessments of colonic permeability. Nevertheless, the lactulose-mannitol dual-probe assay remains the gold-standard for in vivo measurements of small intestinal permeability in human subjects.

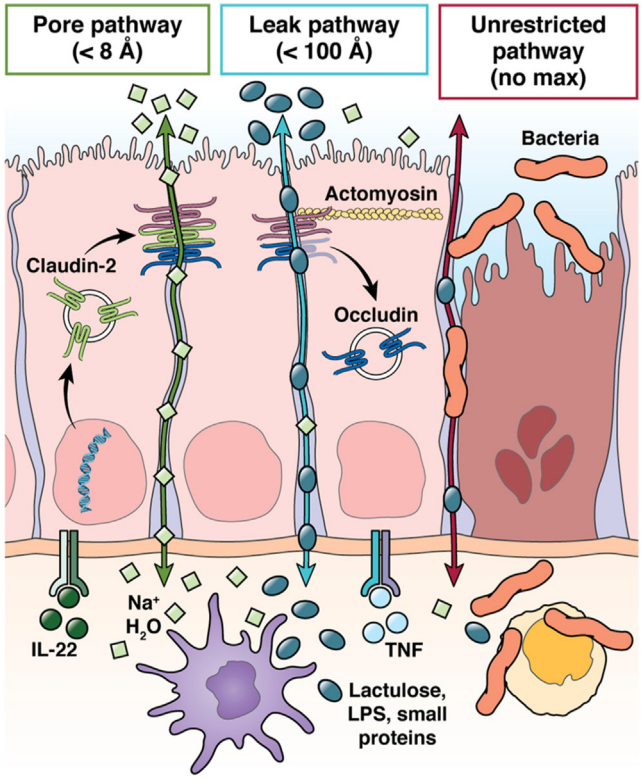

Figure 2. Immune regulation of tight junction–dependent and tight junction–independent passive permeability pathways. In addition to transcellular transport (not shown), 3 distinct routes account for intestinal permeability. These are the trans–tight junction pore (*green*) and leak (*blue*) pathways and the tight junction–independent unrestricted pathway (*red*). The immune system can selectively up-regulate pore pathway permeability by IL22-induced up-regulation of claudin-2 (*green*) expression. In contrast, TNF primarily up-regulates leak pathway permeability by activating MLCK transcription and enzymatic activity. Flux via the unrestricted pathway (*red*) occurs at sites of epithelial damage and is independent of tight junctions. LPS, lipopolysaccharide.

Interactions Between Immune Cells and the Epithelial Barrier

The roles of cytokines in directing epithelial barrier loss are well documented. Cytokines including IL6, IL13, and IL22 up-regulate expression of claudin-2, which forms cation-selective paracellular channels that enhance pore pathway permeability (Figure 2).[99,108–110] In contrast, TNF, LIGHT, and IL1β induce epithelial MLCK expression up-regulation that triggers occludin removal from the tight junction and increases leak pathway permeability.[103–106,111–116] TGFβ has been reported to reduce paracellular permeability through incompletely defined mechanisms.[117–119] $\gamma\delta$ T cells may also support maintenance of the epithelial barrier in mice.[120,121] In the context of damage, adaptive and innate immune cells can signal through cytokine networks to promote epithelial proliferation and restitution, which ultimately leads to barrier restoration. These cytokine networks include IL22, IL23, IL33, IL36, IL10, TGFβ, and, perhaps counterintuitively, TNF and IL6.[23,122–129]

PRR signaling also contributes to mucus layer maintenance and function of specialized cells, eg, Paneth cells.[130,131] Macrophages can regulate the epithelial barrier directly by cytokine secretion and via cross-regulation of other immune cells. For example, microbial sensing through MyD88 in CX3CR1+ mononuclear phagocytes cross-regulates ILC3s, which in turn leads to IL22 production, epithelial claudin-2 expression, increased pore pathway permeability, and protection from microbial-induced colitis.[109,132] Stromal cells contribute to epithelial barrier restoration subsequent to damage. Prostaglandin-endoperoxide synthase 2–expressing stromal cells migrate to the base of crypts in a MyD88-dependent manner, allowing for intestinal epithelial cell (IEC) proliferation after injury.[133] The manner in which immune cells regulate the epithelial barrier is also influenced by dietary factors (eg,

Table 1. Molecules That Have Been Used as Markers of Intestinal Permeability Changes

Orally delivered probes	
Lactulose, 11.5 Å (*d*)	Gold standard (as part of lactulose:mannitol ratio)
Mannitol, 7 Å (*d*)	Gold standard (as part of lactulose:mannitol ratio); sometimes present in foods; ^{13}C-mannitol can be used instead
4 kDa dextran, 28 Å (*d*)	Large quantities required, useful in small animal models
70 kDa dextran, 120 Å (*d*)	Large quantities required, useful in small animal models
Albumin, 7 Å (*d*)	Degraded by intestinal proteases
Creatinine, 5.2 Å (*d*)	Can be used in humans and animals
Cr-EDTA, 13.6 Å (*d*)	Data inconsistent with similar-sized probes
Erythritol , 7.4 Å (*d*)	>90% absorbed, may compete with other sugars
Inulin, 27.8 Å (*d*)	Too little absorbed to be useful
Ovalbumin, 8 Å (*d*)	Degraded by intestinal proteases
PEG400, 3.4 Å (*d*)	Linear molecule, difficult to analyze and interpret
PEG4000, 31.8 Å (*d*)	Linear molecule, difficult to analyze and interpret
PEG900, 16.8 Å (*d*)	Linear molecule, difficult to analyze and interpret
Rhamnose, 9.8 Å (*d*)	Can be metabolized, has been used in place of mannitol
Sucralose, ∼13 Å (*d*)	Alternative to lactulose, not metabolized by colonic bacteria
Tc-DTPA, ∼14 Å (*d*)	Limited data, likely similar to Cr-EDTA
Urea, 3.6 Å (*d*)	Active transcellular transporters exist
Serum/plasma markers	
16s bacterial DNA	Possible surrogate for LPS
CRP	Acute-phase reactant
Citrulline	Marker of epithelial damage, not permeability
Claudin-3	Small changes, uncertain mechanism rarely used
Cortisol	Stress marker
Cytokines (IL-1β, IL-6, IL-8, TNF)	Inflammatory marker
Intestinal FABP	Marker of epithelial damage, not permeability
LPS	Unlikely to be detectable in many cases
LPS binding protein	Acute-phase reactant
Soluble CD14	Marker of monocyte activation
Zonulin	Controversial
α-1AGP	Acute-phase reactant
Fecal/urine markers	
Calprotectin (fecal)	Inflammatory marker, neutrophil product
Claudin-3 (urinary)	Small changes, uncertain mechanism, rarely used
Lactoferrin (fecal)	Inflammatory marker, neutrophil product
Lipocalin-2 (fecal)	Inflammatory marker, neutrophil product

α-1AGP, α-1-acid glycoprotein; CRP, C-reactive protein; DTPA, diethylene-triamine-pentaacetic acid; EDTA, ethylene diamine tetracetic acid; FABP, fatty acid binding protein; LPS, lipopolysaccharide.

fiber, sugars, fat, emulsifiers) and this may be through both direct and indirect mechanisms, including modulation of intestinal microbiota, interactions with PRRs, and cytokine production.[134–144] Therefore, multiple factors modulate the interactions between PRRs and cytokines on immune cell subsets that can contribute to both epithelial health and to the dysregulated barrier observed during disease.

Epithelial PRR Functions

IECs have multiple mechanisms through which they regulate responses to the high density of microbes in the lumen, including through PRR signaling. PRRs are generally expressed at greater levels in colonic compared with small intestinal epithelia and these PRR responses need to be carefully regulated.[145,146] The TLR and IL1β receptor adapter protein MyD88 is critical to epithelial expression of antimicrobial proteins (eg, Regenerating family member 3γ [Reg3γ], Reg3β, RELMβ) that limit penetration of resident and pathogenic bacteria.[147,148] MyD88 is also required for optimal induction of autophagy, which limits dissemination of enteric pathogens.[149] The threshold of PRR signaling is also important in epithelial cells because overly robust PRR signaling can lead to impaired intestinal resident microbial colonization, and, in turn, increased susceptibility to enteric pathogens.[150] Interestingly, PRR expression changes with development. For example, intestinal epithelial TLR5 expression is much lower in neonates relative to older individuals.[146] This may contribute to the altered microbiome of neonates and susceptibility of neonates to certain infections.

Epithelial cells reduce responses to microbial products through down-regulating key PRR-associated signaling molecules (eg, IRAK-1[151]) or up-regulating molecules inhibiting PRR signaling (eg, Tollip, SIGIRR).[150,152] In fact, the failure to properly regulate such pathways after birth may contribute to necrotizing enterocolitis pathogenesis.[151] Some studies have demonstrated segregation of PRRs on

epithelial surfaces and/or differential outcomes depending on the surface mediating the PRR response as another mechanism by which epithelial cells regulate responses to resident microbes.[153,154]

Epithelial Cross-Talk to Immune Cells and Regulated Transfer of Microbial Communication

Contrary to conventional wisdom, increased intestinal permeability is insufficient to cause disease in both human subjects and animal models.[109,155–162] However, studies using genetically modified animal models have shown that increases in either pore or leak pathway permeabilities are sufficient to augment immune-mediated systemic and intestinal disease.[163] Moreover, either pharmacologic restoration of pore or leak pathway barrier function is therapeutically effective in experimental IBD.[111,157] Although similar experiments cannot be performed in humans, recent data show that, in healthy first-degree relatives of patients with CD, increased permeability is a risk factor for later IBD development.[162] In patients with established CD, increased permeability during remission is a risk factor for disease reactivation.[164,165] Notably, environmental factors including stress and microbiome perturbations can modulate intestinal permeability.[166–170] Thus, extensive evidence links increased intestinal permeability to pathogenesis and progression of intestinal disease.

Epithelial cells provide instruction to immune cells residing within intestinal lymphoid tissues; this instruction contributes to homeostatic pathways in health but can also promote inflammatory pathways. As an increasing spectrum of epithelial cell subsets is defined through single-cell sequencing and other approaches,[171] the specialized functions of these subsets provide insight into important cross-talk mechanisms. Under homeostatic conditions, IECs can condition myeloid cells (eg, dendritic cells, CX3CR1+ antigen-presenting cells) to a more "noninflammatory" and/or "regulatory" phenotype.[172,173] Epithelial cells secrete cytokines, such as IL33 and thymic stromal lymphopoietin (TSLP), which then regulate underlying immune cells. For example, IL33 can promote colonic Treg function[174] and a feedback loop to ILC2s through the amphiregulin–epidermal growth factor receptor pathway, which then reduces inflammation and restores epithelial cell function during injury.[175] TSLP secreted from epithelial cells can condition "noninflammatory" dendritic cells that secrete IL10 and promote a Th2 response on microbial challenges; TSLP expression can be reduced in epithelial cells from patients with CD.[172] Studies in recently described intestinal tuft cells have shown that they express IL25, which promotes ILC2 homeostasis and during helminth infection allows for ILC2-mediated secretion of Th2-associated cytokines, which then promotes differentiation of tuft cells and goblet cells along with mucus production.[176–178] Although mucus is traditionally considered to protect the underlying epithelium from luminal bacteria and food antigens, glycans in the mucus can also instruct underlying myeloid cells with tolerogenic signals.[179] Additional epithelial cell mechanisms (eg, retinoic acid receptor β, APRIL) can promote regulation of Th17 cells, CD4+ T-cell homing, and IgA-producing B cells.[180,181] Importantly, increased intestinal permeability can also increase antimicrobial and regulatory mechanisms in both innate and adaptive immune cells, which ultimately can improve outcomes with acute injury.[16,119,182] Although increased intestinal epithelial permeability frequently increases recruitment of a range of intestinal immune cells, in some cases this increased permeability can also lead to reduced recruitment of select immune cell subsets (eg, neutrophils).[183]

Epithelial cells play a key role in transferring microbial products and luminal substances, which then condition the underlying immune system (eg, segmented filamentous bacteria and bacterial strains from patients with ulcerative colitis [UC] can promote Th17 cells).[184,185] Specialized epithelial subsets such as goblet cells can transfer intestinal luminal antigens,[186] with MyD88 regulating the transfer of antigens by goblet cells.[187] In addition, lamina propria myeloid cell extensions between epithelial cells and into the intestinal lumen enable sampling of luminal antigens; these can depend on PRR signaling (eg, MyD88), and are increased during injury.[188–190] In some cases the epithelial regulation of underlying cells can then lead to communication back to the epithelial cells. For example, during *Citrobacter rodentium* infection, goblet cell–derived RELM-β recruits CD4+ T cells and promotes IL22 production, which in turn leads to IEC proliferation and reduced mucosal pathology.[191] Epithelial cells also communicate with ILC3s, which can in turn modulate epithelial outcomes.[192] In aggregate, the specific PRRs stimulated, the strength and environment of this stimulation, and the cell subsets undergoing this stimulation influence the direct responses, cytokines secreted, and cross-talk between the intestinal epithelium and immune cell subsets.

IBD-Associated Genes Regulating PRRs, Cytokines, and Epithelial Cells

The success in IBD genetic discoveries has provided an important opportunity to better understand IBD pathogenesis; a number of IBD-associated genes have been found to regulate PRR-initiated outcomes and cytokines, and, in turn, epithelial and immune cell interactions in the intestine. More than 240 genetic loci have been identified to alter IBD susceptibility.[32,193–195] Consistent with the importance of balancing PRR regulation, genetic variants leading to either a relative decrease or increase in PRR signaling can modulate IBD risk (Figure 1). Variants leading to a loss-of-function in the CD-associated gene *NOD2* were an early example of risk genes leading to a loss in PRR-initiated pathways;[196,197] they can lead to a loss of both antimicrobial mechanisms and tolerance. Additional IBD-risk genes conferring a loss-of-function in PRR-initiated outcomes include *ATG16L1*, *ICOSL*, *LACC1*, *INAVA*, *IL18RAP,* and *RNF186.*[32,198–206] Further highlighting the importance of antimicrobial mechanisms are numerous rare coding genetic variants associated with early-onset IBD and leading to

a loss in antimicrobial pathways.[207–212] On the other hand, genetic variants leading to increased PRR-initiated signaling and cytokines from innate cells can also increase risk for IBD, with examples including *IL23R*, *IRF5*, *TNFSF15*, *TPL2* (*MAP3K8*), *JAK2*, *STAT3/5*, *STAT1/4*, *IL10R*, *PTPN2*, *PTPN22*, and *MTMR3.*[32,213–225] Consistent with the important role for myeloid cells in regulating cytokine secretion during intestinal injury and cytokines in turn regulating barrier function through both direct and indirect mechanisms, IBD genes such as *PTPN2*, *TNFSF15*, and *IRF5* can regulate barrier function and/or mucosal healing through their role in myeloid cells.[79,226,227]

Genes conferring altered risk for IBD can do so through regulating multiple distinct cell types. For example, IBD-associated variants in *PTPN2*, *IL23R*, *IRF5*, *ATG16L1*, *RNF186*, and *INAVA* can regulate myeloid cell, T-cell, and/or epithelial cell outcomes.[201,202,204–206,228–240] In some cases a given gene can differentially regulate inflammatory outcomes in distinct cell types. For example, the increased TPL2 expression in macrophages with the IBD-risk variant leads to increased inflammatory cytokines/inflammation.[215] In contrast, TPL2 expression in intestinal myofibroblasts is essential for optimal levels of the PGE2 pathway, which protects from epithelial injury.[241] In other cases modulation of the risk gene expression in 2 different cell types may be cooperative. For example, decreased INAVA expression with the IBD-risk variant in human macrophages leads to decreased PRR-induced outcomes with a reduced ability to clear intracellular bacteria,[201] and reduced INAVA expression in epithelial cells leads to reduced epithelial barrier function.[206,233]

Various rare IBD-risk coding variants can lead to altered enterocyte function (eg, apoptosis, adhesion, and permeability), including TTC7A and CDH1.[242,243] NOX1 regulates epithelial brush border reactive oxygen species in colonic crypts.[244] In some cases, studies have further defined the regulation of these genes in vivo through epithelial cell–intrinsic regulation of the gene. Epithelial cell–intrinsic XBP1 deletion leads to altered endoplasmic reticulum stress and dysregulated responses to microbial challenges.[234,245] A number of IBD-associated genes regulate cytokine pathways in epithelial cells, including *PTPN2.*[246–248] Either ATG16L1 deletion or the ATG16L1 T300A CD risk variant leading to a loss in autophagy can lead to increased endoplasmic reticulum stress, abnormal morphology, and reduced bacterial clearance in Paneth cells.[202,234–237] The cooperation of ATG16L1 dysfunction with environmental factors such as viral infection promotes the intestinal inflammation and altered Paneth cell phenotypes observed in mice.[202,249] In human studies, combined expression of *ATG16L1* and *NOD2* IBD-risk alleles cooperate to promote abnormal Paneth cell phenotypes,[250] as do *ATG16L1* risk alleles and environmental interactions, such as smoking.[251] In another example of cooperative interactions, whereas deficiency of either A20 (regulates both PRR and TNF signaling) or ABIN-1 in epithelial cells leads to minimal IEC loss, combined A20 and ABIN-1 deficiency in epithelial cells leads to IEC death and increased lethality in mice.[252] Taken together, the IBD genetic discoveries have highlighted pathways leading to cross-talk between PRRs, cytokines, and epithelial cells to promote both health and disease.

IBD Therapies and Regulation of Barrier Function and PRRs

Clinical trials targeting pathways implicated in IBD pathogenesis provide an important opportunity to understand how these pathways function in human disease. In some cases, these clinical trials have highlighted potential unintended effects of blocking select cytokines in epithelial function, including in epithelial proliferation, barrier integrity, and antimicrobial protein expression.

Mucosal Healing and Biologic Therapy: Pathogenetic Mechanisms of Barrier Dysfunction Targeted by Therapy

Anti-TNF therapy ushered in the modern era of IBD treatment and demonstrated that effective therapy could, and ideally should, heal mucosal ulcerations and dampen endoscopically detectable inflammation (Figure 3). More recently, the definition of mucosal healing has been extended to include resolution of histologic inflammation. TNF inhibition promotes mucosal healing by multiple pathways. These include prevention of TNF-induced increases in leak and unrestricted pathway permeabilities. For example, disruption of TNF-induced leak pathway regulation has proven effective, and superior to anti-TNF, in experimental immune-mediated IBD.[111,156]

TNF has pleiotropic effects including proliferation, differentiation, inflammation, and cell death depending on the local concentration, tissue type, or cellular context. TNF can promote proliferation and expansion of naïve and pathogenic effector T-cell populations. In epithelial cells, TNF can trigger proliferation, increase paracellular permeability, or induce apoptosis. To some extent, these likely represent activities of different receptors, as TNF can transactivate epidermal growth factor receptor and ErbB2 and can also signal directly via 2 distinct TNF receptors.[253] Signaling via TNFR1, the low-affinity TNF receptor, is initiated by high local TNF concentrations and induces caspase-3–dependent epithelial apoptosis.[254,255] In contrast, signaling via the high-affinity TNF receptor TNFR2, which can be activated by low TNF concentrations, activates epithelial proliferation, migration, and barrier regulation.[156,256–258] Thus, TNF can increase leak pathway permeability via TNFR2 activation and unrestricted pathway permeability via TNFR1. Effective anti-TNF antibodies in IBD have been suggested to promote expansion of Tregs while inducing apoptosis of lamina propria effector T cells.[259,260] Moreover, similar to various cytokines, TNF amplifies PRR-initiated signaling such that anti-TNF therapies likely also reduce PRR-initiated inflammatory pathways in intestinal tissues. Despite the efficacy of TNF blockade in IBD, only one third of patients are in remission after 1 year.[68] In some of these patients, disease may be driven by oncostatin M,[261] an IL6 family member

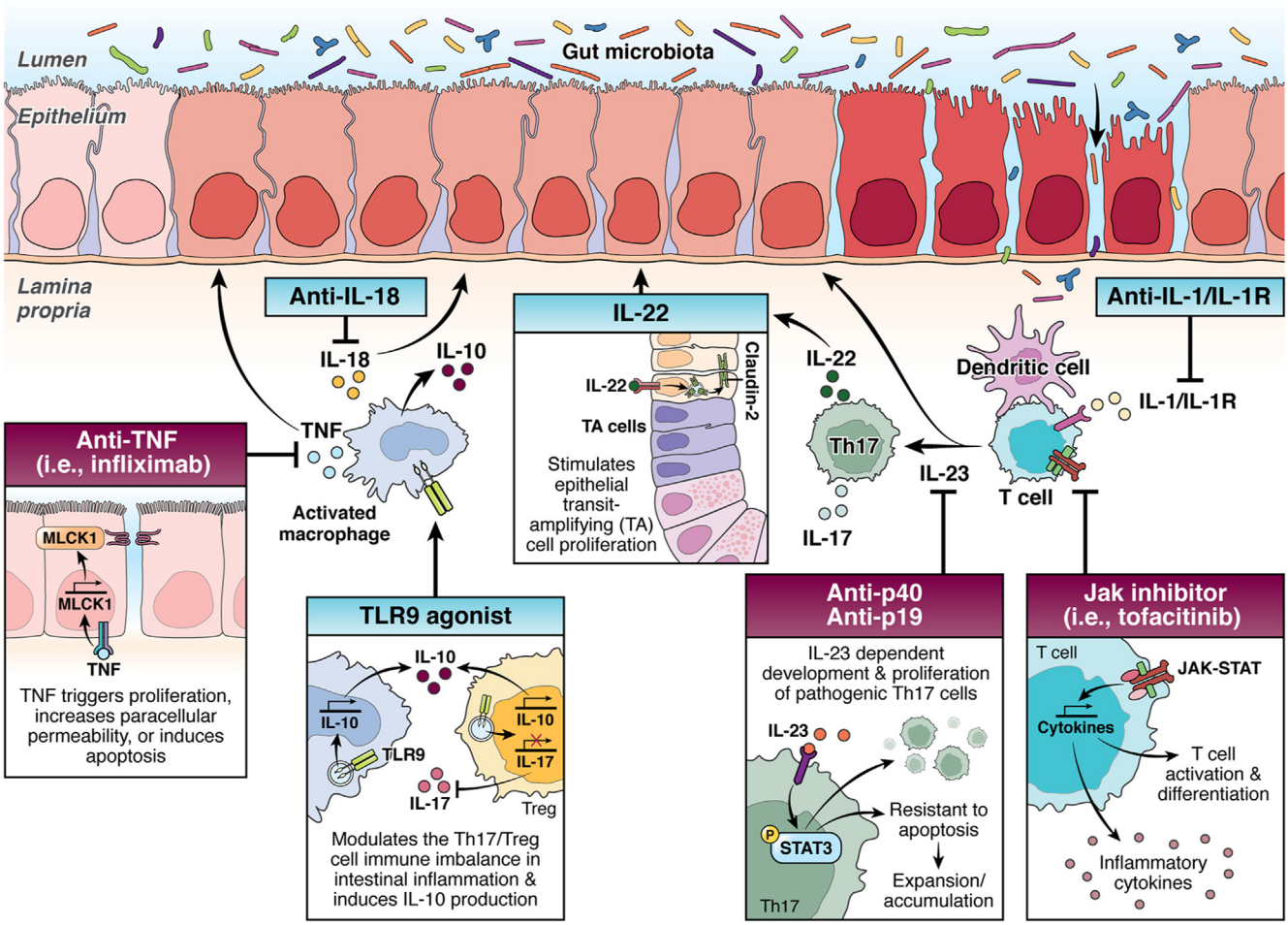

Figure 3. IBD therapies and regulation of PRRs, cytokines, and barrier function. Various current (*red boxes*) and investigational (*blue boxes*) therapies in patients with IBD that target cytokine pathways can also impact epithelial function, in some cases with unintended effects. PRR-initiated signaling leads to cytokine secretion and cytokines, which in turn cooperate with PRR pathways, ultimately affecting a wide range of intestinal cell subsets. To successfully achieve mucosal healing and remission, a multipronged approach aimed at strategically reducing inflammatory cytokine networks while simultaneously improving epithelial barrier function and maintaining beneficial PRR-mediated outcomes is necessary.

that may amplify production of chemokines, cytokines, and endothelial adhesion proteins, thereby enhancing leukocyte recruitment. This pathway may synergize with those activated by TNF, such that some patients may benefit from combination therapies targeting both TNF and oncostatin M.

Patients with IBD have increased levels of IL1β and its receptor.[262–265] There is an ongoing study of anakinra, an IL1R antagonist, in patients with acute severe UC.[266] Some children with very early–onset IBD have inherited mutations in the IL10 receptor, make increased levels of IL1β, and respond to IL1 antagonists including anakinra.[267] IL18 is an IL1 superfamily member and is also up-regulated in lamina propria mononuclear cells and serum of patients with CD.[268–271] Blocking IL18[272] and epithelial-specific deletion of IL18R or IL18[273] reduced experimental colitis severity. IL18 antagonists are being considered for CD. However, it is important to note that the NLR family has complex roles in the intestine leading to both protective[274,275] and inflammatory[276,277] effects, such that deficiency and/or blockade of NLR family members, as well as IL1β and IL18, can also promote intestinal inflammation due to key roles in regulating intestinal microbiota and epithelial integrity.[278–280] Moreover, the IBD-associated variants in the *IL18RAP/IL18R1/IL1R1* region conferring risk for IBD lead to reduced expression of these receptors in myeloid cells,[199] indicating that a reduction in these pathways can promote human IBD. Given the complexity of these pathways in intestinal inflammation, it may be necessary to identify those subsets of patients benefiting from targeting these pathways.

Therapies With Potential Adverse Effects on Mucosal Function

The IL23/Th17 pathway has been an important therapeutic target in patients with IBD. An antibody to the shared anti-IL12p40 subunit, ustekinumab, is Food and Drug Administration–approved for patients with CD and patients with UC. Multiple anti-IL23p19 agents are currently in clinical trials for patients with IBD

and demonstrate efficacy. However, the IL23/Th17 pathway has important roles in epithelial cell regulation. $IL23R^{-/-}RAG^{-/-}$ mice demonstrate more severe dextran sodium sulfate-induced colitis with delayed epithelial recovery in the context of reduced IL22.[281] Mice with IL23R deleted from epithelial cells similarly demonstrate more severe experimental colitis with reduced IL22 and reduced antimicrobial proteins (eg, Reg3β); complementation of either IL22 or Reg3β improved outcomes.[282] Therefore, it is possible that in a subset of patients, blocking IL23 may adversely impact its contributions to epithelial cells and thereby reduce therapeutic efficacy. IL17 was hypothesized to be a Th17-associated cytokine mediating the intestinal inflammation in patients with IBD. However, trials blocking either IL17 (Secukinumab)[283] or the IL17 receptor (brodalumab)[284] did not show efficacy in phase 2 trials in patients with CD. Animal studies highlighted important roles for IL17 in epithelial barrier function,[285,286] thereby suggesting that this essential role for IL17 in epithelial cell function outweighed the benefits of blocking IL17.

Administration of exogenous IL22 is also being investigated as a therapy for patients with IBD. IL22 can promote repair of epithelial damage by stimulating epithelial transit-amplifying cell proliferation in vitro and in vivo.[287,288] Moreover, IL22-induced up-regulation of claudin-2 and, as a consequence, pore pathway permeability is critical to enteric pathogen clearance.[109] However, both claudin-2 up-regulation and IL22 therapy have also been shown to promote intestinal inflammation in experimental colitis,[157,289] and sustained high levels of IL22 can both reduce stem cell numbers and promote increased susceptibility to colon cancer.[279,287] These divergent roles of IL22 highlight a complex underlying regulatory network.[126] It may, therefore, be critical to carefully titrate IL22 if it is to become an effective therapy.

As multiple different cytokines cooperate to mediate inflammation, another therapeutic approach in patients with IBD has been to target signaling pathways shared among more than 1 cytokine. The JAK-STAT pathway is one such shared pathway. JAK blockade leads to decreased T-cell activation and differentiation.[290] However, JAK blockade also has complex roles in epithelial function and in PRR-initiated pathways in innate cells where autocrine/paracrine cytokines play an important role. These might serve to counteract some of the benefits of its blockade in T cells or lead to unintended consequences. In vitro studies reducing JAK expression in innate immune cells show that low levels of reduction decrease PRR-induced proinflammatory and anti-inflammatory cytokines. However, there is a threshold of JAK expression and activation below which the reduction in anti-inflammatory cytokines results in a failure of the negative feedback loop required to suppress proinflammatory cytokines.[216,222] Therefore, as JAK inhibitor doses are increased beyond this threshold, the increased inflammatory cytokines secreted from PRR-stimulated innate cells may lead to unfavorable outcomes with increased inflammation.[216,222] In epithelial cells, JAK3 is required for optimal epithelial proliferation in vitro[291] and enterocytic and secretory epithelial lineage differentiation in vivo.[292] As such, JAK3 deficiency can lead to more severe experimental colitis.[292] Complete deletion and epithelial-specific deletion in mice of another JAK family member, TYK2, can also increase severity of experimental colitis; TYK2 deficiency leads to reduced epithelial proliferation and antimicrobial protein production in response to IL22, as well as altered intestinal microbial composition.[293] Multiple other proteins regulating epithelial proliferation[123,253,291,294] can signal through JAK proteins. In contrast to these studies, other studies have shown that pretreatment with JAK inhibitors prevents interferonγ-induced epithelial barrier permeability disruption in vitro[295] and that JAK inhibition can improve barrier function in vivo.[296] How JAK inhibitors regulate epithelial cell outcomes in patients with IBD, and how this might change with specificity of the JAK inhibitors used, has yet to be determined.

TLR Antagonists and Agonists as Therapy for IBD

Although PRRs can contribute to inflammatory pathways, they also mediate essential functions in intestinal tissues, such that therapeutic targeting of PRR signaling is challenging. TLR4 antagonists have been developed, primarily as potential treatments for sepsis.[297] TLR4 expression is increased in the intestine of patients with IBD,[298,299] and TLR4 inhibitors can reduce inflammation in some experimental colitis models but they have detrimental effects on epithelial repair.[300] In yet other reports TLR4 agonists can improve experimental colitis outcomes.[23] Likewise, TLR2 has both protective[23,301] and exacerbating[302] contributions to experimental colitis. TLR9 has similarly demonstrated mixed outcomes in regulating experimental colitis.[26,303] Studies in lamina propria mononuclear cells from patients with UC demonstrated that a TLR9 agonist, cobitolimod, modulates the Th17/Treg cell immune imbalance in intestinal inflammation and induces IL10 production by macrophages and T cells thereby reducing inflammation.[304] A phase 2b clinical trial of cobitolimod in UC showed efficacy.[305] Stimulator of interferon genes (STING) has also been shown to play a role in experimental IBD models;[306] stimulator of interferon genes inhibitors are being developed for cancer and may be considered for therapeutic repurposing for patients with IBD. For circumstances where TLR blockade may be beneficial, soluble forms of TLRs may prove to be an additional approach to attenuate signaling through TLR pathways. Signaling molecules shared across PRRs may also be promising targets for IBD,[307,308] as may direct administration of select microbial products. The challenge will be the specificity and threshold at which to modulate PRR pathways for both local intestinal regulation and systemic effects that may alter susceptibility to infections.

Summary

PRRs and their regulation of a variety of cell types can have pro- or anti-inflammatory functions in IBD. This

principle is also true with respect to cytokine signaling by immune cells or epithelial cells. Extremes in antagonism of inflammatory pathways may lead to decreased epithelial barrier function as well as microbial invasion. Using combinations of strategies directed at reducing—not eliminating—inflammatory cytokine networks and improving the leak in the epithelial barrier will be essential to achieving improved clinical outcomes in IBD.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at https://doi.org/10.1053/j.gastro.2021.12.288.

References

1. Gury-BenAri M, Thaiss CA, Serafini N, et al. The spectrum and regulatory landscape of intestinal innate lymphoid cells are shaped by the microbiome. Cell 2016;166:1231–1246.
2. Kang B, Alvarado LJ, Kim T, et al. Commensal microbiota drive the functional diversification of colon macrophages. Mucosal Immunol 2020;13:216–229.
3. Bogunovic M, Ginhoux F, Helft J, et al. Origin of the lamina propria dendritic cell network. Immunity 2009;31:513–525.
4. Mortha A, Chudnovskiy A, Hashimoto D, et al. Microbiota-dependent crosstalk between macrophages and ILC3 promotes intestinal homeostasis. Science 2014;343:1249288.
5. Lavin Y, Mortha A, Rahman A, et al. Regulation of macrophage development and function in peripheral tissues. Nat Rev Immunol 2015;15:731–744.
6. Bain CC, Bravo-Blas A, Scott CL, et al. Constant replenishment from circulating monocytes maintains the macrophage pool in the intestine of adult mice. Nat Immunol 2014;15:929–937.
7. Bain CC, Scott CL, Uronen-Hansson H, et al. Resident and pro-inflammatory macrophages in the colon represent alternative context-dependent fates of the same Ly6Chi monocyte precursors. Mucosal Immunol 2013;6:498–510.
8. Zigmond E, Varol C, Farache J, et al. Ly6C hi monocytes in the inflamed colon give rise to proinflammatory effector cells and migratory antigen-presenting cells. Immunity 2012;37:1076–1090.
9. Farro G, Stakenborg M, Gomez-Pinilla PJ, et al. CCR2-dependent monocyte-derived macrophages resolve inflammation and restore gut motility in postoperative ileus. Gut 2017;66:2098–2109.
10. Seo SU, Kuffa P, Kitamoto S, et al. Intestinal macrophages arising from CCR2(+) monocytes control pathogen infection by activating innate lymphoid cells. Nat Commun 2015;6:8010.
11. Rivollier A, He J, Kole A, et al. Inflammation switches the differentiation program of Ly6Chi monocytes from anti-inflammatory macrophages to inflammatory dendritic cells in the colon. J Exp Med 2012;209:139–155.
12. Smythies LE, Sellers M, Clements RH, et al. Human intestinal macrophages display profound inflammatory anergy despite avid phagocytic and bacteriocidal activity. J Clin Invest 2005;115:66–75.
13. Denning TL, Wang YC, Patel SR, et al. Lamina propria macrophages and dendritic cells differentially induce regulatory and interleukin 17-producing T cell responses. Nat Immunol 2007;8:1086–1094.
14. Foster SL, Hargreaves DC, Medzhitov R. Gene-specific control of inflammation by TLR-induced chromatin modifications. Nature 2007;447:972–978.
15. Hedl M, Li J, Cho JH, et al. Chronic stimulation of Nod2 mediates tolerance to bacterial products. Proc Natl Acad Sci U S A 2007;104:19440–19445.
16. Lahiri A, Abraham C. Activation of pattern recognition receptors up-regulates metallothioneins, thereby increasing intracellular accumulation of zinc, autophagy, and bacterial clearance by macrophages. Gastroenterology 2014;147:835–846.
17. Vijay-Kumar M, Aitken JD, Carvalho FA, et al. Metabolic syndrome and altered gut microbiota in mice lacking Toll-like receptor 5. Science 2010;328:228–231.
18. Vijay-Kumar M, Sanders CJ, Taylor RT, et al. Deletion of TLR5 results in spontaneous colitis in mice. J Clin Invest 2007;117:3909–3921.
19. Carvalho FA, Nalbantoglu I, Ortega-Fernandez S, et al. Interleukin-1beta (IL-1beta) promotes susceptibility of Toll-like receptor 5 (TLR5) deficient mice to colitis. Gut 2012;61:373–384.
20. Matharu KS, Mizoguchi E, Cotoner CA, et al. Toll-like receptor 4-mediated regulation of spontaneous Helicobacter-dependent colitis in IL-10-deficient mice. Gastroenterology 2009;137:1380–1390.
21. Ivanov II, Honda K. Intestinal commensal microbes as immune modulators. Cell Host Microbe 2012;12:496–508.
22. Rakoff-Nahoum S, Hao L, Medzhitov R. Role of toll-like receptors in spontaneous commensal-dependent colitis. Immunity 2006;25:319.
23. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, et al. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. Cell 2004;118:229–241.
24. Allen IC, Wilson JE, Schneider M, et al. NLRP12 suppresses colon inflammation and tumorigenesis through the negative regulation of noncanonical NF-kappaB signaling. Immunity 2012;36:742–754.
25. Chen L, Wilson JE, Koenigsknecht MJ, et al. NLRP12 attenuates colon inflammation by maintaining colonic microbial diversity and promoting protective commensal bacterial growth. Nat Immunol 2017;18:541–551.
26. Rachmilewitz D, Katakura K, Karmeli F, et al. Toll-like receptor 9 signaling mediates the anti-inflammatory effects of probiotics in murine experimental colitis. Gastroenterology 2004;126:520–528.
27. Watanabe T, Asano N, Murray PJ, et al. Muramyl dipeptide activation of nucleotide-binding oligomerization domain 2 protects mice from experimental colitis. J Clin Invest 2008;118:545–559.
28. Watanabe T, Asano N, Meng G, et al. NOD2 down-regulates colonic inflammation by IRF4-mediated

inhibition of K63-linked polyubiquitination of RICK and TRAF6. Mucosal Immunol 2014;7:1312–1325.
29. Yang Z, Fuss IJ, Watanabe T, et al. NOD2 transgenic mice exhibit enhanced MDP-mediated down-regulation of TLR2 responses and resistance to colitis induction. Gastroenterology 2007;133:1510–1521.
30. Han X, Uchida K, Jurickova I, et al. Granulocyte-macrophage colony-stimulating factor autoantibodies in murine ileitis and progressive ileal Crohn's disease. Gastroenterology 2009;136:1261–1271.
31. Caruso R, Mathes T, Martens EC, et al. A specific gene-microbe interaction drives the development of Crohn's disease-like colitis in mice. Sci Immunol 2019; 4:eaaw4341.
32. Jostins L, Ripke S, Weersma RK, et al. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. Nature 2012;491:119–124.
33. Shiloh MU, MacMicking JD, Nicholson S, et al. Phenotype of mice and macrophages deficient in both phagocyte oxidase and inducible nitric oxide synthase. Immunity 1999;10:29–38.
34. Lebeis SL, Bommarius B, Parkos CA, et al. TLR signaling mediated by MyD88 is required for a protective innate immune response by neutrophils to Citrobacter rodentium. J Immunol 2007;179:566–577.
35. Satoh-Takayama N, Vosshenrich CA, Lesjean-Pottier S, et al. Microbial flora drives interleukin 22 production in intestinal NKp46+ cells that provide innate mucosal immune defense. Immunity 2008;29:958–970.
36. Sonnenberg GF, Monticelli LA, Alenghat T, et al. Innate lymphoid cells promote anatomical containment of lymphoid-resident commensal bacteria. Science 2012; 336:1321–1325.
37. Hepworth MR, Monticelli LA, Fung TC, et al. Innate lymphoid cells regulate CD4+ T-cell responses to intestinal commensal bacteria. Nature 2013;498:113–117.
38. Buonocore S, Ahern PP, Uhlig HH, et al. Innate lymphoid cells drive interleukin-23-dependent innate intestinal pathology. Nature 2010;464:1371–1375.
39. Geremia A, Arancibia-Carcamo CV, Fleming MP, et al. IL-23-responsive innate lymphoid cells are increased in inflammatory bowel disease. J Exp Med 2011; 208:1127–1133.
40. Arpaia N, Godec J, Lau L, et al. TLR signaling is required for Salmonella Typhimurium virulence. Cell 2011; 144:675–688.
41. Sivick KE, Arpaia N, Reiner GL, et al. Toll-like receptor-deficient mice reveal how innate immune signaling influences Salmonella virulence strategies. Cell Host Microbe 2014;15:203–213.
42. Schulthess J, Pandey S, Capitani M, et al. The short chain fatty acid butyrate imprints an antimicrobial program in macrophages. Immunity 2019;50:432–445.
43. Chang PV, Hao L, Offermanns S, et al. The microbial metabolite butyrate regulates intestinal macrophage function via histone deacetylase inhibition. Proc Natl Acad Sci U S A 2014;111:2247–2252.
44. Hedl M, Abraham C. Secretory mediators regulate Nod2-mediated tolerance in human macrophages. Gastroenterology 2011;140:231–241.
45. Zheng S, Abraham C. NF-kappaB1 inhibits NOD2-induced cytokine secretion through ATF3-dependent mechanisms. Mol Cell Biol 2013;33:4857–4871.
46. Zheng S, Hedl M, Abraham C. TAM receptor-dependent regulation of SOCS3 and MAPKs contributes to proinflammatory cytokine downregulation following chronic NOD2 stimulation of human macrophages. J Immunol 2015;194:1928–1937.
47. Zheng S, Hedl M, Abraham C. Twist1 and Twist2 contribute to cytokine downregulation following chronic NOD2 stimulation of human macrophages through the coordinated regulation of transcriptional repressors and activators. J Immunol 2015;195:217–226.
48. Sun R, Hedl M, Abraham C. Twist1 and Twist2 induce human macrophage memory upon chronic innate receptor treatment by HDAC-mediated deacetylation of cytokine promoters. J Immunol 2019;202: 3297–3308.
49. Bist P, Cheong WS, Ng A, et al. E3 Ubiquitin ligase ZNRF4 negatively regulates NOD2 signalling and induces tolerance to MDP. Nat Commun 2017; 8:15865.
50. Kobayashi K, Hernandez LD, Galan JE, et al. IRAK-M is a negative regulator of Toll-like receptor signaling. Cell 2002;110:191–202.
51. Hedl M, Abraham C. Negative regulation of human mononuclear phagocyte function. Mucosal Immunol 2013;6:205–223.
52. Hitotsumatsu O, Ahmad RC, Tavares R, et al. The ubiquitin-editing enzyme A20 restricts nucleotide-binding oligomerization domain containing 2-triggered signals. Immunity 2008;28:381–390.
53. Hammer GE, Turer EE, Taylor KE, et al. Expression of A20 by dendritic cells preserves immune homeostasis and prevents colitis and spondyloarthritis. Nat Immunol 2011;12:1184–1193.
54. Danne C, Ryzhakov G, Martinez-Lopez M, et al. A large polysaccharide produced by helicobacter hepaticus induces an anti-inflammatory gene signature in macrophages. Cell Host Microbe 2017;22:733–745.
55. Hayashi A, Sato T, Kamada N, et al. A single strain of Clostridium butyricum induces intestinal IL-10-producing macrophages to suppress acute experimental colitis in mice. Cell Host Microbe 2013; 13:711–722.
56. Huang FP, Platt N, Wykes M, et al. A discrete subpopulation of dendritic cells transports apoptotic intestinal epithelial cells to T cell areas of mesenteric lymph nodes. J Exp Med 2000;191:435–444.
57. Cummings RJ, Barbet G, Bongers G, et al. Different tissue phagocytes sample apoptotic cells to direct distinct homeostasis programs. Nature 2016; 539:565–569.
58. Roberts AW, Lee BL, Deguine J, et al. Tissue-resident macrophages are locally programmed for silent clearance of apoptotic cells. Immunity 2017;47:913–927.
59. Shouval DS, Biswas A, Goettel JA, et al. Interleukin-10 receptor signaling in innate immune cells regulates mucosal immune tolerance and anti-inflammatory macrophage function. Immunity 2014;40:706–719.

60. Zigmond E, Bernshtein B, Friedlander G, et al. Macrophage-restricted interleukin-10 receptor deficiency, but not IL-10 deficiency, causes severe spontaneous colitis. Immunity 2014;40:720–733.
61. Fenton TM, Kelly A, Shuttleworth EE, et al. Inflammatory cues enhance TGFbeta activation by distinct subsets of human intestinal dendritic cells via integrin alphavbeta8. Mucosal Immunol 2017;10:624–634.
62. Aschenbrenner D, Quaranta M, Banerjee S, et al. Deconvolution of monocyte responses in inflammatory bowel disease reveals an IL-1 cytokine network that regulates IL-23 in genetic and acquired IL-10 resistance. Gut 2021;70:1023–1036.
63. Monteleone G, Kumberova A, Croft NM, et al. Blocking Smad7 restores TGF-beta1 signaling in chronic inflammatory bowel disease. J Clin Invest 2001;108:601–609.
64. Abraham C, Medzhitov R. Interactions between the host innate immune system and microbes in inflammatory bowel disease. Gastroenterology 2011;140:1729–1737.
65. Arnold IC, Mathisen S, Schulthess J, et al. CD11c(+) monocyte/macrophages promote chronic Helicobacter hepaticus-induced intestinal inflammation through the production of IL-23. Mucosal Immunol 2016;9:352–363.
66. Asquith MJ, Boulard O, Powrie F, et al. Pathogenic and protective roles of MyD88 in leukocytes and epithelial cells in mouse models of inflammatory bowel disease. Gastroenterology 2010;139:519–529.
67. Abraham C, Cho JH. Inflammatory bowel disease. N Engl J Med 2009;361:2066–2078.
68. Abraham C, Dulai PS, Vermeire S, et al. Lessons learned from trials targeting cytokine pathways in patients with inflammatory bowel diseases. Gastroenterology 2017;152:374–388.
69. Iwata M, Hirakiyama A, Eshima Y, et al. Retinoic acid imprints gut-homing specificity on T cells. Immunity 2004;21:527–538.
70. Hegazy AN, West NR, Stubbington MJT, et al. Circulating and tissue-resident CD4(+) T cells with reactivity to intestinal microbiota are abundant in healthy individuals and function is altered during inflammation. Gastroenterology 2017;153:1320–1337.
71. Weigmann B, Neurath MF. Th9 cells in inflammatory bowel diseases. Semin Immunopathol 2017;39:89–95.
72. Neurath MF. Cytokines in inflammatory bowel disease. Nat Rev Immunol 2014;14:329–342.
73. Liu H, Komai-Koma M, Xu D, et al. Toll-like receptor 2 signaling modulates the functions of CD4+ CD25+ regulatory T cells. Proc Natl Acad Sci U S A 2006;103:7048–7053.
74. Fukata M, Breglio K, Chen A, et al. The myeloid differentiation factor 88 (MyD88) is required for CD4+ T cell effector function in a murine model of inflammatory bowel disease. J Immunol 2008;180:1886–1894.
75. Negishi H, Yanai H, Nakajima A, et al. Cross-interference of RLR and TLR signaling pathways modulates antibacterial T cell responses. Nat Immunol 2012;13:659–666.
76. Pawlak M, Ho AW, Kuchroo VK. Cytokines and transcription factors in the differentiation of CD4(+) T helper cell subsets and induction of tissue inflammation and autoimmunity. Curr Opin Immunol 2020;67:57–67.
77. Han D, Walsh MC, Cejas PJ, et al. Dendritic cell expression of the signaling molecule TRAF6 is critical for gut microbiota-dependent immune tolerance. Immunity 2013;38:1211–1222.
78. Kang JW, Yan J, Ranjan K, et al. Myeloid cell expression of LACC1 is required for bacterial clearance and control of intestinal inflammation. Gastroenterology 2020;159:1051–1067.
79. Pandey SP, Yan J, Turner JR, et al. Reducing IRF5 expression attenuates colitis in mice, but impairs the clearance of intestinal pathogens. Mucosal Immunol 2019;12:874–887.
80. Diehl GE, Longman RS, Zhang JX, et al. Microbiota restricts trafficking of bacteria to mesenteric lymph nodes by CX(3)CR1(hi) cells. Nature 2013;494:116–120.
81. Flannigan KL, Ngo VL, Geem D, et al. IL-17A-mediated neutrophil recruitment limits expansion of segmented filamentous bacteria. Mucosal Immunol 2017;10:673–684.
82. Atarashi K, Tanoue T, Shima T, et al. Induction of colonic regulatory T cells by indigenous Clostridium species. Science 2011;331:337–341.
83. Ahern PP, Schiering C, Buonocore S, et al. Interleukin-23 drives intestinal inflammation through direct activity on T cells. Immunity 2010;33:279–288.
84. Lee Y, Awasthi A, Yosef N, et al. Induction and molecular signature of pathogenic TH17 cells. Nat Immunol 2012;13:991–999.
85. Britton GJ, Contijoch EJ, Mogno I, et al. Microbiotas from humans with inflammatory bowel disease alter the balance of gut Th17 and RORgammat(+) regulatory T cells and exacerbate colitis in mice. Immunity 2019;50:212–224.
86. Nagayama M, Yano T, Atarashi K, et al. TH1 cell-inducing Escherichia coli strain identified from the small intestinal mucosa of patients with Crohn's disease. Gut Microbes 2020;12:1788898.
87. Nakamoto N, Sasaki N, Aoki R, et al. Gut pathobionts underlie intestinal barrier dysfunction and liver T helper 17 cell immune response in primary sclerosing cholangitis. Nat Microbiol 2019;4:492–503.
88. Hang S, Paik D, Yao L, et al. Bile acid metabolites control TH17 and Treg cell differentiation. Nature 2019;576:143–148.
89. Furusawa Y, Obata Y, Fukuda S, et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. Nature 2013;504:446–450.
90. Sanders CJ, Yu Y, Moore DA 3rd, et al. Humoral immune response to flagellin requires T cells and activation of innate immunity. J Immunol 2006;177:2810–2818.
91. Uematsu S, Fujimoto K, Jang MH, et al. Regulation of humoral and cellular gut immunity by lamina propria dendritic cells expressing Toll-like receptor 5. Nat Immunol 2008;9:769–776.
92. Kubinak JL, Petersen C, Stephens WZ, et al. MyD88 signaling in T cells directs IgA-mediated control of the microbiota to promote health. Cell Host Microbe 2015;17:153–163.

93. Cullender TC, Chassaing B, Janzon A, et al. Innate and adaptive immunity interact to quench microbiome flagellar motility in the gut. Cell Host Microbe 2013; 14:571–581.
94. Zeng MY, Cisalpino D, Varadarajan S, et al. Gut microbiota-induced immunoglobulin G controls systemic infection by symbiotic bacteria and pathogens. Immunity 2016;44:647–658.
95. Mishima Y, Oka A, Liu B, et al. Microbiota maintain colonic homeostasis by activating TLR2/MyD88/PI3K signaling in IL-10-producing regulatory B cells. J Clin Invest 2019;129:3702–3716.
96. Clayburgh DR, Musch MW, Leitges M, et al. Coordinated epithelial NHE3 inhibition and barrier dysfunction are required for TNF-mediated diarrhea in vivo. J Clin Invest 2006;116:2682–2694.
97. Anderson JM, Van Itallie CM. Physiology and function of the tight junction. Cold Spring Harb Perspect Biol 2009; 1:a002584.
98. Turner JR. Intestinal mucosal barrier function in health and disease. Nat Rev Immunol 2009;9:799–809.
99. Weber CR, Raleigh DR, Su L, et al. Epithelial myosin light chain kinase activation induces mucosal interleukin-13 expression to alter tight junction ion selectivity. J Biol Chem 2010;285:12037–12046.
100. Weber CR, Liang GH, Wang Y, et al. Claudin-2-dependent paracellular channels are dynamically gated. Elife 2015;4:e09906.
101. Yu AS, Cheng MH, Angelow S, et al. Molecular basis for cation selectivity in claudin-2-based paracellular pores: identification of an electrostatic interaction site. J Gen Physiol 2009;133:111–127.
102. Li J, Zhuo M, Pei L, et al. Comprehensive cysteine-scanning mutagenesis reveals claudin-2 pore-lining residues with different intrapore locations. J Biol Chem 2014;289:6475–6484.
103. Zolotarevsky Y, Hecht G, Koutsouris A, et al. A membrane-permeant peptide that inhibits MLC kinase restores barrier function in in vitro models of intestinal disease. Gastroenterology 2002;123:163–172.
104. Clayburgh DR, Barrett TA, Tang Y, et al. Epithelial myosin light chain kinase-dependent barrier dysfunction mediates T cell activation-induced diarrhea in vivo. J Clin Invest 2005;115:2702–2715.
105. Marchiando AM, Shen L, Graham WV, et al. Caveolin-1-dependent occludin endocytosis is required for TNF-induced tight junction regulation in vivo. J Cell Biol 2010;189:111–126.
106. Buschmann MM, Shen L, Rajapakse H, et al. Occludin OCEL-domain interactions are required for maintenance and regulation of the tight junction barrier to macromolecular flux. Mol Biol Cell 2013;24:3056–3068.
107. Grover M, Camilleri M, Hines J, et al. 13) C mannitol as a novel biomarker for measurement of intestinal permeability. Neurogastroenterol Motil 2016;28:1114–1119.
108. 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.
109. Tsai PY, Zhang B, He WQ, et al. IL-22 upregulates epithelial claudin-2 to drive diarrhea and enteric pathogen clearance. Cell Host Microbe 2017;21:671–681.
110. Suzuki T, Yoshinaga N, Tanabe S. Interleukin-6 (IL-6) regulates claudin-2 expression and tight junction permeability in intestinal epithelium. J Biol Chem 2011; 286:31263–31271.
111. Graham WV, He W, Marchiando AM, et al. Intracellular MLCK1 diversion reverses barrier loss to restore mucosal homeostasis. Nat Med 2019;25:690–700.
112. Graham WV, Wang F, Clayburgh DR, et al. Tumor necrosis factor-induced long myosin light chain kinase transcription is regulated by differentiation-dependent signaling events. Characterization of the human long myosin light chain kinase promoter. J Biol Chem 2006; 281:26205–26215.
113. Wang J, Anders RA, Wang Y, et al. The critical role of LIGHT in promoting intestinal inflammation and Crohn's disease. J Immunol 2005;174:8173–8182.
114. 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.
115. Schwarz BT, Wang F, Shen L, et al. LIGHT signals directly to intestinal epithelia to cause barrier dysfunction via cytoskeletal and endocytic mechanisms. Gastroenterology 2007;132:2383–2394.
116. Al-Sadi R, Ye D, Dokladny K, et al. Mechanism of IL-1beta-induced increase in intestinal epithelial tight junction permeability. J Immunol 2008;180:5653–5661.
117. Howe KL, Reardon C, Wang A, et al. Transforming growth factor-beta regulation of epithelial tight junction proteins enhances barrier function and blocks enterohemorrhagic Escherichia coli O157:H7-induced increased permeability. Am J Pathol 2005; 167:1587–1597.
118. Planchon SM, Martins CA, Guerrant RL, et al. Regulation of intestinal epithelial barrier function by TGF-beta 1. Evidence for its role in abrogating the effect of a T cell cytokine. J Immunol 1994;153:5730–5739.
119. Boirivant M, Amendola A, Butera A, et al. A transient breach in the epithelial barrier leads to regulatory T-cell generation and resistance to experimental colitis. Gastroenterology 2008;135:1612–1623.
120. Edelblum KL, Shen L, Weber CR, et al. Dynamic migration of gammadelta intraepithelial lymphocytes requires occludin. Proc Natl Acad Sci U S A 2012; 109:7097–7102.
121. Dalton JE, Cruickshank SM, Egan CE, et al. Intraepithelial gammadelta+ lymphocytes maintain the integrity of intestinal epithelial tight junctions in response to infection. Gastroenterology 2006;131:818–829.
122. Jeffery V, Goldson AJ, Dainty JR, et al. IL-6 signaling regulates small intestinal crypt homeostasis. J Immunol 2017;199:304–311.
123. Kuhn KA, Manieri NA, Liu TC, et al. IL-6 stimulates intestinal epithelial proliferation and repair after injury. PLoS One 2014;9:e114195.
124. Bradford EM, Ryu SH, Singh AP, et al. Epithelial TNF receptor signaling promotes mucosal repair in

inflammatory bowel disease. J Immunol 2017;199:1886–1897.
125. Birkl D, Quiros M, Garcia-Hernandez V, et al. TNFalpha promotes mucosal wound repair through enhanced platelet activating factor receptor signaling in the epithelium. Mucosal Immunol 2019;12:909–918.
126. Ngo VL, Abo H, Maxim E, et al. A cytokine network involving IL-36gamma, IL-23, and IL-22 promotes antimicrobial defense and recovery from intestinal barrier damage. Proc Natl Acad Sci U S A 2018;115:E5076–E5085.
127. Lopetuso LR, De Salvo C, Pastorelli L, et al. IL-33 promotes recovery from acute colitis by inducing miR-320 to stimulate epithelial restitution and repair. Proc Natl Acad Sci U S A 2018;115:E9362–E9370.
128. Miyoshi H, Ajima R, Luo CT, et al. Wnt5a potentiates TGF-beta signaling to promote colonic crypt regeneration after tissue injury. Science 2012;338:108–113.
129. Quiros M, Nishio H, Neumann PA, et al. Macrophage-derived IL-10 mediates mucosal repair by epithelial WISP-1 signaling. J Clin Invest 2017;127:3510–3520.
130. Kamdar K, Johnson AMF, Chac D, et al. Innate recognition of the microbiota by TLR1 promotes epithelial homeostasis and prevents chronic inflammation. J Immunol 2018;201:230–242.
131. Hu S, Peng L, Kwak YT, et al. The DNA sensor AIM2 maintains intestinal homeostasis via regulation of epithelial antimicrobial host defense. Cell Rep 2015;13:1922–1936.
132. Longman RS, Diehl GE, Victorio DA, et al. CX(3)CR1(+) mononuclear phagocytes support colitis-associated innate lymphoid cell production of IL-22. J Exp Med 2014;211:1571–1583.
133. Brown SL, Riehl TE, Walker MR, et al. Myd88-dependent positioning of Ptgs2-expressing stromal cells maintains colonic epithelial proliferation during injury. J Clin Invest 2007;117:258–269.
134. Llewellyn SR, Britton GJ, Contijoch EJ, et al. Interactions between diet and the intestinal microbiota alter intestinal permeability and colitis severity in mice. Gastroenterology 2018;154:1037–1046.
135. Singh V, Yeoh BS, Walker RE, et al. Microbiota fermentation-NLRP3 axis shapes the impact of dietary fibres on intestinal inflammation. Gut 2019;68:1801–1812.
136. Khan S, Waliullah S, Godfrey V, et al. Dietary simple sugars alter microbial ecology in the gut and promote colitis in mice. Sci Transl Med 2020;12:eaay6218.
137. Zou J, Chassaing B, Singh V, et al. Fiber-mediated nourishment of gut microbiota protects against diet-induced obesity by restoring IL-22-mediated colonic health. Cell Host Microbe 2018;23:41–53.
138. Chassaing B, Koren O, Goodrich JK, et al. Dietary emulsifiers impact the mouse gut microbiota promoting colitis and metabolic syndrome. Nature 2015;519:92–96.
139. Tuganbaev T, Mor U, Bashiardes S, et al. Diet diurnally regulates small intestinal microbiome-epithelial-immune homeostasis and enteritis. Cell 2020;182:1441–1459.
140. Mukherji A, Kobiita A, Ye T, et al. Homeostasis in intestinal epithelium is orchestrated by the circadian clock and microbiota cues transduced by TLRs. Cell 2013;153:812–827.
141. Kuang Z, Wang Y, Li Y, et al. The intestinal microbiota programs diurnal rhythms in host metabolism through histone deacetylase 3. Science 2019;365:1428–1434.
142. Desai MS, Seekatz AM, Koropatkin NM, et al. A dietary fiber-deprived gut microbiota degrades the colonic mucus barrier and enhances pathogen susceptibility. Cell 2016;167:1339–1353.e21.
143. Kau AL, Planer JD, Liu J, et al. Functional characterization of IgA-targeted bacterial taxa from undernourished Malawian children that produce diet-dependent enteropathy. Sci Transl Med 2015;7:276ra24.
144. Thaiss CA, Levy M, Grosheva I, et al. Hyperglycemia drives intestinal barrier dysfunction and risk for enteric infection. Science 2018;359:1376–1383.
145. Fulde M, Sommer F, Chassaing B, et al. Neonatal selection by Toll-like receptor 5 influences long-term gut microbiota composition. Nature 2018;560:489–493.
146. Price AE, Shamardani K, Lugo KA, et al. A map of toll-like receptor expression in the intestinal epithelium reveals distinct spatial, cell type-specific, and temporal patterns. Immunity 2018;49:560–575.
147. Vaishnava S, Behrendt CL, Ismail AS, et al. Paneth cells directly sense gut commensals and maintain homeostasis at the intestinal host-microbial interface. Proc Natl Acad Sci U S A 2008;105:20858–20863.
148. Vaishnava S, Yamamoto M, Severson KM, et al. The antibacterial lectin RegIIIgamma promotes the spatial segregation of microbiota and host in the intestine. Science 2011;334:255–258.
149. Benjamin JL, Sumpter R Jr, Levine B, et al. Intestinal epithelial autophagy is essential for host defense against invasive bacteria. Cell Host Microbe 2013;13:723–734.
150. Sham HP, Yu EY, Gulen MF, et al. SIGIRR, a negative regulator of TLR/IL-1R signalling promotes Microbiota dependent resistance to colonization by enteric bacterial pathogens. PLoS Pathog 2013;9:e1003539.

Received August 13, 2021. Accepted December 10, 2021.

Correspondence
Address correspondence to: Clara Abraham, MD, Yale University, 333 Cedar Street, 1080 LMP, New Haven, Connecticut 06520. e-mail: clara.abraham@yale.edu.

Conflicts of interest
Maria T. Abreu has served as a trainer or lecturer for Prime CME, Janssen Pharmaceuticals, Focus Medical Communications, Cornerstones Health, Inc, and Imedex and as a consultant or advisor to Boehringer Ingelheim Pharmaceuticals, Gilead, Prometheus Biosciences, Takeda, UCB Biopharma SRL, Eli Lilly, Bellatrix Pharmaceuticals, Abbvie, and Bristol Myers Squibb. Jerrold R. Turner is a founder and shareholder of Thelium Therapeutics and has served as a consultant for Entrinsic, Immunic, Johnson & Johnson, Kallyope, and 89Bio. Clara Abraham discloses no conflicts.

Funding
Supported by Department of Defense (PR181271 to Jerrold R. Turner) and National Institutes of Health grants (R01DK61931 and R01DK68271 to Jerrold R. Turner, R01DK099097 and R01AI120369 to Clara Abraham, R01DK099076 to Maria T. Abreu) and the Harvard Digestive Disease Center (National Institutes of Health grant P30DK034854).

Supplementary References

151. Lotz M, Gutle D, Walther S, et al. Postnatal acquisition of endotoxin tolerance in intestinal epithelial cells. J Exp Med 2006;203:973–984.
152. Melmed G, Thomas LS, Lee N, et al. Human intestinal epithelial cells are broadly unresponsive to Toll-like receptor 2-dependent bacterial ligands: implications for host-microbial interactions in the gut. J Immunol 2003;170:1406–1415.
153. Lee J, Mo JH, Katakura K, et al. Maintenance of colonic homeostasis by distinctive apical TLR9 signalling in intestinal epithelial cells. Nat Cell Biol 2006; 8:1327–1336.
154. Gewirtz AT, Navas TA, Lyons S, et al. Cutting edge: bacterial flagellin activates basolaterally expressed TLR5 to induce epithelial proinflammatory gene expression. J Immunol 2001;167:1882–1885.
155. Nalle SC, Kwak HA, Edelblum KL, et al. Recipient NK cell inactivation and intestinal barrier loss are required for MHC-matched graft-versus-host disease. Sci Transl Med 2014;6:243ra87.
156. Su L, Nalle SC, Shen L, et al. TNFR2 activates MLCK-dependent tight junction dysregulation to cause apoptosis-mediated barrier loss and experimental colitis. Gastroenterology 2013;145:407–415.
157. Raju P, Shashikanth N, Tsai PY, et al. Inactivation of paracellular cation-selective claudin-2 channels attenuates immune-mediated experimental colitis in mice. J Clin Invest 2020;130:5197–5208.
158. Vetrano S, Rescigno M, Cera MR, et al. Unique role of junctional adhesion molecule-a in maintaining mucosal homeostasis in inflammatory bowel disease. Gastroenterology 2008;135:173–184.
159. Laukoetter MG, Nava P, Lee WY, et al. JAM-A regulates permeability and inflammation in the intestine in vivo. J Exp Med 2007;204:3067–3076.
160. Hollander D, Vadheim CM, Brettholz E, et al. Increased intestinal permeability in patients with Crohn's disease and their relatives. A possible etiologic factor. Ann Intern Med 1986;105:883–885.
161. May GR, Sutherland LM, Meddings JB. Lactulose/mannitol permeability is increased in relatives of patients with Crohn's disease. Gastroenterology 1992; 102:A934.
162. Turpin W, Lee SH, Raygoza Garay JA, et al. Increased intestinal permeability is associated with later development of Crohn's disease. Gastroenterology 2020; 159:2092–2100.
163. Nalle SC, Zuo L, Ong M, et al. Graft-versus-host disease propagation depends on increased intestinal epithelial tight junction permeability. J Clin Invest 2019;129:902–914.
164. Wyatt J, Vogelsang H, Hubl W, et al. Intestinal permeability and the prediction of relapse in Crohn's disease. Lancet 1993;341:1437–1439.
165. D'Inca R, Di Leo V, Corrao G, et al. Intestinal permeability test as a predictor of clinical course in Crohn's disease. Am J Gastroenterol 1999;94:2956–2960.
166. Meddings JB, Swain MG. Environmental stress-induced gastrointestinal permeability is mediated by endogenous glucocorticoids in the rat. Gastroenterology 2000;119:1019–1028.
167. Bitton A, Dobkin P, Edwardes MD, et al. Predicting relapse in Crohn's disease: a biopsychosocial model. Gut 2008;57:1386–1392.
168. Edelblum KL, Sharon G, Singh G, et al. The microbiome activates CD4 T-cell-mediated immunity to compensate for increased intestinal permeability. Cell Mol Gastroenterol Hepatol 2017;4:285–297.
169. Wu WL, Adame MD, Liou CW, et al. Microbiota regulate social behaviour via stress response neurons in the brain. Nature 2021;595:409–414.
170. Sharon G, Cruz NJ, Kang DW, et al. Human gut microbiota from autism spectrum disorder promote behavioral symptoms in mice. Cell 2019; 177:1600–1618.
171. Haber AL, Biton M, Rogel N, et al. A single-cell survey of the small intestinal epithelium. Nature 2017; 551:333–339.
172. 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.
173. Kim M, Galan C, Hill AA, et al. Critical role for the microbiota in CX3CR1(+) intestinal mononuclear phagocyte regulation of intestinal T cell responses. Immunity 2018;49:151–163.
174. Schiering C, Krausgruber T, Chomka A, et al. The alarmin IL-33 promotes regulatory T-cell function in the intestine. Nature 2014;513:564–568.
175. Monticelli LA, Osborne LC, Noti M, et al. IL-33 promotes an innate immune pathway of intestinal tissue protection dependent on amphiregulin-EGFR interactions. Proc Natl Acad Sci U S A 2015; 112:10762–10767.
176. von Moltke J, Ji M, Liang HE, et al. Tuft-cell-derived IL-25 regulates an intestinal ILC2-epithelial response circuit. Nature 2016;529:221–225.
177. Gerbe F, Sidot E, Smyth DJ, et al. Intestinal epithelial tuft cells initiate type 2 mucosal immunity to helminth parasites. Nature 2016;529:226–230.
178. Howitt MR, Lavoie S, Michaud M, et al. Tuft cells, taste-chemosensory cells, orchestrate parasite type 2 immunity in the gut. Science 2016;351:1329–1333.
179. Shan M, Gentile M, Yeiser JR, et al. Mucus enhances gut homeostasis and oral tolerance by delivering immunoregulatory signals. Science 2013; 342:447–453.
180. Gattu S, Bang YJ, Pendse M, et al. Epithelial retinoic acid receptor beta regulates serum amyloid A expression and vitamin A-dependent intestinal immunity. Proc Natl Acad Sci U S A 2019; 116:10911–10916.
181. 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.

182. Khounlotham M, Kim W, Peatman E, et al. Compromised intestinal epithelial barrier induces adaptive immune compensation that protects from colitis. Immunity 2012;37:563–573.
183. Luissint AC, Williams HC, Kim W, et al. Macrophage-dependent neutrophil recruitment is impaired under conditions of increased intestinal permeability in JAM-A-deficient mice. Mucosal Immunol 2019;12:668–678.
184. Ladinsky MS, Araujo LP, Zhang X, et al. Endocytosis of commensal antigens by intestinal epithelial cells regulates mucosal T cell homeostasis. Science 2019; 363:eaat4042.
185. Atarashi K, Tanoue T, Ando M, et al. Th17 cell induction by adhesion of microbes to intestinal epithelial cells. Cell 2015;163:367–380.
186. McDole JR, Wheeler LW, McDonald KG, et al. Goblet cells deliver luminal antigen to CD103+ dendritic cells in the small intestine. Nature 2012;483:345–349.
187. Knoop KA, McDonald KG, McCrate S, et al. Microbial sensing by goblet cells controls immune surveillance of luminal antigens in the colon. Mucosal Immunol 2015;8:198–210.
188. Niess JH, Brand S, Gu X, et al. CX3CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance. Science 2005;307:254–258.
189. Chieppa M, Rescigno M, Huang AY, et al. Dynamic imaging of dendritic cell extension into the small bowel lumen in response to epithelial cell TLR engagement. J Exp Med 2006;203:2841–2852.
190. 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.
191. Bergstrom KS, Morampudi V, Chan JM, et al. Goblet cell derived RELM-beta recruits CD4+ T cells during infectious colitis to promote protective intestinal epithelial cell proliferation. PLoS Pathog 2015;11: e1005108.
192. Giacomin PR, Moy RH, Noti M, et al. Epithelial-intrinsic IKKalpha expression regulates group 3 innate lymphoid cell responses and antibacterial immunity. J Exp Med 2015;212:1513–1528.
193. Liu JZ, van Sommeren S, Huang H, et al. Association analyses identify 38 susceptibility loci for inflammatory bowel disease and highlight shared genetic risk across populations. Nat Genet 2015;47:979–986.
194. Somineni HK, Nagpal S, Venkateswaran S, et al. Whole-genome sequencing of African Americans implicates differential genetic architecture in inflammatory bowel disease. Am J Hum Genet 2021; 108:431–445.
195. Huang H, Fang M, Jostins L, et al. Fine-mapping inflammatory bowel disease loci to single-variant resolution. Nature 2017;547:173–178.
196. 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.
197. 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.
198. Hedl M, Lahiri A, Ning K, et al. Pattern recognition receptor signaling in human dendritic cells is enhanced by ICOS ligand and modulated by the Crohn's disease ICOSLG risk allele. Immunity 2014; 40:734–746.
199. Hedl M, Zheng S, Abraham C. The IL18RAP region disease polymorphism decreases IL-18RAP/IL- 18R1/IL-1R1 expression and signaling through innate receptor-initiated pathways. J Immunol 2014; 192:5924–5932.
200. Lahiri A, Hedl M, Yan J, et al. Human LACC1 increases innate receptor-induced responses and a LACC1 disease-risk variant modulates these outcomes. Nat Commun 2017;8:15614.
201. Yan J, Hedl M, Abraham C. An inflammatory bowel disease-risk variant in INAVA decreases pattern recognition receptor-induced outcomes. J Clin Invest 2017;127:2192–2205.
202. Cadwell K, Liu JY, Brown SL, et al. A key role for autophagy and the autophagy gene Atg16l1 in mouse and human intestinal Paneth cells. Nature 2008; 456:259–263.
203. Cader MZ, Boroviak K, Zhang Q, et al. C13orf31 (FAMIN) is a central regulator of immunometabolic function. Nat Immunol 2016;17:1046–1056.
204. Ranjan K, Hedl M, Abraham C. The E3 ubiquitin ligase RNF186 and RNF186 risk variants regulate innate receptor-induced outcomes. Proc Natl Acad Sci U S A 2021;118:e2013500118.
205. Ranjan K, Hedl M, Sinha S, et al. Ubiquitination of ATF6 by disease-associated RNF186 promotes the innate receptor-induced unfolded protein response. J Clin Invest 2021;131:e145472.
206. Luong P, Hedl M, Yan J, et al. INAVA-ARNO complexes bridge mucosal barrier function with inflammatory signaling. Elife 2018;7:e38539.
207. Muise AM, Snapper SB, Kugathasan S. The age of gene discovery in very early onset inflammatory bowel disease. Gastroenterology 2012;143:285–288.
208. Muise AM, Walters T, Xu W, et al. Single nucleotide polymorphisms that increase expression of the guanosine triphosphatase RAC1 are associated with ulcerative colitis. Gastroenterology 2011;141:633–641.
209. Muise AM, Xu W, Guo CH, et al. NADPH oxidase complex and IBD candidate gene studies: identification of a rare variant in NCF2 that results in reduced binding to RAC2. Gut 2012;61:1028–1035.
210. Li Q, Lee CH, Peters LA, et al. Variants in TRIM22 that affect NOD2 signaling are associated with very-early-onset inflammatory bowel disease. Gastroenterology 2016;150:1196–1207.
211. Serra EG, Schwerd T, Moutsianas L, et al. Somatic mosaicism and common genetic variation contribute to the risk of very-early-onset inflammatory bowel disease. Nat Commun 2020;11:995.
212. Uhlig HH, Schwerd T, Koletzko S, et al. The diagnostic approach to monogenic very early onset inflammatory bowel disease. Gastroenterology 2014;147:990–1007.

213. Hedl M, Abraham C. IRF5 risk polymorphisms contribute to inter-individual variance in pattern recognition receptor-mediated cytokine secretion in human monocyte-derived cells. J Immunol 2012; 188:5348–5356.
214. Hedl M, Abraham C. A TNFSF15 disease-risk polymorphism increases pattern-recognition receptor-induced signaling through caspase-8-induced IL-1. Proc Natl Acad Sci U S A 2014;111:13451–13456.
215. Hedl M, Abraham C. A TPL2 (MAP3K8) disease-risk polymorphism increases TPL2 expression thereby leading to increased pattern recognition receptor-initiated caspase-1 and caspase-8 activation, signalling and cytokine secretion. Gut 2015;65:1799–1811.
216. Hedl M, Proctor DD, Abraham C. JAK2 disease-risk variants are gain of function and JAK signaling threshold determines innate receptor-induced proinflammatory cytokine secretion in macrophages. J Immunol 2016;197:3695–3704.
217. Hedl M, Yan J, Abraham C. IRF5 and IRF5 disease-risk variants increase glycolysis and human M1 macrophage polarization by regulating proximal signaling and Akt2 activation. Cell Rep 2016;16:2442–2455.
218. Lahiri A, Hedl M, Abraham C. MTMR3 risk allele enhances innate receptor-induced signaling and cytokines by decreasing autophagy and increasing caspase-1 activation. Proc Natl Acad Sci U S A 2015;112:10461–10466.
219. Sun R, Hedl M, Abraham C. IL23 induces IL23R recycling and amplifies innate receptor-induced signalling and cytokines in human macrophages, and the IBD-protective IL23R R381Q variant modulates these outcomes. Gut 2019;69:264–273.
220. Spalinger MR, Kasper S, Gottier C, et al. NLRP3 tyrosine phosphorylation is controlled by protein tyrosine phosphatase PTPN22. J Clin Invest 2016; 126:1783–1800.
221. Hedl M, Sun R, Huang C, et al. STAT3 and STAT5 signaling thresholds determine distinct regulation for innate receptor-induced inflammatory cytokines, and STAT3/STAT5 disease variants modulate these outcomes. J Immunol 2019;203:3325–3338.
222. Hedl M, Sun R, Abraham C. Disease risk-associated genetic variants in STAT1 and STAT4 function in a complementary manner to increase pattern-recognition receptor-induced outcomes in human macrophages. J Immunol 2020;205:1406–1418.
223. Sun R, Hedl M, Abraham C. TNFSF15 promotes antimicrobial pathways in human macrophages and these are modulated by TNFSF15 disease-risk variants. Cell Mol Gastroenterol Hepatol 2021; 11:249–272.
224. Chuang LS, Villaverde N, Hui KY, et al. A frameshift in CSF2RB predominant among Ashkenazi Jews increases risk for Crohn's Disease and reduces monocyte signaling via GM-CSF. Gastroenterology 2016; 151:710–723.
225. Scharl M, Mwinyi J, Fischbeck A, et al. Crohn's disease-associated polymorphism within the PTPN2 gene affects muramyl-dipeptide-induced cytokine secretion and autophagy. Inflamm Bowel Dis 2012; 18:900–912.
226. Spalinger MR, Sayoc-Becerra A, Santos AN, et al. PTPN2 regulates interactions between macrophages and intestinal epithelial cells to promote intestinal barrier function. Gastroenterology 2020; 159:1763–1777.
227. Castellanos JG, Woo V, Viladomiu M, et al. Microbiota-induced TNF-like ligand 1A drives Group 3 innate lymphoid cell-mediated barrier protection and intestinal T cell activation during colitis. Immunity 2018;49:1077–1089.
228. Sarin R, Wu X, Abraham C. Inflammatory disease protective R381Q IL23 receptor polymorphism results in decreased primary CD4+ and CD8+ human T-cell functional responses. Proc Natl Acad Sci U S A 2011; 108:9560–9565.
229. Di Meglio P, Di Cesare A, Laggner U, et al. The IL23R R381Q gene variant protects against immune-mediated diseases by impairing IL-23-induced Th17 effector response in humans. PLoS One 2011;6: e17160.
230. Pidasheva S, Trifari S, Phillips A, et al. Functional studies on the IBD susceptibility gene IL23R implicate reduced receptor function in the protective genetic variant R381Q. PLoS One 2011;6:e25038.
231. Yan J, Pandey SP, Barnes BJ, et al. T cell-intrinsic IRF5 regulates T cell signaling, migration, and differentiation and promotes intestinal inflammation. Cell Rep 2020;31:107820.
232. Spalinger MR, Kasper S, Chassard C, et al. PTPN2 controls differentiation of CD4(+) T cells and limits intestinal inflammation and intestinal dysbiosis. Mucosal Immunol 2015;8:918–929.
233. Manzanillo P, Mouchess M, Ota N, et al. Inflammatory bowel disease susceptibility gene C1ORF106 regulates intestinal epithelial permeability. Immunohorizons 2018;2:164–171.
234. Adolph TE, Tomczak MF, Niederreiter L, et al. Paneth cells as a site of origin for intestinal inflammation. Nature 2013;503:272–276.
235. Conway KL, Kuballa P, Song JH, et al. Atg16l1 is required for autophagy in intestinal epithelial cells and protection of mice from Salmonella infection. Gastroenterology 2013;145:1347–1357.
236. Deuring JJ, Fuhler GM, Konstantinov SR, et al. Genomic ATG16L1 risk allele-restricted Paneth cell ER stress in quiescent Crohn's disease. Gut 2014; 63:1081–1091.
237. Tschurtschenthaler M, Adolph TE, Ashcroft JW, et al. Defective ATG16L1-mediated removal of IRE1alpha drives Crohn's disease-like ileitis. J Exp Med 2017; 214:401–422.
238. Homer CR, Richmond AL, Rebert NA, et al. ATG16L1 and NOD2 interact in an autophagy- dependent, antibacterial pathway implicated in Crohn's disease pathogenesis. Gastroenterology 2010; 139:1630–1641.
239. Travassos LH, Carneiro LA, Ramjeet M, et al. Nod1 and Nod2 direct autophagy by recruiting ATG16L1 to

the plasma membrane at the site of bacterial entry. Nat Immunol 2010;11:55–62.
240. Fujimoto K, Kinoshita M, Tanaka H, et al. Regulation of intestinal homeostasis by the ulcerative colitis-associated gene RNF186. Mucosal Immunol 2017; 10:446–459.
241. Roulis M, Nikolaou C, Kotsaki E, et al. Intestinal myofibroblast-specific Tpl2-Cox-2-PGE2 pathway links innate sensing to epithelial homeostasis. Proc Natl Acad Sci U S A 2014;111:E4658–E4667.
242. Avitzur Y, Guo C, Mastropaolo LA, et al. Mutations in tetratricopeptide repeat domain 7A result in a severe form of very early onset inflammatory bowel disease. Gastroenterology 2014;146:1028–1039.
243. Muise AM, Walters TD, Glowacka WK, et al. Polymorphisms in E-cadherin (CDH1) result in a mislocalised cytoplasmic protein that is associated with Crohn's disease. Gut 2009;58:1121–1127.
244. Schwerd T, Bryant RV, Pandey S, et al. NOX1 loss-of-function genetic variants in patients with inflammatory bowel disease. Mucosal Immunol 2018;11:562–574.
245. Kaser A, Lee AH, Franke A, et al. XBP1 links ER stress to intestinal inflammation and confers genetic risk for human inflammatory bowel disease. Cell 2008; 134:743–756.
246. Scharl M, Paul G, Weber A, et al. Protection of epithelial barrier function by the Crohn's disease associated gene protein tyrosine phosphatase n2. Gastroenterology 2009;137:2030–2040.e5.
247. Scharl M, Wojtal KA, Becker HM, et al. Protein tyrosine phosphatase nonreceptor type 2 regulates autophagosome formation in human intestinal cells. Inflamm Bowel Dis 2012;18:1287–1302.
248. Spalinger MR, Manzini R, Hering L, et al. PTPN2 regulates inflammasome activation and controls onset of intestinal inflammation and colon cancer. Cell Rep 2018;22:1835–1848.
249. Cadwell K, Patel KK, Maloney NS, et al. Virus-plus-susceptibility gene interaction determines Crohn's disease gene Atg16L1 phenotypes in intestine. Cell 2010;141:1135–1145.
250. VanDussen KL, Liu TC, Li D, et al. Genetic variants synthesize to produce paneth cell phenotypes that define subtypes of Crohn's disease. Gastroenterology 2014;146:200–209.
251. Liu TC, Kern JT, VanDussen KL, et al. Interaction between smoking and ATG16L1T300A triggers Paneth cell defects in Crohn's disease. J Clin Invest 2018; 128:5110–5122.
252. Kattah MG, Shao L, Rosli YY, et al. A20 and ABIN-1 synergistically preserve intestinal epithelial cell survival. J Exp Med 2018;215:1839–1852.
253. Yamaoka T, Yan F, Cao H, et al. Transactivation of EGF receptor and ErbB2 protects intestinal epithelial cells from TNF-induced apoptosis. Proc Natl Acad Sci U S A 2008;105:11772–11777.
254. Jung H, Leal-Ekman JS, Lu Q, et al. Atg14 protects the intestinal epithelium from TNF-triggered villus atrophy. Autophagy 2019;15:1990–2001.
255. Nenci A, Becker C, Wullaert A, et al. Epithelial NEMO links innate immunity to chronic intestinal inflammation. Nature 2007;446:557–561.
256. Kaiser GC, Polk DB. Tumor necrosis factor alpha regulates proliferation in a mouse intestinal cell line. Gastroenterology 1997;112:1231–1240.
257. Mizoguchi E, Mizoguchi A, Takedatsu H, et al. Role of tumor necrosis factor receptor 2 (TNFR2) in colonic epithelial hyperplasia and chronic intestinal inflammation in mice. Gastroenterology 2002;122:134–144.
258. Popivanova BK, Kitamura K, Wu Y, et al. Blocking TNF-alpha in mice reduces colorectal carcinogenesis associated with chronic colitis. J Clin Invest 2008; 118:560–570.
259. Nguyen DX, Ehrenstein MR. Anti-TNF drives regulatory T cell expansion by paradoxically promoting membrane TNF-TNF-RII binding in rheumatoid arthritis. J Exp Med 2016;213:1241–1253.
260. Van den Brande JM, Braat H, van den Brink GR, et al. Infliximab but not etanercept induces apoptosis in lamina propria T-lymphocytes from patients with Crohn's disease. Gastroenterology 2003; 124:1774–1785.
261. West NR, Hegazy AN, Owens BMJ, et al. Oncostatin M drives intestinal inflammation and predicts response to tumor necrosis factor-neutralizing therapy in patients with inflammatory bowel disease. Nat Med 2017;23:579–589.
262. Mitsialis V, Wall S, Liu P, et al. Single-cell analyses of colon and blood reveal distinct immune cell signatures of ulcerative colitis and Crohn's disease. Gastroenterology 2020;159:591–608.e10.
263. Casini-Raggi V, Kam L, Chong YJ, et al. Mucosal imbalance of IL-1 and IL-1 receptor antagonist in inflammatory bowel disease. A novel mechanism of chronic intestinal inflammation. J Immunol 1995; 154:2434–2440.
264. Martin JC, Chang C, Boschetti G, et al. Single-cell analysis of Crohn's disease lesions identifies a pathogenic cellular module associated with resistance to anti-TNF therapy. Cell 2019;178:1493–1508.e20.
265. Ludwiczek O, Vannier E, Borggraefe I, et al. Imbalance between interleukin-1 agonists and antagonists: relationship to severity of inflammatory bowel disease. Clin Exp Immunol 2004;138:323–329.
266. Thomas MG, Bayliss C, Bond S, et al. Trial summary and protocol for a phase II randomised placebo-controlled double-blinded trial of Interleukin 1 blockade in acute severe colitis: the IASO trial. BMJ Open 2019;9:e023765.
267. Shouval DS, Biswas A, Kang YH, et al. Interleukin 1beta mediates intestinal inflammation in mice and patients with interleukin 10 receptor deficiency. Gastroenterology 2016;151:1100–1104.
268. Mokry LE, Zhou S, Guo C, et al. Interleukin-18 as a drug repositioning opportunity for inflammatory bowel disease: a Mendelian randomization study. Sci Rep 2019;9:9386.

269. Kanai T, Watanabe M, Okazawa A, et al. Interleukin 18 is a potent proliferative factor for intestinal mucosal lymphocytes in Crohn's disease. Gastroenterology 2000;119:1514–1523.
270. Monteleone G, Trapasso F, Parrello T, et al. Bioactive IL-18 expression is up-regulated in Crohn's disease. J Immunol 1999;163:143–147.
271. Pizarro TT, Michie MH, Bentz M, et al. IL-18, a novel immunoregulatory cytokine, is up-regulated in Crohn's disease: expression and localization in intestinal mucosal cells. J Immunol 1999;162:6829–6835.
272. Sivakumar PV, Westrich GM, Kanaly S, et al. Interleukin 18 is a primary mediator of the inflammation associated with dextran sulphate sodium induced colitis: blocking interleukin 18 attenuates intestinal damage. Gut 2002;50:812–820.
273. Nowarski R, Jackson R, Gagliani N, et al. Epithelial IL-18 equilibrium controls barrier function in colitis. Cell 2015;163:1444–1456.
274. Williams TM, Leeth RA, Rothschild DE, et al. The NLRP1 inflammasome attenuates colitis and colitis-associated tumorigenesis. J Immunol 2015; 194:3369–3380.
275. Yao X, Zhang C, Xing Y, et al. Remodelling of the gut microbiota by hyperactive NLRP3 induces regulatory T cells to maintain homeostasis. Nat Commun 2017; 8:1896.
276. Tye H, Yu CH, Simms LA, et al. NLRP1 restricts butyrate producing commensals to exacerbate inflammatory bowel disease. Nat Commun 2018; 9:3728.
277. Bauer C, Duewell P, Mayer C, et al. Colitis induced in mice with dextran sulfate sodium (DSS) is mediated by the NLRP3 inflammasome. Gut 2010;59:1192–1199.
278. Elinav E, Strowig T, Kau AL, et al. NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. Cell 2011;145:745–757.
279. Huber S, Gagliani N, Zenewicz LA, et al. The IL-22—IL-22BP axis is regulated by the inflammasome and modulates tumorigenesis in the intestine. Nature 2012;491:259–263.
280. Zaki MH, Boyd KL, Vogel P, et al. The NLRP3 inflammasome protects against loss of epithelial integrity and mortality during experimental colitis. Immunity 2010;32:379–391.
281. Cox JH, Kljavin NM, Ota N, et al. Opposing consequences of IL-23 signaling mediated by innate and adaptive cells in chemically induced colitis in mice. Mucosal Immunol 2012;5:99–109.
282. Aden K, Rehman A, Falk-Paulsen M, et al. Epithelial IL-23R signaling licenses protective IL-22 responses in intestinal inflammation. Cell Rep 2016;16:2208–2218.
283. Hueber W, Sands BE, Lewitzky S, et al. Secukinumab, a human anti-IL-17A monoclonal antibody, for moderate to severe Crohn's disease: unexpected results of a randomised, double-blind placebo-controlled trial. Gut 2012;61:1693–1700.
284. Mozaffari S, Nikfar S, Abdollahi M. Inflammatory bowel disease therapies discontinued between 2009 and 2014. Expert Opin Investig Drugs 2015;24:949–956.
285. Maxwell JR, Zhang Y, Brown WA, et al. Differential roles for interleukin-23 and interleukin-17 in intestinal immunoregulation. Immunity 2015;43:739–750.
286. Lee JS, Tato CM, Joyce-Shaikh B, et al. Interleukin-23-independent IL-17 production regulates intestinal epithelial permeability. Immunity 2015;43:727–738.
287. Zha JM, Li HS, Lin Q, et al. Interleukin 22 expands transit-amplifying cells while depleting Lgr5(+) stem cells via inhibition of Wnt and notch signaling. Cell Mol Gastroenterol Hepatol 2019;7:255–274.
288. Lindemans CA, Calafiore M, Mertelsmann AM, et al. Interleukin-22 promotes intestinal-stem- cell-mediated epithelial regeneration. Nature 2015;528:560–564.
289. Gunasekera DC, Ma J, Vacharathit V, et al. The development of colitis in Il10(-/-) mice is dependent on IL-22. Mucosal Immunol 2020;13:493–506.
290. O'Shea JJ, Schwartz DM, Villarino AV, et al. The JAK-STAT pathway: impact on human disease and therapeutic intervention. Annu Rev Med 2015;66: 311–328.
291. Kumar N, Mishra J, Narang VS, et al. Janus kinase 3 regulates interleukin 2-induced mucosal wound repair through tyrosine phosphorylation of villin. J Biol Chem 2007;282:30341–30345.
292. Mishra J, Verma RK, Alpini G, et al. Role of Janus kinase 3 in mucosal differentiation and predisposition to colitis. J Biol Chem 2013;288:31795–31806.
293. Hainzl E, Stockinger S, Rauch I, et al. Intestinal Epithelial cell tyrosine kinase 2 transduces IL-22 signals to protect from acute colitis. J Immunol 2015; 195:5011–5024.
294. Koon HW, Zhao D, Zhan Y, et al. Substance P stimulates cyclooxygenase-2 and prostaglandin E2 expression through JAK-STAT activation in human colonic epithelial cells. J Immunol 2006; 176:5050–5059.
295. Sayoc-Becerra A, Krishnan M, Fan S, et al. The JAK-inhibitor tofacitinib rescues human intestinal epithelial cells and colonoids from cytokine-induced barrier dysfunction. Inflamm Bowel Dis 2020;26:407–422.
296. Spalinger MR, Sayoc-Becerra A, Ordookhanian C, et al. The JAK inhibitor tofacitinib rescues intestinal barrier defects caused by disrupted epithelial-macrophage interactions. J Crohns Colitis 2021; 15:471–484.
297. Savva A, Roger T. Targeting toll-like receptors: promising therapeutic strategies for the management of sepsis-associated pathology and infectious diseases. Front Immunol 2013;4:387.
298. Cario E, Podolsky DK. Differential alteration in intestinal epithelial cell expression of toll-like receptor 3 (TLR3) and TLR4 in inflammatory bowel disease. Infect Immun 2000;68:7010–7017.
299. Fukata M, Chen A, Vamadevan AS, et al. Toll-like receptor-4 promotes the development of colitis-associated colorectal tumors. Gastroenterology 2007;133:1869–1881.
300. Ungaro R, Fukata M, Hsu D, et al. A novel Toll-like receptor 4 antagonist antibody ameliorates inflammation but impairs mucosal healing in murine colitis.

Am J Physiol Gastrointest Liver Physiol 2009; 296:G1167–G1179.

301. Cario E, Gerken G, Podolsky DK. Toll-like receptor 2 controls mucosal inflammation by regulating epithelial barrier function. Gastroenterology 2007; 132:1359–1374.
302. Shmuel-Galia L, Aychek T, Fink A, et al. Neutralization of pro-inflammatory monocytes by targeting TLR2 dimerization ameliorates colitis. EMBO J 2016; 35:685–698.
303. Obermeier F, Dunger N, Strauch UG, et al. CpG motifs of bacterial DNA essentially contribute to the perpetuation of chronic intestinal inflammation. Gastroenterology 2005;129:913–927.
304. Schmitt H, Ulmschneider J, Billmeier U, et al. The TLR9 agonist Cobitolimod induces IL10- producing wound healing macrophages and regulatory T cells in ulcerative colitis. J Crohns Colitis 2020;14:508–524.
305. Atreya R, Peyrin-Biroulet L, Klymenko A, et al. Cobitolimod for moderate-to-severe, left-sided ulcerative colitis (CONDUCT): a phase 2b randomised, double-blind, placebo-controlled, dose- ranging induction trial. Lancet Gastroenterol Hepatol 2020;5:1063–1075.
306. Ahn J, Son S, Oliveira SC, et al. STING-dependent signaling underlies IL-10 controlled inflammatory colitis. Cell Rep 2017;21:3873–3884.
307. Jun JC, Cominelli F, Abbott DW. RIP2 activity in inflammatory disease and implications for novel therapeutics. J Leukoc Biol 2013;94:927–932.
308. Wang Z, Wesche H, Stevens T, et al. IRAK-4 inhibitors for inflammation. Curr Top Med Chem 2009; 9:724–737.