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Immunology of Inflammatory Bowel Disease and Molecular Targets for Biologics

Maneesh Dave, MBBS, MPH, Konstantinos A. Papadakis, MD, PhD, and William A. Faubion Jr, MD*

Division of Gastroenterology and Hepatology, Mayo Clinic, 200 First Street Southwest, Rochester, MN 55905, USA

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INTRODUCTION

Inflammatory bowel disease (IBD), specifically Crohn disease (CD) and ulcerative colitis (UC), are autoimmune diseases whose incidence and prevalence are increasing worldwide.¹ Over the last few decades, substantial progress has been made in understanding the pathophysiology of IBD, which has been translated into newer, more effective therapies (biologics) that have reduced flares, brought more patients into remission, and improved the quality of life of patients with IBD.^{2–4} IBD is considered to be an immune-mediated disease that involves a complex pattern/interplay of host genetics and environmental influences.⁵ Our knowledge of the immune system and its homeostatic imbalance is derived from mouse models of colitis and human studies involving clinical and laboratory experiments.

The immune system evolved in multicellular organisms/metazoans as a defense mechanism against pathogens like bacteria, protozoa, parasites, and fungi.⁶ The human immune system can be broadly categorized into innate and adaptive based on the differences in timing of the response and specificity. The immune system comes in contact with a foreign challenge, which could be food, commensal flora, microbial pathogens, and xenobiotics at different sites like the skin, mucous membrane of lungs, gastrointestinal tract, and so forth. The human gastrointestinal tract, with a total surface area roughly equal to that of a tennis court (400 m²), serves as the largest area of interface with the external environment. The gut mucosal immune system, which interacts with this large antigenic load, thus, has the most varied immune cells in the body. In a disease-free host, there is a fine balance between a protective and deleterious response of the immune system, which becomes perturbed in patients with IBD. To understand these perturbations in IBD that produce a disease state, it is necessary to first understand how the intestinal immune system works. In this review, the authors divide their article into subsections of innate and adaptive immunity and link it with the currently identified abnormalities in these pathways in IBD. In addition, the authors have

*Corresponding author. faubion.william@mayo.edu.

summarized in Table 1 the current and emerging therapies for IBD that target specific molecules in the immune system.

INNATE INTESTINAL IMMUNITY

Epithelial Barrier

The gastrointestinal tract has a continuous layer of single epithelial cells that are derived from a common progenitor LGR5+ intestinal stem cell.⁷ The epithelial cells comprise enterocytes (intestinal absorptive cells), goblet cells, neuroendocrine cells, Paneth cells, and microfold (M) cells.⁷ The epithelial cells are sealed with intercellular tight junctions that serve a barrier function and regulate the trafficking of macromolecules between the luminal environment and the host.⁸ The tight junctions are composed of a meshwork of proteins like occludin, claudin family members, and the junctional adhesion molecule,⁸ with zonulin as one of the physiologic modulators (of tight junctions) that controls intestinal permeability.⁹ The luminal surface is covered by a thick layer of mucus, which is produced by goblet cells. Mucus is rich in secreted immunoglobulin A (IgA) antibodies,¹⁰ proteins with antibacterial activity (ie, α - and β -defensins),¹¹ and proteolytic and glycolytic enzymes¹² that form the first line of defense against invasion by foreign pathogens. In spite of this barrier, gut bacteria and luminal antigens do enter the subepithelial lamina propria (an extracellular matrix compartment that contains a variety of immune and nonimmune cells), with some entering through unwanted breaks but most through the specialized, follicle-associated epithelium (FAE) that overlies the organized lymphoid tissue of the gut (gut-associated lymphoid tissue [GALT]). GALT consists of organized lymphoid compartments (Peyer patches, mesenteric lymph nodes, isolated lymphoid follicles, cryptopatches) and dispersed lymphoid cells in lamina propria and intraepithelial spaces.¹³ The FAE overlies the large lymphoid aggregates called Peyer patches and, compared with other parts of intestinal epithelium, is devoid of goblet cells and has a lower concentration of brush border enzymes. The FAE also contains unique highly specialized epithelial cells called M cells that actively pinocytose or endocytose selected luminal macromolecules into underlying lymphoid follicles, thus, facilitating transepithelial transport.¹³ M cells, in comparison with enterocytes, have irregular microvilli, decreased alkaline phosphatase activity,¹⁴ and no IgA secretory component.¹⁵ Immediately below the FAE is the subepithelial dome region that overlies the lymphoid follicle containing large numbers of dendritic cells (DCs). The antigens and microorganisms transported through M cells are captured by these DCs, which then present antigenic peptides to interfollicular T cells or naïve T cells within the mesenteric lymph nodes (Fig. 1). Paneth cells are epithelial cells found at the base of the small intestinal crypt that can sense luminal microbiota and antigens to secrete antimicrobial peptides and contribute to innate host immunity.^{16,17}

Innate Immune Cells

The cells of the innate immune system, in contrast to the adaptive immune system, have a rapid and less specific response to invading microorganisms or toxic macromolecules. This response is mediated by pathogen recognition receptors (PRR), which are membrane-bound (ie, Toll-like receptors [TLRs], C-type lectin receptor) or cytoplasmic (ie, nucleotide-binding oligomerization domain family members [NODs], retinoic acid-inducible gene 1-

like receptor). These receptors are germ-line encoded, invariable, and predetermined to recognize a repertoire of bacterially/virally associated carbohydrate and lipid structures leading to the rapidity of response. The microbial molecules recognized by these receptors are called pathogen-associated molecular patterns (PAMPs) (eg, lipopolysaccharide and peptidoglycan). The PAMPs are highly conserved molecules on microbes as they are central to survival. These receptors also recognize endogenous or self-danger signals (eg, heat-shock proteins, uric acid), which are known as damage-associated molecular pattern molecules. The professional phagocytic cells (macrophages, dendritic cells and neutrophils), eosinophils, and lymphoid cells have immediate bactericidal activity; in the following paragraphs, the authors present data on these cells and their functions.

Macrophages

Macrophages are phagocytic cells present throughout the gastrointestinal mucosa, with the GALT containing the largest reservoir of macrophages within the adult mouse.¹⁸ Macrophages are derived from peripheral blood monocytes and are recruited to the intestine by chemoattractants, chemokines, and bacterial degradation products. Macrophages mature after migrating to intestinal mucosa,¹⁹ in contrast to neutrophils that are produced and lost in large numbers each day. In the noninflamed mucosa, macrophages differ from peripheral monocytes in their downregulated proinflammatory function, characterized by hyporesponsiveness to TLR ligands, diminished ability to prime adaptive immune responses, but still preserved capacity for phagocytosis and intracellular killing.¹⁹ However, in the setting of pathogen invasion and inflammation, intestinal macrophages freshly recruited from blood monocytes rapidly convert to a proinflammatory phenotype by ligation of their PRR. This proinflammatory phenotype is marked by abundant cytokine production (interleukin [IL]-1, IL-6, IL-8 and tumor necrosis factor [TNF]-alpha) and accessory cell function, instead of a primarily scavenger function. Once activated, the macrophages express receptors specific for opsonized particles and pathogens, complement, and common bacterial proteins (ie, mannose receptor, TLR, NOD). Pathogens are recognized by these receptors, which lead to their phagocytosis and subsequent intracellular killing. In addition, the activated macrophages secrete cytokines, which are proteins that affect the function of other cells in the absence of cell contact. Transforming growth factor beta (TGF- β), a cytokine produced by activated macrophages, is a potent chemoattractant for other macrophages and neutrophils and, hence, augments the recruitment of these cells to sites of inflammation.²⁰ Cytokine secretion not only augments the phagocytic intracellular killing but also serves as a critical link between the 2 arms (ie, innate and adaptive of the immune system) and plays an important role in gut homeostasis and inflammation.²¹ For simplicity, macrophages had been broadly divided into inflammatory M1 and wound-healing M2; but recently, this view is being replaced by one of considerable plasticity of macrophages wherein they acquire distinct functions of host defense, wound healing, and immune regulation based on environmental cues they encounter. So they are considered a highly heterogeneous population of cells with a continuum of activation states.^{22,23}

DCs

DCs are phagocytic cells, which, like the macrophages, originate from blood monocytes or a common DC progenitor in the bone marrow. DCs have the most potent ability to initiate

adaptive immune responses against pathogens. Current evidence supports that intestinal DCs continuously migrate in an immature or tolerogenic state, form scavenging apoptotic cells, and acquire antigens sampled either directly from the lumen²⁴ or shuttled from the lumen through M cells (see Fig. 1).²⁵ After the acquisition of antigen, DCs process it within, load the antigenic peptide onto major histocompatibility complex (MHC) class II molecules, and display this complex on its cell surface. DCs then migrate to the draining mesenteric lymph nodes where they present a particular MHC-peptide complex to T cells bearing the T cell receptor (TCR) specific for the antigen being presented. In a normal physiologic state, these antigen-loaded DCs (intestinal DCs) express low levels of costimulatory molecules and cytokines and, on contact with T cells, preferentially stimulate their differentiation into T regulatory cells (Tregs) that produce antiinflammatory cytokines IL-4, IL-10, and TGF- β . In contrast, when intestinal DCs are activated in a proinflammatory microenvironment, they migrate to T cell areas of the GALT, where they induce effector rather than tolerogenic T-cell responses. The DCs isolated from these sites express high levels of costimulatory molecules and adhesion molecules and produce large amounts of cytokines.²⁶ The intestinal DCs can then induce the mucosal homing receptor $\alpha 4\beta 7$ and the chemokine receptor CCR9 on T cells, suggesting that they can instruct a T cell to home back to the original site of the DC's activation.^{27,28}

Atypical lymphocytes

The atypical lymphocytes $\gamma\delta$ T cells and natural killer T cells (NKT) are also a part of the innate immune response. T cells normally express alpha and beta TCR chains; but when they express gamma and delta TCR chains ($\gamma\delta$ T cells), they are considered atypical lymphocytes.²⁹ These $\gamma\delta$ T cells only exist in significant numbers in the epithelial tissues and constitute greater than 10% of small intestinal intraepithelial lymphocytes, with the remaining majority being CD8+ T cells.²⁹ The $\gamma\delta$ T cells, unlike the conventional T cells, do not depend on the thymus for development³⁰ and do not recognize antigen in association with MHC class I or II.³¹ The $\gamma\delta$ T cells perform different functions, including acting as effectors against pathogens and tumors and as professional antigen-presenting cells (APCs); but their role depends on tissue distribution and the local microenvironment.^{32,33} NKT cells are a subset of T cells that mature in the thymus and recognize lipid antigen (presumably bacterial) presented on the MHC-like complex Cd1d.³⁴ On activation, they secrete large quantities of proinflammatory cytokines and readily kill infected cells or tumor cells. NKT cells that produce large amounts of proinflammatory cytokine IL-13 have been found in the intestine of patients with UC³⁵ and are also the mediators of inflammation in the oxazolone-induced murine model of colitis, which resembles UC.⁵

EVIDENCE FOR INNATE IMMUNE CELLULAR DYSFUNCTION IN THE PATHOGENESIS OF IBD

There are multiple lines of evidence that indicate a major role for innate immune cells in the perpetuation of human chronic intestinal inflammation. The most recent meta-analysis of genome-wide association studies (GWAS) in IBD has implicated multiple susceptibility genes involved in innate mucosal defense (NOD2, CARD9, REL, SLC1A) and antigen presentation (ERAP2, LNPEP). Indeed, one of the strongest genetic associations to date with

small bowel CD is the loss-of-function polymorphisms in the bacterial sensing gene CARD15/NOD2.^{36,37} The dysfunction secondary to NOD2 mutation has been reported in Paneth cells³⁸ and monocytes.³⁹ It seems that dysregulation in the detection of and/or responsiveness to enteric bacteria by innate immune and epithelial cells promotes chronic inflammation in this subgroup of patients. More evidence for their role rests in the efficacy of immune therapy targeted against proinflammatory cytokines produced by innate immune cells. Activated macrophages produce large quantities of TNF- α and IL-12, which are 2 cytokines responsible for the recruitment and activation of pathogenic effector T cells. The efficacy of anti-TNF- α therapy in IBD is well established,^{40,41} and promising anti-IL-12/23 studies are ongoing.^{42,43}

Another feature in patients with CD is a defective inflammatory response to injury and bacterial products, as evidenced by decreased neutrophil infiltration, lower production of proinflammatory interleukins (IL-8 and IL-1 β), and reduced vascular flow as compared with healthy individuals.⁴⁴ In addition to defective acute response, there is failure of clearance of bacteria and inflammatory debris, which indicates a disruption in the autophagy pathway. Autophagy (or self-eating) is a process that involves degradation and recycling of intracellular contents and removal of intracellular microbes and is mediated by lysosomes.⁴⁵ The GWAS first pointed to the role of autophagy in CD pathogenesis, with many CD-associated genetic loci lying within the category of autophagy homeostatic function (eg, ATG16L1 and IRGM).^{46,47} Homozygosity for the ATG16L1 risk allele contributes to Paneth cell dysfunction in mice and humans, but mice do not develop spontaneous intestinal inflammation. Another pathway interconnected with autophagy and that affects intestinal epithelial cells of patients with IBD is unfolded protein response (UPR) from endoplasmic reticulum (ER) stress. Most of the secreted and membrane proteins enter the ER where they fold and assemble with only properly folded proteins exiting the ER via vesicles.⁴⁸ When the unfolded proteins accumulate in the ER, it may lead to ER stress and activation of signaling pathways collectively known as UPR that help mitigate the stress.⁴⁸ Not only the intestines of patients with IBD have evidence of endoplasmic reticulum (ER) stress⁴⁹ but multiple ER stress-related genes like XBP1 have been associated with IBD.⁵⁰ Genetic deletion of XBP1 in the intestinal epithelial cells of mice results in the depletion of Paneth cells, inflammatory hyperresponsiveness to TNF- α , and spontaneous enteritis.⁵¹

EVIDENCE FOR BARRIER DYSFUNCTION AND EPITHELIAL INJURY IN THE PATHOGENESIS OF IBD

Numerous studies have shown that disruption of the epithelial barrier may either initiate or perpetuate chronic intestinal inflammation.^{46–49} Abnormal intestinal permeability has been established among patients with CD^{52,53} and their healthy first-degree relatives^{54,55} and may represent a primary abnormality predisposing to excessive antigen uptake, continuous immune stimulation, and eventually mucosal inflammation. Animal models like SAMP1/YitFc have increased intestinal permeability before the development of spontaneous ileitis,⁵⁶ and GWAS studies have identified as a CD risk locus MUC19, whose product comprises the intestinal mucous layer.⁵⁷ Barrier dysfunction has been directly observed by confocal endomicroscopy in a cohort of patients with IBD, and this dysfunction is predictive of IBD

relapse within 12 months of microscopy.⁵⁸ The etiologic factors for barrier dysfunction in CD may be environmental or genetic.⁵⁹ Diet and nonsteroidal antiinflammatory drugs can affect gut permeability and are variably associated with IBD.^{60–63} Furthermore, polymorphic variants in several IBD-associated genes (DLG5, JAK2, HNF4A) seem to primarily affect epithelial permeability and may lead to inappropriate exposure of the mucosal immune system to luminal antigens.^{50,64,65} Additionally, epithelial cell death, particularly of Paneth cells, has been demonstrated to induce terminal ileal inflammation in mice with conditional deletion of caspase-8 in the intestinal epithelium and is associated with CD in humans.⁶⁶

ADAPTIVE INTESTINAL IMMUNITY

T Cells

CD4+ and CD8+ T cells bearing the conventional $\alpha\beta$ TCR are nearly equally represented in the intestinal lamina propria. The intraepithelial space contains unusual T-cell populations that bear the $\gamma\delta$ TCR and CD8+ cells with $\alpha\alpha$ homodimeric expression of TCR. In human and experimental models of IBD, the activated CD4+ TCR $\alpha\beta$ T cells play an essential role in the disease pathogenesis. On maturation in the thymus, naïve T cells (cells that have not yet been exposed to an antigen) circulate in the lymphatic tissues in search of cognate MHC-peptide complex. Naïve T cells constitutively express the selectin CD62L (L-selectin) and the chemokine receptor 7 (CCR7), whereas the endothelial cells of high endothelial venules (HEV) in lymph nodes express vascular addressins like CD34, glycosylation-dependent cell adhesion molecule-1 (GlyCAM-1), and the chemokine ligand 21.⁶⁷ The interaction of CD62L and CD34 (among other glycosylated endothelial molecules) promotes a rolling action of the T cell across the endothelial surface. The chemokines present at the luminal surface of HEV activate the surface adhesion molecules like leukocyte function-associated antigen 1 (LFA-1) on T cells. The binding of LFA-1 (on T cells) to the receptors intercellular adhesion molecules (ICAM-1 and ICAM-2) on endothelial cells promotes firm adhesion of T cells and their crossing of the endothelial lining into the lymphoid tissue. Within the underlying lymphoid tissue are DCs loaded with antigen that also secrete chemokines like chemokine (C-C motif) ligand 19 (CCL19, a ligand for CCR7 on T cells) that retain T cells in lymph nodes. Naïve T cells, after entering the lymphoid tissue, scan the surfaces of DCs for a recognizable MHC-peptide complex. If none is found, the cells exit the lymph node, return to the circulation, and reenter other nodes to repeat the process. T cells egress the lymph node (LN) secondary to a concentration gradient of sphingosine-1-phosphate (S1P), which is kept low inside the LN and is at a high concentration in blood and efferent lymphatics. Once a naïve T cell encounters a cognate antigen through its TCR, it gets activated. The complete T-cell activation requires 2 signals: signal 1 is mediated by TCR stimulation, and signal 2 is a costimulatory signal provided by accessory pathways. Costimulation via CD28 (on T cells) and B7.1 or B7.2 (on APC) results in T-cell activation, whereas the costimulation mediated by cytotoxic T-lymphocyte antigen 4 (CTLA-4) (on T cells) by B7.1 or B7.2 results in T cell inhibition. If TCR signaling occurs in the absence of costimulation, it results in anergic T cells (ie, lack of response on reexposure to the same antigen in the future). On activation, CD4+ T cells secrete a particular set of cytokines that determine the type of ensuing immune response and the phenotype of the mature effector T

cells. New data continue to enhance our understanding of this complex event, with the most recent data summarized as follows. CD4⁺ T cells activated in the presence of IL-12 and the absence of IL-4 acquire a T helper 1 (Th1) phenotype, resulting in interferon- γ (IFN- γ)–producing cells effective in the control of intracellular pathogens. CD4⁺ T cells activated in the presence of IL-4 acquire a Th2 phenotype, generating IL-4–, IL-5–, and IL-13–producing cells effective in the clearance of parasitic infections and allergic responses. Activation in the presence of IL-6, TGF- β , and IL-23 results in cells expressing the Th17 phenotype (ie, IL-17 and IL-6 producing T cells responsible for acute inflammation and recruitment of granulocytes) (Fig. 2).^{68,69} The activation of T cells and their clonal expansion in lymphoid tissue takes 4 to 5 days; during this process, the T cells downregulate their surface S1P1 receptor to prevent egress from the LN.⁷⁰ As T-cell activation wanes and on maturation, the surface S1P1 receptor is upregulated and the effector T cells leave the lymph node returning to the circulation (higher concentration of S1P).⁷¹ On activation, T cells express unique adhesion molecules that direct the T cells to the sites of inflammation⁷²; indeed, T cells primed by mucosal DCs are destined to return to the gut through the upregulation of 2 surface molecules, $\alpha 4\beta 7$ integrin and the chemokine receptor CCR9.^{28,72} The integrin $\alpha 4$ binds to vascular adhesion molecule 1 (VCAM-1), a molecule expressed on vascular endothelium only at sites of inflammation. The $\alpha 4\beta 7$ integrin recognizes vascular endothelial molecule mucosal addressin adhesion molecule-1, which is upregulated at sites of intestinal inflammation in IBD and, hence, directs migration of effector T cells to the intestinal lamina propria.^{73,74} Within the intestinal tissue, recognition of cognate antigen by effector T cells results in the production of proinflammatory cytokines like TNF- α . TNF- α in turn stimulates endothelial cells to upregulate adhesion molecules like E-selectin (recruits monocytes and neutrophils) and VCAM-1 and ICAM-1 (both of which recruit activated T cells). In addition, TNF- α and IFN- γ alter the blood flow, endothelial cell shape, and vascular permeability to enhance the migration of inflammatory cells into the tissue. The inflammatory cascades set in motion by activated T cells lead to significant structural alterations in the inflamed tissue, including ulceration, which eventually triggers the signs and symptoms characteristic of active IBD.

Tregs

In both mice and humans, there is a subpopulation of CD4⁺ T cells with regulatory potential that restrain not only effector T cells but also innate inflammatory leukocytes.⁷⁵ Although there are many different subpopulations of Tregs, the 3 major and not necessarily mutually exclusive subtypes of Tregs are as follows: the naturally occurring CD4⁺CD25⁺ cell, the IL-10– and TGF- β –producing Tr1 cell, and the TGF- β –producing Th3 cell derived through oral tolerance.⁷⁶ The most-studied Treg cell type is the CD4⁺CD25⁺ T cell expressing the transcription factor forkhead box protein P3 (FOXP3). This cell type has been conclusively shown to suppress mucosal inflammation and murine colitis^{77,78}; however, its function in patients with IBD is less well understood, as patients with IBD have more FOXP3⁺ Tregs at the site of intestinal inflammation⁷⁹ and retain potent suppressor activity *ex vivo*.^{80,81} There is evidence that effector T cells obtained from patients with IBD are refractory to suppression by Tregs, but this area requires further study.^{82,83}

B Cells

B cells have a membrane-bound immunoglobulin that serves as the B cell receptor for an antigen. A terminally differentiated B cell called plasma cell secretes an immunoglobulin of the same antigen specificity (known as antibody). In GALT, B cells work in collaboration with the epithelium to export secretory IgA (sIgA) and, to some extent, secretory IgM to enhance the mucosal defense from intestinal pathogens. Mucosal B cells exhibit a predominant IgA class switch and, on differentiation to mature plasma cells, produce approximately 3 g of sIgA per day.⁸⁴ More than 80% of all human plasma cells are found in the gut, and nearly all of these plasma cells produce IgA.⁸⁵ Mucosal plasma cells produce primarily dimeric or polymeric forms of IgA. The joining or J chain of polymeric IgA spontaneously interacts with the polymeric immunoglobulin receptor expressed on the basolateral surface of epithelial cells facilitating exportation of the sIgA to the gastrointestinal lumen.⁸⁶ Once in the lumen, the sIgA functions mainly to inhibit the adhesion of viruses and bacteria to the epithelium by agglutinating the bacteria and other antigens, trapping them in the mucous layer, and facilitating their removal from the host. sIgA also promotes M cell-mediated bacterial uptake and target presentation to intestinal DCs and macrophages to provide a positive feedback loop to enhance secretory immunity.⁸⁷

EVIDENCE FOR ADAPTIVE IMMUNE CELLULAR DYSFUNCTION IN THE PATHOGENESIS OF IBD

A multitude of studies strongly support the view that activated CD4+ T cells are a central feature of human IBD. First, T cell-driven animal models of colitis mimic human IBD in both histologic features and responsiveness to similar treatment regimens.^{35,88–90} In human IBD, Th17 cells that secrete IL-17 and IL-22 are increased in inflamed patients with CD,^{91,92} and an IL-23 receptor variant associated with CD impairs the IL-23-induced Th17 effector function and is a protective genetic variant.⁹³ In addition, the established and emerging therapies for human IBD are directed toward the destruction of activated effector T cells or the blockade of T cell-derived proinflammatory cytokines.⁹⁴ Fourth, clonal populations of T cells (ie, expansion of T cells in response to persistent and specific antigens) have been described in patients with CD in comparison to normal controls.⁹⁵ In an animal model of IBD, similar T-cell clones that specifically recognize endogenous gut bacteria have been described.⁹⁶ These T-cell clones have pathogenic potential and mediate colitis when transferred into noncolitic recipient mice.⁹⁶ Finally, patients with IBD have antibodies directed against particular microbial antigens that point to a pathogenic adaptive immune responses against the intestinal flora.⁹⁷

PUTTING IT ALL TOGETHER: INTEGRATING GUT MICROBES, EPITHELIAL CELLS, LYMPHOCYTES, AND HOST GENETICS IN THE PATHOGENESIS OF IBD

Our knowledge of the human gut microbiota has made rapid strides in the last decade,⁹⁸ and the role of the microbiome in both immune system development and function is being increasingly understood.⁹⁹ Indeed, the presence of intestinal bacteria is required for the

development of inflammation in most animal models of IBD^{100,101}; most patients with IBD have antibodies to bacterial antigens. The data from GWAS have yielded important and comprehensive new insights into the pathogenesis of IBD (as an example autophagy pathway) and have implicated the host-microbiota interaction as the driver of disease. Fig. 3 summarizes the important susceptibility loci identified through GWAS that contribute to the unique and/or combined risk for CD and/or UC.

Similar to the innate immune cells, intestinal epithelial cells constitutively express PRRs like TLR2 and 4 to sense gut bacteria.¹⁰² The interaction between the commensal flora and TLRs enhances the integrity of the intestinal barrier and has been shown to protect against a chemical (dextran sodium sulfate [DSS]) epithelial injury model of colitis.^{103,104} The epithelial cells also have NOD1 and NOD2 (intracellular PRRs), with the NOD2 protein shown to have an antibacterial effect in infected epithelial cell lines.¹⁰⁵ Paneth cells in terminal ileum are a very abundant source of NOD2 protein; polymorphisms in NOD2, especially in Paneth cells, have been shown to decrease the production of alpha defensins by these cells.^{106,107} Paneth cells from NOD2^{-/-} mice have impaired antimicrobial function; but these mice develop granulomatous inflammation of the ileum only on inoculation with *Helicobacter hepaticus*, highlighting the role of host genetics and microbial interactions in the development of IBD.¹⁰⁸ Another classic example of gene-environment interaction in IBD is the ATG16L1 allele (T300A) variant, a CD risk-associated allele, which is present in approximately 50% of the Caucasian population but does not cause IBD in a vast majority. The ATG16L1 allele (T300 A in humans and T316A in mice) variant has been shown to affect Paneth cell function in ATG16L1 hypomorph mice (ATG16L1^{HM}) that are infected with a murine norovirus (MNV CR6).¹⁰⁹ The ATG16L1^{HM} mice derived in an enhanced barrier facility (absence of MNV CR6) do not develop Paneth cell abnormalities. The ATG16L1^{HM} mice infected with MNV CR6 only have enhanced susceptibility but do not develop spontaneous intestinal inflammation. A recent study showed that in the presence of cellular stress, caused by starvation or infection with a bacteria like *Yersinia enterocolitica*, resulted in Caspase-3-mediated degradation of the ATG16L1 protein. This degradation of ATG16L1 resulted in impaired autophagy and production of proinflammatory cytokines like TNF- α and IL-1 β .¹¹⁰ The signaling pathways downstream of TLRs, NOD2 lead to dysfunction of autophagy, ER stress response and activation of nuclear factor- κ B responsive genes that mediate inflammatory response. An excellent paper provides an in-depth review of these pathways.¹¹¹

The pathogenic and commensal flora interacts with immune cells of the GALT in multiple ways (see Fig. 1). First, infected epithelial cells may undergo apoptosis (either spontaneously or killed by effector immune cells); apoptotic fragments containing bacteria may be ingested by resident phagocytic cells (macrophages and DCs) and subsequently presented to host T cells.¹¹² Second, DCs and macrophages may acquire bacteria directly from the environment and present bacterial antigen on MHC class II to CD4⁺ T cells. Third, Tregs may contact bacterial antigens directly through their TLRs in the absence of APCs and modify their function.¹¹³ It has been demonstrated that DCs are activated in the mucosa of patients with CD, with upregulation of their PRR in contrast to controls.²⁶ In the *Tbx21*^{-/-} X *Rag 2*^{-/-} ulcerative colitis (TRUC) model, DCs that are deficient in

transcription factor T bet secrete large amounts of TNF- α which results in increased epithelial permeability and injury leading to colitis.¹¹⁴ The colitis is transmitted from the affected mother to not just her progeny but also wild-type mice (genetically intact) progeny cross-fostered from birth, thus, highlighting the role of colitogenic flora in the development of colitis.¹¹⁴

It is thought that the reduction in infectious diseases and reduced exposure to microbes in the industrialized world has led to an increase in the incidence of immunologically mediated diseases, such as IBD, type I diabetes, and asthma, over the last few decades.¹¹⁵ An elegant study has shown that germ-free (GF) mice¹¹⁶ have increased invariant NKT (iNKT) cells in colonic mucosa as compared with specific pathogen-free mice; this results in more severe oxazolone-induced colitis in GF mice, which is the opposite of murine models that depend on adaptive immunity (reduced disease severity in GF mice).¹¹⁶ This change in NKT cells is stable throughout life; in fact, colonization with conventional microbiota protects against colitis in neonatal GF mice but not in adult mice.¹¹⁶ This study highlights the role of early life exposure to microbes as important in developing adult iNKT cell tolerance.

The adaptive immune system first emerged in vertebrates.^{99,117} The main characteristics of the adaptive immune system (ie, ability to respond to a wide variety of antigens and to have long-lived memory to those antigens) also facilitated the coevolution of a diverse and more complex microbiota as compared with invertebrates.⁹⁹ A more diverse microbiota is advantageous for the vertebrates who extend their metabolic capacity (intestinal microbiota has 100 times the number of human genes) and can harvest energy from a wider selection of foods. The adaptive arm of the immune system allows tailoring of the immune response toward microbiota as either symbiosis or one of host defense. Dysregulation of this crosstalk can lead to autoimmune diseases like IBD. An example of the dynamic interaction of microbiota with adaptive immunity is in the role of Th17 cells in IBD. Patients with CD have been shown to have increased Th17 cells; hence, anti-IL-17 antibody (secukinumab) was tried as a therapeutic strategy. Unexpectedly, secukinumab resulted in the worsening of CD and more adverse events in treated patients, which was attributed to the complex biology of Th17 cells.¹¹⁸ Segmented filamentous bacteria have been shown to be potent inducers of Th17 cells¹¹⁹ that are in turn important for protecting the host from bacterial and fungal infections. In fact, the role of Th17 cells in limiting fungal infections like *Candida albicans* and the increased number of mucocutaneous candidiasis noted in the clinical trial in patients treated with anti-IL-17 antibody have been proposed as a reason for the adverse study outcomes.¹²⁰

CD4+ T cell on activation by bacterial antigen recognition interacts with B cells (CD40) through their cell-surface molecule CD40 ligand. This interaction results in the activation of B cells leading to their proliferation and initiation of antibody secretion. Subsequently a cascade of secondary events unfurls that involves production of reactive oxygen metabolites which are directly toxic to cells¹²¹ and proteolytic enzymes like matrix metalloproteinases that digest the extracellular matrix. The necrosis and tissue damage from these secondary events leads to the characteristic morphological features of IBD.

SUMMARY AND FUTURE DIRECTIONS

As discussed earlier, most of the recent advances in IBD, including biologic therapies, have resulted from studies of mucosal immunity in the normal and inflamed intestine.¹²² Both murine models of IBD and human studies have shown dysfunction of the epithelial barrier, innate immune cells, and adaptive T cells in the pathogenesis of IBD. Recent advances, including the data from GWAS and microbiome, have started to unravel the complex interaction between host genetics and environmental influences in the pathogenesis of IBD. The insight gained from the study of the aberrant immune system in IBD has led to the identification of molecular targets in the immune system for the design of drugs, some of which are already being used in clinical practice (like TNF- α antibodies and $\alpha 4$ integrin inhibitors), with many others in various phases of development. Unfortunately, most of the clinical trials in IBD have not performed extensive immune phenotyping of patients.

Despite the increased knowledge gained from the aforementioned studies, many aspects of mucosal immunity remain unclear in patients with IBD. As an example, the clinical phenotype may be similar between two patients with IBD; but the underlying aberrations in the immune system, whether it is the presence of susceptibility genes like NOD2, altered microbiota, or a primary defect in innate or adaptive immune system, could be very different. Even in the same patient, the underlying immunopathology may change over time regardless of the initial triggering events. This gap in knowledge can likely be attributed to many reasons, one of which is the limitations of available technology to study the dynamic and complex immune system of patients with IBD. In addition, IBD is identified and characterized based on morphology, an approach that has enhanced our understanding of the natural history of IBD but is also limited by its difficulty to characterize the inherent biologic variability between and within patients with IBD.

Recently, significant progress has been made in high-throughput technologies like genomic sequencing and mass cytometry (cytometry by time-of-flight [CyTOF]) that provide multiparametric data, which can be used to not just define the various immune cells states but also assess how these interact with each other in variety of states.^{123,124} The high-throughput technologies coupled with informatics and a systems immunology approach may lead to a new molecular-based definition of complex diseases like IBD.^{125–127} These approaches will enhance our understanding of the complex heterogeneity of immune cells and the immunoregulatory pathways and provide a tool to monitor and probe the aberrant immune system of patients with IBD not just at diagnosis but also during the later stages of the disease. A more detailed knowledge of the complexity of immune system in IBD will improve classification of IBD, in developing more representative animal models, and the design of new biologic therapies for a more personalized treatment approach.

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REFERENCES

1. Molodecky NA, Soon IS, Rabi DM, et al. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterology*. 2012; 142:46–54. e42. quiz: e30. [PubMed: 22001864]
2. Terdiman JP, Gruss CB, Heidelbaugh JJ, et al. American Gastroenterological Association Institute guideline on the use of thiopurines, methotrexate, and anti-TNF-alpha biologic drugs for the induction and maintenance of remission in inflammatory Crohn's disease. *Gastroenterology*. 2013; 145:1459–63. [PubMed: 24267474]
3. Vogelaar L, Spijker AV, van der Woude CJ. The impact of biologics on health-related quality of life in patients with inflammatory bowel disease. *Clin Exp Gastroenterol*. 2009; 2:101–9. [PubMed: 21694833]
4. Bernstein CN, Loftus EV Jr, Ng SC, et al. Hospitalisations and surgery in Crohn's disease. *Gut*. 2012; 61:622–9. [PubMed: 22267595]
5. Abraham C, Cho JH. Inflammatory bowel disease. *N Engl J Med*. 2009; 361:2066–78. [PubMed: 19923578]
6. Murphy, K. Janeway's immunobiology. Garland Science; New York: 2011.
7. Barker N, van Es JH, Kuipers J, et al. Identification of stem cells in small intestine and colon by marker gene Lgr5. *Nature*. 2007; 449:1003–7. [PubMed: 17934449]
8. Fasano A, Shea-Donohue T. Mechanisms of disease: the role of intestinal barrier function in the pathogenesis of gastrointestinal autoimmune diseases. *Nat Clin Pract Gastroenterol Hepatol*. 2005; 2:416–22. [PubMed: 16265432]
9. Tripathi A, Lammers KM, Goldblum S, et al. Identification of human zonulin, a physiological modulator of tight junctions, as prehaptoglobin-2. *Proc Natl Acad Sci U S A*. 2009; 106:16799–804. [PubMed: 19805376]
10. Fagarasan S, Honjo T. Intestinal IgA synthesis: regulation of front-line body defences. *Nat Rev Immunol*. 2003; 3:63–72. [PubMed: 12511876]
11. Ganz T. Defensins: antimicrobial peptides of innate immunity. *Nat Rev Immunol*. 2003; 3:710–20. [PubMed: 12949495]
12. Harwig SS, Tan L, Qu XD, et al. Bactericidal properties of murine intestinal phospholipase A2. *J Clin Invest*. 1995; 95:603–10. [PubMed: 7860744]
13. Newberry RD, Lorenz RG. Organizing a mucosal defense. *Immunol Rev*. 2005; 206:6–21. [PubMed: 16048539]
14. Owen RL, Bhalla DK. Cytochemical analysis of alkaline phosphatase and esterase activities and of lectin-binding and anionic sites in rat and mouse Peyer's patch M cells. *Am J Anat*. 1983; 168:199–212. [PubMed: 6650435]
15. Pappo J, Owen RL. Absence of secretory component expression by epithelial cells overlying rabbit gut-associated lymphoid tissue. *Gastroenterology*. 1988; 95:1173–7. [PubMed: 2458985]
16. Ayabe T, Satchell DP, Wilson CL, et al. Secretion of microbicidal alpha-defensins by intestinal Paneth cells in response to bacteria. *Nat Immunol*. 2000; 1:113–8. [PubMed: 11248802]
17. 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; 105:20858–63. [PubMed: 19075245]
18. Lee SH, Starkey PM, Gordon S. Quantitative analysis of total macrophage content in adult mouse tissues. Immunochemical studies with monoclonal antibody F4/80. *J Exp Med*. 1985; 161:475–89. [PubMed: 3973536]
19. Smith PD, Ochsenbauer-Jambor C, Smythies LE. Intestinal macrophages: unique effector cells of the innate immune system. *Immunol Rev*. 2005; 206:149–59. [PubMed: 16048547]
20. Wahl SM, Hunt DA, Wakefield LM, et al. Transforming growth factor type beta induces monocyte chemotaxis and growth factor production. *Proc Natl Acad Sci U S A*. 1987; 84:5788–92. [PubMed: 2886992]
21. Bamias G, Corridoni D, Pizarro TT, et al. New insights into the dichotomous role of innate cytokines in gut homeostasis and inflammation. *Cytokine*. 2012; 59:451–9. [PubMed: 22795953]

22. Steinbach EC, Plevy SE. The role of macrophages and dendritic cells in the initiation of inflammation in IBD. *Inflamm Bowel Dis*. 2014; 20:166–75. [PubMed: 23974993]
23. Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. *Nat Rev Immunol*. 2008; 8:958–69. [PubMed: 19029990]
24. Niess JH, Brand S, Gu X, et al. CX3CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance. *Science*. 2005; 307:254–8. [PubMed: 15653504]
25. Lelouard H, Fallet M, de Bovis B, et al. Peyer's patch dendritic cells sample antigens by extending dendrites through M cell-specific transcellular pores. *Gastroenterology*. 2012; 142:592–601. e3. [PubMed: 22155637]
26. Hart AL, Al-Hassi HO, Rigby RJ, et al. Characteristics of intestinal dendritic cells in inflammatory bowel diseases. *Gastroenterology*. 2005; 129:50–65. [PubMed: 16012934]
27. Menning A, Loddenkemper C, Westendorf AM, et al. Retinoic acid-induced gut tropism improves the protective capacity of Treg in acute but not in chronic gut inflammation. *Eur J Immunol*. 2010; 40:2539–48. [PubMed: 20690178]
28. Mora JR, Bono MR, Manjunath N, et al. Selective imprinting of gut-homing T cells by Peyer's patch dendritic cells. *Nature*. 2003; 424:88–93. [PubMed: 12840763]
29. Hayday A, Theodoridis E, Ramsburg E, et al. Intraepithelial lymphocytes: exploring the Third Way in immunology. *Nat Immunol*. 2001; 2:997–1003. [PubMed: 11685222]
30. Kanamori Y, Ishimaru K, Nanno M, et al. Identification of novel lymphoid tissues in murine intestinal mucosa where clusters of c-kit+ IL-7R+ Thy1+ lymphohemopoietic progenitors develop. *J Exp Med*. 1996; 184:1449–59. [PubMed: 8879216]
31. Groh V, Steinle A, Bauer S, et al. Recognition of stress-induced MHC molecules by intestinal epithelial gammadelta T cells. *Science*. 1998; 279:1737–40. [PubMed: 9497295]
32. Thedrez A, Sabourin C, Gertner J, et al. Self/non-self discrimination by human gammadelta T cells: simple solutions for a complex issue? *Immunol Rev*. 2007; 215:123–35. [PubMed: 17291284]
33. Carding SR, Egan PJ. Gammadelta T cells: functional plasticity and heterogeneity. *Nat Rev Immunol*. 2002; 2:336–45. [PubMed: 12033739]
34. Godfrey DI, MacDonald HR, Kronenberg M, et al. NKT cells: what's in a name? *Nat Rev Immunol*. 2004; 4:231–7. [PubMed: 15039760]
35. Fuss IJ, Heller F, Boirivant M, et al. Nonclassical CD1d-restricted NK T cells that produce IL-13 characterize an atypical Th2 response in ulcerative colitis. *J Clin Invest*. 2004; 113:1490–7. [PubMed: 15146247]
36. Ogura Y, Bonen DK, Inohara N, et al. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature*. 2001; 411:603–6. [PubMed: 11385577]
37. 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. [PubMed: 11385576]
38. Kobayashi KS, Chamaillard M, Ogura Y, et al. Nod2-dependent regulation of innate and adaptive immunity in the intestinal tract. *Science*. 2005; 307:731–4. [PubMed: 15692051]
39. Maeda S, Hsu LC, Liu H, et al. Nod2 mutation in Crohn's disease potentiates NF-kappaB activity and IL-1beta processing. *Science*. 2005; 307:734–8. [PubMed: 15692052]
40. Targan SR, Hanauer SB, van Deventer SJ, et al. A short-term study of chimeric monoclonal antibody cA2 to tumor necrosis factor alpha for Crohn's disease. Crohn's Disease cA2 Study Group. *N Engl J Med*. 1997; 337:1029–35. [PubMed: 9321530]
41. Rutgeerts P, Sandborn WJ, Feagan BG, et al. Infliximab for induction and maintenance therapy for ulcerative colitis. *N Engl J Med*. 2005; 353:2462–76. [PubMed: 16339095]
42. Orenstein R. Anti-interleukin-12 antibody for active Crohn's disease. *N Engl J Med*. 2005; 352:627–8. [PubMed: 15703432]
43. Sandborn WJ, Gasink C, Gao LL, et al. Ustekinumab induction and maintenance therapy in refractory Crohn's disease. *N Engl J Med*. 2012; 367:1519–28. [PubMed: 23075178]
44. Marks DJ, Harbord MW, MacAllister R, et al. Defective acute inflammation in Crohn's disease: a clinical investigation. *Lancet*. 2006; 367:668–78. [PubMed: 16503465]

45. Boya P, Reggiori F, Codogno P. Emerging regulation and functions of autophagy. *Nat Cell Biol.* 2013; 15:713–20. [PubMed: 23817233]
46. Rioux JD, Xavier RJ, Taylor KD, et al. Genome-wide association study identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis. *Nat Genet.* 2007; 39:596–604. [PubMed: 17435756]
47. McCarroll SA, Huett A, Kuballa P, et al. Deletion polymorphism upstream of IRGM associated with altered IRGM expression and Crohn's disease. *Nat Genet.* 2008; 40:1107–12. [PubMed: 19165925]
48. Walter P, Ron D. The unfolded protein response: from stress pathway to homeostatic regulation. *Science.* 2011; 334:1081–6. [PubMed: 22116877]
49. Treton X, Pedruzzi E, Cazals-Hatem D, et al. Altered endoplasmic reticulum stress affects translation in inactive colon tissue from patients with ulcerative colitis. *Gastroenterology.* 2011; 141:1024–35. [PubMed: 21699776]
50. Khor B, Gardet A, Xavier RJ. Genetics and pathogenesis of inflammatory bowel disease. *Nature.* 2011; 474:307–17. [PubMed: 21677747]
51. 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; 134:743–56. [PubMed: 18775308]
52. Jenkins RT, Ramage JK, Jones DB, et al. Small bowel and colonic permeability to ⁵¹Cr-EDTA in patients with active inflammatory bowel disease. *Clin Invest Med.* 1988; 11:151–5. [PubMed: 3135136]
53. Wyatt J, Oberhuber G, Pongratz S, et al. Increased gastric and intestinal permeability in patients with Crohn's disease. *Am J Gastroenterol.* 1997; 92:1891–6. [PubMed: 9382060]
54. 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; 105:883–5. [PubMed: 3777713]
55. Teahon K, Smethurst P, Levi AJ, et al. Intestinal permeability in patients with Crohn's disease and their first degree relatives. *Gut.* 1992; 33:320–3. [PubMed: 1568650]
56. Olson TS, Reuter BK, Scott KG, et al. The primary defect in experimental ileitis originates from a nonhematopoietic source. *J Exp Med.* 2006; 203:541–52. [PubMed: 16505137]
57. Jostins L, Ripke S, Weersma RK, et al. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature.* 2012; 491:119–24. [PubMed: 23128233]
58. Kiesslich R, Duckworth CA, Moussata D, et al. Local barrier dysfunction identified by confocal laser endomicroscopy predicts relapse in inflammatory bowel disease. *Gut.* 2012; 61(8):1146–53. [PubMed: 22115910]
59. McGuckin MA, Eri R, Simms LA, et al. Intestinal barrier dysfunction in inflammatory bowel diseases. *Inflamm Bowel Dis.* 2009; 15:100–13. [PubMed: 18623167]
60. Sigthorsson G, Tibble J, Hayllar J, et al. Intestinal permeability and inflammation in patients on NSAIDs. *Gut.* 1998; 43:506–11. [PubMed: 9824578]
61. Ananthakrishnan AN, Higuchi LM, Huang ES, et al. Aspirin, nonsteroidal anti-inflammatory drug use, and risk for Crohn disease and ulcerative colitis: a cohort study. *Ann Intern Med.* 2012; 156:350–9. [PubMed: 22393130]
62. Roberts CL, Keita AV, Duncan SH, et al. Translocation of Crohn's disease *Escherichia coli* across M-cells: contrasting effects of soluble plant fibres and emulsifiers. *Gut.* 2010; 59:1331–9. [PubMed: 20813719]
63. Ananthakrishnan AN, Khalili H, Konijeti GG, et al. A prospective study of long-term intake of dietary fiber and risk of Crohn's disease and ulcerative colitis. *Gastroenterology.* 2013; 145:970–7. [PubMed: 23912083]
64. Prager M, Buttner J, Haas V, et al. The JAK2 variant rs10758669 in Crohn's disease: altering the intestinal barrier as one mechanism of action. *Int J Colorectal Dis.* 2012; 27(5):565–73. [PubMed: 22065112]
65. Stoll M, Corneliussen B, Costello CM, et al. Genetic variation in DLG5 is associated with inflammatory bowel disease. *Nat Genet.* 2004; 36:476–80. [PubMed: 15107852]
66. Gunther C, Martini E, Wittkopf N, et al. Caspase-8 regulates TNF-alpha-induced epithelial necroptosis and terminal ileitis. *Nature.* 2011; 477:335–9. [PubMed: 21921917]

67. Gunn MD, Tangemann K, Tam C, et al. A chemokine expressed in lymphoid high endothelial venules promotes the adhesion and chemotaxis of naive T lymphocytes. *Proc Natl Acad Sci U S A*. 1998; 95:258–63. [PubMed: 9419363]
68. Weaver CT, Harrington LE, Mangan PR, et al. Th17: an effector CD4 T cell line-age with regulatory T cell ties. *Immunity*. 2006; 24:677–88. [PubMed: 16782025]
69. Iwakura Y, Ishigame H. The IL-23/IL-17 axis in inflammation. *J Clin Invest*. 2006; 116:1218–22. [PubMed: 16670765]
70. Matloubian M, Lo CG, Cinamon G, et al. Lymphocyte egress from thymus and peripheral lymphoid organs is dependent on S1P receptor 1. *Nature*. 2004; 427:355–60. [PubMed: 14737169]
71. Shioh LR, Rosen DB, Brdickova N, et al. CD69 acts downstream of interferon- α/β to inhibit S1P1 and lymphocyte egress from lymphoid organs. *Nature*. 2006; 440:540–4. [PubMed: 16525420]
72. Johansson-Lindbom B, Svensson M, Pabst O, et al. Functional specialization of gut CD103+ dendritic cells in the regulation of tissue-selective T cell homing. *J Exp Med*. 2005; 202:1063–73. [PubMed: 16216890]
73. Berlin C, Berg EL, Briskin MJ, et al. Alpha 4 beta 7 integrin mediates lymphocyte binding to the mucosal vascular addressin MAdCAM-1. *Cell*. 1993; 74:185–95. [PubMed: 7687523]
74. Briskin M, Winsor-Hines D, Shyjan A, et al. Human mucosal addressin cell adhesion molecule-1 is preferentially expressed in intestinal tract and associated lymphoid tissue. *Am J Pathol*. 1997; 151:97–110. [PubMed: 9212736]
75. Maloy KJ, Powrie F. Intestinal homeostasis and its breakdown in inflammatory bowel disease. *Nature*. 2011; 474:298–306. [PubMed: 21677746]
76. Shevach EM. From vanilla to 28 flavors: multiple varieties of T regulatory cells. *Immunity*. 2006; 25:195–201. [PubMed: 16920638]
77. Mottet C, Uhlig HH, Powrie F. Cutting edge: cure of colitis by CD4+CD25+ regulatory T cells. *J Immunol*. 2003; 170:3939–43. [PubMed: 12682220]
78. Sakaguchi S, Sakaguchi N, Asano M, et al. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor α -chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J Immunol*. 1995; 155:1151–64. [PubMed: 7636184]
79. Maul J, Loddenkemper C, Mundt P, et al. Peripheral and intestinal regulatory CD4+ CD25(high) T cells in inflammatory bowel disease. *Gastroenterology*. 2005; 128:1868–78. [PubMed: 15940622]
80. Saruta M, Yu QT, Fleshner PR, et al. Characterization of FOXP3+CD4+ regulatory T cells in Crohn's disease. *Clin Immunol*. 2007; 125:281–90. [PubMed: 17897887]
81. Yu QT, Saruta M, Avanesyan A, et al. Expression and functional characterization of FOXP3+ CD4+ regulatory T cells in ulcerative colitis. *Inflamm Bowel Dis*. 2007; 13:191–9. [PubMed: 17206665]
82. Fantini MC, Rizzo A, Fina D, et al. Smad7 controls resistance of colitogenic T cells to regulatory T cell-mediated suppression. *Gastroenterology*. 2009; 136:1308–16. e1–3. [PubMed: 19192480]
83. Nguyen DD, Snapper SB. Targeting Smads to restore transforming growth factor- β signaling and regulatory T-cell function in inflammatory bowel disease. *Gastroenterology*. 2009; 136:1161–4. [PubMed: 19236965]
84. Conley ME, Delacroix DL. Intravascular and mucosal immunoglobulin A: two separate but related systems of immune defense? *Ann Intern Med*. 1987; 106:892–9. [PubMed: 3579073]
85. Brandtzaeg P, Farstad IN, Johansen FE, et al. The B-cell system of human mucosae and exocrine glands. *Immunol Rev*. 1999; 171:45–87. [PubMed: 10582165]
86. Brandtzaeg P, Prydz H. Direct evidence for an integrated function of J chain and secretory component in epithelial transport of immunoglobulins. *Nature*. 1984; 311:71–3. [PubMed: 6433206]
87. Macpherson AJ, Uhr T. Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria. *Science*. 2004; 303:1662–5. [PubMed: 15016999]
88. Powrie F, Leach MW, Mauze S, et al. Inhibition of Th1 responses prevents inflammatory bowel disease in scid mice reconstituted with CD45RBhi CD4+ T cells. *Immunity*. 1994; 1:553–62. [PubMed: 7600284]

89. Sugawara K, Olson TS, Moskaluk CA, et al. Linkage to peroxisome proliferator-activated receptor-gamma in SAMP1/YitFc mice and in human Crohn's disease. *Gastroenterology*. 2005; 128:351–60. [PubMed: 15685547]
90. Totsuka T, Kanai T, Uraushihara K, et al. Therapeutic effect of anti-OX40L and anti-TNF-alpha MABs in a murine model of chronic colitis. *Am J Physiol Gastrointest Liver Physiol*. 2003; 284:10.
91. Hovhannisyan Z, Treatman J, Littman DR, et al. Characterization of interleukin-17-producing regulatory T cells in inflamed intestinal mucosa from patients with inflammatory bowel diseases. *Gastroenterology*. 2011; 140:957–65. [PubMed: 21147109]
92. Pariente B, Mocan I, Camus M, et al. Activation of the receptor NKG2D leads to production of Th17 cytokines in CD4+ T cells of patients with Crohn's disease. *Gastroenterology*. 2011; 141:217–26. 226.e1–2. [PubMed: 21600899]
93. 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. [PubMed: 21364948]
94. Danese S. New therapies for inflammatory bowel disease: from the bench to the bedside. *Gut*. 2012; 61:918–32. [PubMed: 22115827]
95. Probert CS, Chott A, Turner JR, et al. Persistent clonal expansions of peripheral blood CD4+ lymphocytes in chronic inflammatory bowel disease. *J Immunol*. 1996; 157:3183–91. [PubMed: 8816432]
96. Cong Y, Brandwein SL, McCabe RP, et al. CD4+ T cells reactive to enteric bacterial antigens in spontaneously colitic C3H/HeJBir mice: increased T helper cell type 1 response and ability to transfer disease. *J Exp Med*. 1998; 187:855–64. [PubMed: 9500788]
97. Lodes MJ, Cong Y, Elson CO, et al. Bacterial flagellin is a dominant antigen in Crohn disease. *J Clin Invest*. 2004; 113:1296–306. [PubMed: 15124021]
98. Dave M, Higgins PD, Middha S, et al. The human gut microbiome: current knowledge, challenges, and future directions. *Transl Res*. 2012; 160:246–57. [PubMed: 22683238]
99. Maynard CL, Elson CO, Hatton RD, et al. Reciprocal interactions of the intestinal microbiota and immune system. *Nature*. 2012; 489:231–41. [PubMed: 22972296]
100. Chassaing B, Darfeuille-Michaud A. The commensal microbiota and enteropathogens in the pathogenesis of inflammatory bowel diseases. *Gastroenterology*. 2011; 140:1720–8. [PubMed: 21530738]
101. Nell S, Suerbaum S, Josenhans C. The impact of the microbiota on the pathogenesis of IBD: lessons from mouse infection models. *Nat Rev Microbiol*. 2010; 8:564–77. [PubMed: 20622892]
102. Sansonetti PJ. War and peace at mucosal surfaces. *Nat Rev Immunol*. 2004; 4:953–64. [PubMed: 15573130]
103. Cario E, Gerken G, Podolsky DK. Toll-like receptor 2 enhances ZO-1-associated intestinal epithelial barrier integrity via protein kinase C. *Gastroenterology*. 2004; 127:224–38. [PubMed: 15236188]
104. 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; 118:229–41. [PubMed: 15260992]
105. Hisamatsu T, Suzuki M, Reinecker HC, et al. CARD15/NOD2 functions as an antibacterial factor in human intestinal epithelial cells. *Gastroenterology*. 2003; 124:993–1000. [PubMed: 12671896]
106. Wehkamp J, Harder J, Weichenthal M, et al. NOD2 (CARD15) mutations in Crohn's disease are associated with diminished mucosal alpha-defensin expression. *Gut*. 2004; 53:1658–64. [PubMed: 15479689]
107. Lala S, Ogura Y, Osborne C, et al. Crohn's disease and the NOD2 gene: a role for Paneth cells. *Gastroenterology*. 2003; 125:47–57. [PubMed: 12851870]
108. Biswas A, Liu YJ, Hao L, et al. Induction and rescue of Nod2-dependent Th1-driven granulomatous inflammation of the ileum. *Proc Natl Acad Sci U S A*. 2010; 107:14739–44. [PubMed: 20679225]

109. Cadwell K, Patel KK, Maloney NS, et al. Virus-plus-susceptibility gene interaction determines Crohn's disease gene Atg16L1 phenotypes in intestine. *Cell*. 2010; 141:1135–45. [PubMed: 20602997]
110. Murthy A, Li Y, Peng I, et al. A Crohn's disease variant in Atg16l1 enhances its degradation by caspase 3. *Nature*. 2014; 506:456–62. [PubMed: 24553140]
111. Hamilton MJ, Snapper SB, Blumberg RS. Update on biologic pathways in inflammatory bowel disease and their therapeutic relevance. *J Gastroenterol*. 2012; 47(1):1–8. [PubMed: 22215058]
112. Bevan MJ. Cross-priming. *Nat Immunol*. 2006; 7:363–5. [PubMed: 16550200]
113. Suttmoller RP, Morgan ME, Netea MG, et al. Toll-like receptors on regulatory T cells: expanding immune regulation. *Trends Immunol*. 2006; 27:387–93. [PubMed: 16814607]
114. Garrett WS, Lord GM, Punit S, et al. Communicable ulcerative colitis induced by T-bet deficiency in the innate immune system. *Cell*. 2007; 131:33–45. [PubMed: 17923086]
115. Bach JF. The effect of infections on susceptibility to autoimmune and allergic diseases. *N Engl J Med*. 2002; 347:911–20. [PubMed: 12239261]
116. Olszak T, An D, Zeissig S, et al. Microbial exposure during early life has persistent effects on natural killer T cell function. *Science*. 2012; 336:489–93. [PubMed: 22442383]
117. McFall-Ngai M. Adaptive immunity: care for the community. *Nature*. 2007; 445:153. [PubMed: 17215830]
118. 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; 61:1693–700. [PubMed: 22595313]
119. Ivanov II, Atarashi K, Manel N, et al. Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell*. 2009; 139:485–98. [PubMed: 19836068]
120. Colombel JF, Sendid B, Jouault T, et al. Secukinumab failure in Crohn's disease: the yeast connection? *Gut*. 2013; 62:800–1. [PubMed: 23232049]
121. Pavlick KP, Laroux FS, Fuseler J, et al. Role of reactive metabolites of oxygen and nitrogen in inflammatory bowel disease. *Free Radic Biol Med*. 2002; 33:311–22. [PubMed: 12126753]
122. Macdonald TT, Monteleone G. Immunity, inflammation, and allergy in the gut. *Science*. 2005; 307:1920–5. [PubMed: 15790845]
123. Newell EW, Sigal N, Bendall SC, et al. Cytometry by time-of-flight shows combinatorial cytokine expression and virus-specific cell niches within a continuum of CD8+ T cell phenotypes. *Immunity*. 2012; 36:142–52. [PubMed: 22265676]
124. Chattopadhyay PK, Gierahn TM, Roederer M, et al. Single-cell technologies for monitoring immune systems. *Nat Immunol*. 2014; 15:128–35. [PubMed: 24448570]
125. Kidd BA, Peters LA, Schadt EE, et al. Unifying immunology with informatics and multiscale biology. *Nat Immunol*. 2014; 15:118–27. [PubMed: 24448569]
126. Virgin HW, Todd JA. Metagenomics and personalized medicine. *Cell*. 2011; 147:44–56. [PubMed: 21962506]
127. Zitnik M, Janjic V, Larminie C, et al. Discovering disease-disease associations by fusing systems-level molecular data. *Sci Rep*. 2013; 3:3202. [PubMed: 24232732]

KEY POINTS

- Most of the recent advances in inflammatory bowel disease (IBD) have resulted from studies of mucosal immunity in the normal and inflamed intestine.
- Both murine models of IBD and human studies have shown dysfunction of the epithelial barrier, innate immune cells, and adaptive T cells in the pathogenesis of IBD.
- The insight gained from the study of the aberrant immune system in IBD has led to the identification of molecular targets in the immune system for the design of drugs, some of which are already being used in clinical practice with many others in various phases of development.
- Despite the increased knowledge gained from animal and human studies, many aspects of mucosal immunity remain unclear in patients with IBD.
- Recently, significant progress has been made in high-throughput technologies like genomic sequencing and mass cytometry that provide multiparametric data which can be used to not just define the various immune cells states but also assess how these interact with each other in a variety of conditions.

Fig. 1.

Basic organizational structure of the small intestine mucosal immune system. On the left of the figure is FAE. The main functional component of the FAE is the M cell, which is specialized in transepithelial antigen transport. DCs and antigen-presenting cells (APC) take up these transported antigens and then present them to naïve T cells. A single layer of epithelial cells that are derived from a common progenitor LGR5+ intestinal stem cell (SC) act as a barrier against the pathogens and foreign antigens. The Paneth cells are found in base of small intestine crypts and secrete antimicrobial peptides (AMP). Goblet cells secrete mucus that limits exposure of intestinal epithelium to bacteria. Immune cells present in the mucosa include macrophages, DCs, T cells, intraepithelial lymphocytes, IgA-secreting plasma cells, atypical lymphocytes, B cells, and stromal cells. The right side of the figure depicts the likely pathways through which bacterial antigens can activate mucosal T cells: (1) apoptosis of infected epithelial cells; (2) luminal sampling and acquisition of bacteria by mucosal DCs; and (3) direct contact of translocated bacteria with T cells mediated by Toll-like receptor (TLR) recognition. APCs like the DC on activation migrate through the efferent lymphatics to the mesenteric lymph nodes, where they interact with naïve T cells and induce 2 surface molecules, $\alpha 4\beta 7$ integrin and the chemokine receptor CCR9, that induce homing of T cells back to the gut (site of activation of DC). MLN, mesenteric lymph node.

Fig. 2.

T-helper (Th) cell differentiation. The naïve T cells differentiate into 3 major types of effector Th cells (Th1, Th2, and Th17). The ultimate functional phenotype of mature effector T cells depends on the type and amount of antigen and the cytokine milieu present at the time of activation. CD was traditionally thought to be the result of aberrant Th1 responses because of increased mucosal levels of interferon γ (IFN- γ), IL-2, and IL-12. However, recent evidence has supported a prominent role for a second Th subset, Th17, as IL-17 and IL-22 are increased in inflamed mucosa of patients with CD. Similarly, UC was thought to be a Th2-mediated disease because of increased mucosal levels of IL-5 and IL-13. However, patients with UC exhibit decreased production of mucosal IL-4 (Th2 cytokine), and natural killer T cells may be responsible for the aberrant production of IL-13. APC, antigen-presenting cell; IL, interleukins; TGF- β , transforming growth factor- β .

Fig. 3. The illustration shows a model for IBD pathways based on GWAS. Genes that are in linkage disequilibrium with GWAS identified single nucleotide polymorphisms (associated with IBD) were classified according to their function in intestinal inflammation. Text color indicates whether the genes are linked to risk loci associated with CD (*black*), UC (*blue*), or both (*red*). The asterisk denotes corresponding coding mutations; cis-eQTL effects are underlined. G, goblet cell; P, Paneth cell. (From Khor B, Gardet A, Xavier RJ. Genetics and pathogenesis of inflammatory bowel disease. Nature 2011;474(7351):309; with permission.)

Table 1

Some of the key biologic molecules in active use or under study for treatment of IBD

Biologic Target	Antibody/Drug	Mechanism of Action	CD, UC, or Both
CCR9	CCX282-B	Inhibition of CCR9	CD
	CCX 025	Inhibition of CCR9	CD
IL-21	PF 05230900	IL-21 receptor antagonist	CD
IL-13	QAX576	IL-13 antagonist	CD
	Anrukinzumab	IL-13 antagonist	UC
	Tralokinumab	IL-13 antagonist	UC
IL-17	Vidofludimus	Inhibitor of IL-17 A and IL-17F	Both
IL-12/23	Ustekinumab	Blockade of IL-12/23	CD
IL-18	GSK1070806	Blockade of soluble IL-18	CD
IL-6 and IL-6R	Tocilizumab	Inhibitor of IL -6	CD
	PF04236921	Inhibitor of IL -6	CD
IP-10	MDX 1100	Blockade of interferon- γ inducible protein (IP-10 or CXCL10)	UC
IRAK4/TRAF6/MyD88	RDP58	Disrupts IRAK4/TRAF6/MyD88 signaling and reduces production of proinflammatory cytokines	Both
JAK3	Tofacitinib	Inhibition of JAK3	Both
MAdCAM-1	PF-547659	Blocks MAdCAM-1	Both
NF- κ B	HE3286	Synthetic steroid that modulates NF- κ B activity	UC
NKG2D	NN8555	Anti-NKG2D receptor monoclonal antibody	CD
PKC	AEB071/Sotrastaurin	PKC inhibitor	UC
T Cell	Laquinimod	Reduces IL-17 level and interferes with migration of T cells	CD
TLR	DIMS0150	Blockade of Toll-like receptor	UC
	BL-7040	Blockade of Toll-like receptor	UC
TNF- α	Infliximab	Neutralization of TNF- α	Both
	Adalimumab	Neutralization of TNF- α	Both
	Certolizumab pegol	Neutralization of TNF- α	CD
	Golimumab	Neutralization of TNF- α	UC
	Debiaerse	Vaccine against TNF- α consisting of a TNF- α derivative TNF- α kinoid	CD
Effector T cells, B cells	Antigen specific Type 1 regulatory cells (OvaSave)	Autologous ova expanded regulatory T cells injected	CD

Biologic Target	Antibody/Drug	Mechanism of Action	CD, UC, or Both
$\alpha 4$ integrin	AJM-300	Blockade of $\alpha 4$ integrin	CD
$\alpha 4$ integrin	Natalizumab	Blockade of $\alpha 4$ integrin	Both
$\alpha 4\beta 7$ integrin	Vedolizumab	Blockade of $\alpha 4\beta 7$ integrin	Both
$\beta 7$ integrin	Etrolizumab (aka rHuMab $\beta 7$)	Anti- $\beta 7$ integrin	UC

Abbreviations: aka, also known as; CCR9, chemokine receptor 9; IL, interleukin; IP, inducible protein; IRAK-4, interleukin-1 receptor-associated kinase 4; JAK3, Janus kinase 3; MAdCAM-1, human mucosal addressin cell adhesion molecule-1; MyD88, myeloid differentiation primary response 88; NF- κ B, nuclear factor- κ B; PKC, protein kinase C; TLR, Toll-like receptor; TNF- α , tumor necrosis factor- α ; TRAF6, TNF receptor-associated factor 6, E3 ubiquitin protein ligase.