REVIEWS

Unravelling the pathogenesis of inflammatory bowel disease

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Recently, substantial advances in the understanding of the molecular pathogenesis of inflammatory bowel disease (IBD) have been made owing to three related lines of investigation. First, IBD has been found to be the most tractable of complex disorders for discovering susceptibility genes, and these have shown the importance of epithelial barrier function, and innate and adaptive immunity in disease pathogenesis. Second, efforts directed towards the identification of environmental factors implicate commensal bacteria (or their products), rather than conventional pathogens, as drivers of dysregulated immunity and IBD. Third, murine models, which exhibit many of the features of ulcerative colitis and seem to be bacteria-driven, have helped unravel the pathogenesis/mucosal immunopathology of IBD.

The major forms of idiopathic IBD, ulcerative colitis and Crohn's disease are chronic inflammatory disorders of the gastrointestinal tract that have been empirically defined by clinical, pathological, endoscopic and radiological features[1]. The onset of IBD typically occurs in the second and third decades of life and a majority of affected individuals progress to relapsing and chronic disease. Family aggregation has long been recognized. First-degree relatives of affected individuals have a relative risk of fivefold or greater. The inheritable component seems stronger in Crohn's disease than in ulcerative colitis[2,3]. It is of interest that in several countries with historically low rates of IBD, a pattern of rising incidence in the past one to two decades, particularly for Crohn's disease, has occurred, suggesting that environmental factors are also involved.

Key features of ulcerative colitis include diffuse mucosal inflammation that extends proximally from the rectum to a varying degree. In conjunction with severe inflammation and the coincident production of a complex mixture of inflammatory mediators, extensive superficial mucosal ulceration develops. Histopathological features include the presence of a significant number of neutrophils within the lamina propria and the crypts, where they form micro-abscesses (Fig. 1). Depletion of goblet cell mucin is also common. Crohn's disease is characterized by aggregation of macrophages that frequently form non-caseating granulomas (Fig. 1). Although any site of the gastrointestinal tract may be affected, involvement of the terminal ileum is most common and the earliest mucosal lesions in Crohn's disease often appear over Peyer's patches. Unlike ulcerative colitis, Crohn's disease may be patchy and segmental, and inflammation typically transmural.

IBD genetics

Genome-wide searches for IBD susceptibility loci performed in the last few years have been highly successful in identifying genes that contribute to disease susceptibility. In initial screening efforts, two groups identified *NOD2* (also designated *CARD15* and *IBD1*) as a susceptibility gene in Crohn's disease, using positional cloning and candidate gene approaches[4,5]. Since then, several additional susceptibility loci have been implicated in inflammatory bowel disease and confirmed by replication: *IBD5*, *IL23R* and *ATG16L1* (refs 6–14). (See Fig. 2 for the full list of genes validated in multiple studies as well as those genes that require additional confirmation). The genetic variants that have been found to confer Crohn's disease risk point to the importance of innate immunity, autophagy and phagocytosis in Crohn's disease pathogenesis. In particular, a number of genes associated with Crohn's disease (*IL23R*, *PTPN2*) are also associated with other autoimmune disorders, suggesting that a subset of Crohn's disease patients share common triggers with these conditions. In addition, multiple disease-associated intergenic segments have been identified and replicated in genome-wide association studies. These intergenic regions implicate new genes and pathways—possibly including genes that are expressed within these regions and others that are remotely regulated to modify the disease phenotype. Further understanding of regulatory elements within non-coding genomic regions and gene–gene interactions will lead to a better understanding of the underlying mechanisms that cause disease. Despite early linkage analysis suggesting an important contribution of the MHC complex to IBD susceptibility, in contrast to rheumatoid arthritis

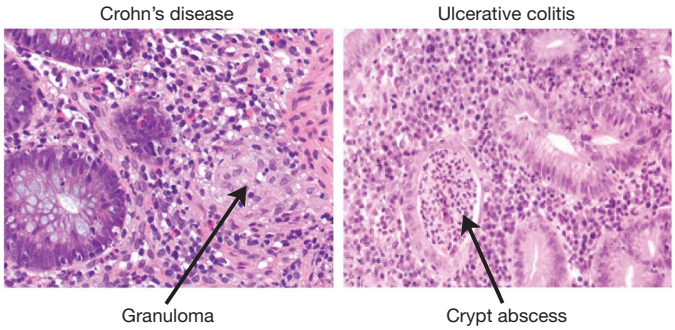

Figure 1 | Histologic hallmarks of IBD: clues to immunopathogenesis. Left panel, Crohn's disease—biopsy from a terminal ileum with active disease. The figure illustrates a discrete granuloma composed of compact macrophages, giant cells and epithelioid cells. Surrounding the nodule there is marked infiltration of lymphoid cells, plasma cells and other inflammatory cells, but there is no necrosis. Right panel, Ulcerative colitis—colonic mucosal biopsy taken from a patient with active disease. The crypt abscess is composed of transmigrated neutrophils and the surrounding epithelium exhibits features of acute mucosal injury.

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and multiple sclerosis, identification of precise genes within the MHC region that confer susceptibility has been problematic. Individual risk genes are discussed below within the context of a consideration of pathophysiologic mechanisms.

Microbes control development of IBD in a susceptible host

Accumulating evidence suggests that the dynamic balance between microbes, particularly commensal flora, and host defensive responses at the mucosal frontier has a pivotal role in the initiation and pathogenesis of chronic IBD. Circumstantial evidence for this inference is provided by the observed therapeutic benefits of antibiotic treatment in, at least, subsets of IBD patient and recent findings suggesting that so-called 'healthy bacteria' or probiotic combinations can ameliorate IBD[15,16]. In addition, the enteric flora of IBD patients has been found more commonly than in control patient groups[17,18] to include strains of *Escherichia coli* that are able to adhere to the epithelium, and with low frequency effect epithelial invasion. The importance of the flora is more directly supported by studies in a variety of murine strains in which 'spontaneous' chronic colitis seems to be entirely dependent on the presence of a luminal flora. Thus, colitis is not observed when any of several of these lines are maintained in a gnotobiotic state, but rapidly emerges when they are reconstituted with bacteria that are considered normal constituents of luminal flora[19,20]. In some instances, it has been possible to induce colitis in a susceptible murine strain with a single species of normal bacteria, for example, *Bacteroides vulgatus* in the *Il10*-deficient mouse[21]. These studies provide compelling evidence that the nature of the host defenses, rather than the biological properties of a luminal bacterial species *per se*, may determine the functional outcome of that interaction.

Unfortunately, our understanding of the microbial flora itself is quite incomplete[22]. Insights into the microbial–host interrelationships are hampered by both the limited knowledge of the diversity and complexity of the microbial flora and the limitation of available tools to delineate these characteristics. Metagenomic and computational analysis of the so-called microbiome may provide a foundation to achieve an understanding of the relevant, functional diversity of the flora in the context of IBD. Understanding the distribution, dynamics and responses to microbial flora in these disease states will probably provide insight into the regional distribution of disease.

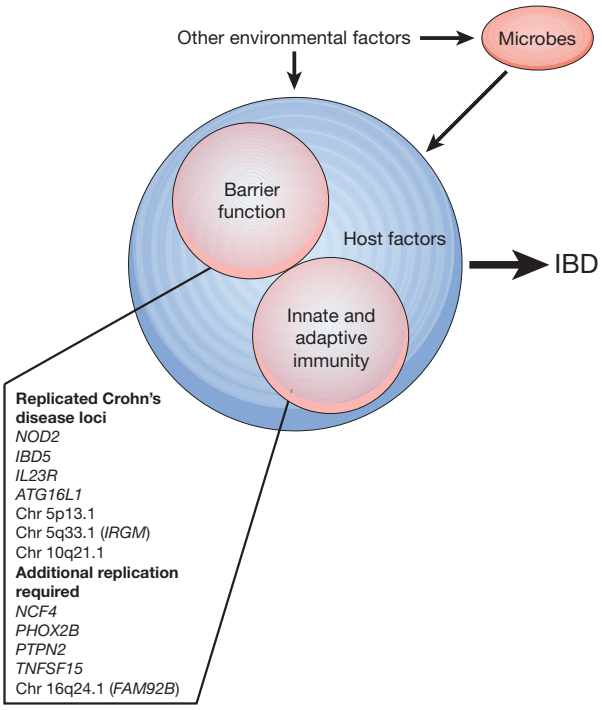


Figure 2 | Patterns of IBD etiopathogenesis. Intestinal inflammation in IBD results from alteration in the interaction between resident microbes and the mucosa. This can result from the influence of environmental factors and/or host factors, which vary depending on genetic inheritance at several susceptibility loci. Genetic factors discovered to date affect barrier function, and innate and adaptive immunity. Replicated Crohn's disease loci and Crohn's disease loci that require additional replication are listed below.

Barrier and mucosal homeostasis

Defending the integrity of the mucosal frontier as a barrier is as essential a function of the mucosa as nutrient and fluid absorption and secretion. The importance of the epithelial barrier in disease predisposition is supported by the finding of abnormal intestinal permeability in some first-degree relatives with Crohn's disease[23–26]. Barrier function is provided by anatomical features that physically impede penetration of macromolecules and intact bacteria. Many molecular details of the pre-epithelial barrier and structure of the epithelial tight junctions that comprise this physical barrier have been defined. The junctions are dynamically regulated in response to cytokines tumour necrosis factor (TNF)-α, interleukin (IL)-17, interferon-γ, chemokines and the underlying immune cell network.

Expression analysis in human IBD biopsies has demonstrated downregulation of junctional complexes (E-cadherin and β-catenin), although the mechanisms involved are unknown[27]. A recent genome-wide association study reported that 5p13.1, a Crohn's disease locus contained within a 1.25-Mb gene desert, is associated with disease susceptibility and the associated alleles correlated with quantitative expression of the prostaglandin receptor EP4 (also known as PTGER4)[9]. EP4 is expressed in intestinal epithelial cells and regulates epithelial barrier function; interestingly, EP4 knockout mice are susceptible to dextran sodium sulphate colitis[28] (Table 1).

The epithelium is in constant communication with luminal flora and the underlying dense network of innate and adaptive immune cells. Intestinal epithelial cells express Toll-like receptors (TLRs), NOD1 and 2, and receptors for different chemokines and antibody-specific Fc[29–31]. In this context, epithelial-cell-specific NF-κB activation or suppression seems to be a nodal point in the suppression and/or recruitment of immune responses in IBD. Of note, some bacteria seem to be able to hijack this process to minimize epithelial cell NF-κB activation[32]. These studies imply that commensals actively dampen intestinal inflammation by inhibition of IKBα degradation, protein ubiquitination and enhanced PPARγ-mediated nuclear export of RelA to terminate signal transduction[33]. Mice with conditional deletion of *NEMO* (also known as *IKBKG*) in intestinal epithelial cells develop spontaneous colitis and the expression of antimicrobial peptides is significantly attenuated[34]. Deletion of IKK-β (encoded by *IKK2*) in intestinal epithelial cells does not trigger spontaneous colitis (as in the case of $NOD2^{-/-}$ mice); however, these mice are unable to initiate pathogen specific T helper (T_H)2 responses to parasitic infection[35]. Together, these observations highlight the importance of NF-κB signalling networks within the intestinal epithelium in sustaining normal mucosal homeostasis and in mediating pathogen-specific responses. These studies point to substantial challenges in the development and use of inhibitors of NF-κB signalling pathways in intestinal inflammation as these pathways have protective as well as deleterious effects.

In addition to columnar epithelial cells, specialized cells are interspersed along the crypt villus axis to enhance protection against microbes and promote repair. Paneth cells reside in the base of the crypt where they secrete antimicrobial peptides, including the α-defensins. Some observations suggest that a reduction in Paneth cell α-defensins may contribute to the pathogenesis of terminal ileal Crohn's disease in patients with mutant NOD2 (refs 36, 37).

Goblet cells are also an important component of the epithelium. They are responsible for production of trefoil peptides, key both to defence and epithelial/mucosal repair[38]. Recent studies have demonstrated that RELMβ, another goblet cell specific protein, is induced

on bacterial colonization, and disruption of this gene reduces the severity of colitis in a dextran sodium sulphate model of colonic injury[39]. MUC2, a major goblet-cell-derived secretory mucin, is differentially expressed in human IBD. Consistent with these observations, *MUC2*$^{-/-}$ mice are deficient in goblet cells and develop spontaneous colitis[40]. A recent study elegantly demonstrates a contribution of intestinal mucus to the suppression of colitis[41]. Overall these data suggest that Paneth cell deficiency increases the risk of Crohn's disease, whereas goblet cells play a part in protection and pathogenesis in colitis[35,39,42,43].

Mucosal monitors

The processes that monitor the luminal contents and titrate the mucosal response are probably central to functional integrity of the mucosa and health. Thus, the mucosal immune system is poised to detect bacteria and antigens at the mucosal surface and to drive an appropriate response. The response must be nuanced between 'tolerant' and 'active' to distinguish between an innocuous commensal (which may even serve as a symbiote) and pathogens, which can invade the epithelium and beyond.

A number of sentinel cell populations in the intestinal mucosa continuously monitor luminal microbes. These include specialized M cells that transport by pinocytosis macromolecules, IgA complexes and microbes that are then picked up by antigen-presenting cells. Peyer's patches, isolated lymphoid follicles and the lamina propria work as inductive sites for the mucosal response, whereas the lamina propria functions as a recognition/effector site[35,44].

Among the subsets of antigen-presenting cells, myeloid-derived dendritic cells are the dominant subtype in the intestinal lamina propria and show considerable functional plasticity depending on the location, state of maturation, and stage of inflammation. Intravital fluorescent microscopy techniques have demonstrated that dendritic cells form an extensive network beneath the intestinal epithelium and project long processes through the interstices of epithelial cells to sample luminal antigens[45]. Sampling of bacteria by resident dendritic cells is mediated in part by a CX3CR1-dependent mechanism that permits direct dendritic-cell–microbe contact[46]. In response to TLR ligands, the immature CD11c^{+} CD11b^{+} dendritic cells produce IL-23, which contributes to development of intestinal inflammation in murine models of colitis. Furthermore, studies using a transgenic *p40* (also known as *Il-12p40* or *IL-12B*) or IL-23 depletion suggest that IL-23 is the dominant driver of regional intestinal inflammation in murine models of intestinal inflammation[47,48]. Recent studies also implicate IL-4, B-lymphocytes and enteric flora in dendritic-cell-mediated granuloma formation in states of chronic intestinal inflammation[49].

Consistent with a baseline state of hyporesponsiveness, intestinal macrophages show attenuated proliferation and chemotactic activity in response to either microbial ligands or host cytokines/chemokines despite possessing the molecular mechanisms to elaborate strong phagocytic and bactericidal responses[50]. Following an inflammatory signal, circulating macrophages migrate to the intestinal mucosa and these cells, unlike resident macrophages, express TREM1/2, Nod-like receptors (NLRs), TLRs capable of rapid response and functional chemotactic receptors. Direct support for the role of macrophages in the regulation of IBD has been obtained from analysis of mice with selective disruption of *STAT3* in their macrophages[51]. Tumour-necrosis factor (TNF) produced by non-lymphoid cells, mostly macrophages, was found to be essential for the development of colitis using the adoptive T-cell model of colitis induction. Recent studies have also shown that depletion of macrophages in the *Il10*$^{-/-}$ mouse prevents development of colitis, which otherwise occurs owing to unregulated production of IL-12 and IL-23 by macrophages[52].

Migration of innate immune cells such as neutrophils, macrophages, and dendritic cells into target mucosal tissues depends on the expression of cytokines, chemokines and adhesion molecules. Furthermore, these cells generate reactive oxygen species that are key effectors of inflammation and tissue injury and also increase epithelial permeability. Recruitment of activated neutrophils, dendritic cells and macrophages into the lamina propria in general amplifies the local immune response, whereas activated natural killer cell recruitment seems to enhance antimicrobial factors, leading to attenuation of inflammation[53]. This regulated homing of cells into the mucosa is tightly choreographed by integrins, chemokine receptors and microbial signals. Modulation of the expression of regional inflammation-induced adhesion molecules may be a useful method of disease intervention.

It is ironic that attention has only relatively recently turned to the role of the evolutionarily ancient set of innate immune responses, which serve as, figuratively, the first line of immune defence. Although there are many components of innate immunity, they are

Table 1 | Mouse models of colitis with altered barrier, innate or adaptive immune responses

Model	Known defects
Multidrug-resistant 1α$^{-/-}$ (also known as *ABCB1*$^{-/-}$)	Altered epithelial barrier
Gαi2$^{-/-}$ (*GNAI2*$^{-/-}$)	Defective epithelial barrier; defective regulatory B cells
Macrophage-PMN *Stat3*$^{-/-}$	Increased response to lipopolysaccharide (LPS); resistance to IL-10 regulation
Bone marrow *Stat3*$^{-/-}$	Increased response to LPS; impairment of innate immune function
A20$^{-/-}$ (*TNFAIP3*$^{-/-}$)	Increased response to LPS
Il10 and *Il10Rb*$^{-/-}$	Lack of Trl (Tr1; T_{reg} cells) activity; lack of TGF-β signalling
NF-κB (*Nfkb1*$^{-/-}$, *Rela*$^{-/-}$)	Increased IL-2 production
TGFb1$^{-/-}$, *TGFbR2*$^{-/-}$	Decreased numbers of regulatory T cells
Cdcs$^{C3H/HeJBir}$ mutant mice	Impaired innate responses to TLR ligands; increased numbers of bacterially reactive T cells
SAMP1/Yit mutant mice	Epithelial cell defects; expanded B-cell population; increased numbers of activated T cells
Il2$^{-/-}$ and *Il2Ra*$^{-/-}$	Decreased numbers of CD4^{+} CD25^{+} T cells
TNFa$^{\Delta ARE-/-}$ (ARE, AU-rich elements)	Increased TNF-α production
CD4^{+}, CD45RB^{high} transfer	Decreased numbers of regulatory T cells
TCRa$^{-/-}$ (T-cell antigen receptor mutant)	Loss of a regulatory B-cell function
WASP$^{-/-}$ (*WAS*$^{-/-}$)	Regulatory T cells
CD40L transgenic mice	Increased numbers of activated T cells
Smad3$^{-/-}$	Decreased numbers of regulatory T cells
Epithelial cell specific deletion of *NEMO*	Barrier function/innate immunity
Dextran sulphate sodium	Direct damage to epithelial barrier
Dextran sulphate sodium/*Tff3*$^{-/-}$	Goblet cell dysfunction; impaired epithelial repair
Dextran sulphate sodium/*Ptger4*$^{-/-}$	Altered epithelial barrier
Muc2$^{-/-}$	Barrier function/mucus defect
N-cadherin mutant	Barrier function

Key lessons learned from IBD models include: (a) a compromised epithelial layer has been shown to be sufficient to result in intestinal inflammation; (b) T cells have been implicated in numerous models presumably promoting an inappropriately activated autoreactive effector T-cell population; (c) a variety of other haematopoietic cells have been shown to be able to mediate or regulate intestinal inflammation; (d) many studies have elucidated the roles of the different cytokines at play in the different models of colitis—chemoattractant cytokines may have a unique role in IBD pathogenesis; and (e) although no specific pathogen has been isolated from the intestinal flora of spontaneous colitis models, the resident enteric flora seems necessary for colitis induction.

all 'hard wired': a defining characteristic that contrasts with the acquired specificity of adaptive responses. Immediacy of response is possible, in part because innate immune sensors/receptors recognize stereotypic molecular structures. It seems clear that the innate immune system evolved as a means to monitor the microbial environment and to limit infection by invasive organisms.

Distinct classes of receptors that recognize microbial molecular patterns are central to innate immunity. To date, more than 11 TLRs, 23 NLRs, several C-type lectin-like molecules, and β-glucan receptors have been identified. Although initial studies suggested that TLRs/NLRs were expressed in macrophages and dendritic cells, recent studies have demonstrated that innate immune receptors are broadly expressed in many cell types, including the intestinal epithelium. Many TLRs are probably non-functional at the apical side of intestinal epithelial cells, and, at baseline, NLR (also known variously as NOD or CARD) proteins might be the most important active sentinels for bacterial molecular patterns in these cells. Engagement of TLRs by microbial components triggers the activation of signalling cascades leading to the induction of genes involved in antimicrobial defence. A recent study also proposes that ligands for NOD2 may synergize with TLR2 for the production of IL-12p70 and IL-23 (ref. 54).

In the past few years, mutations in both TLRs and NLRs have been found to be associated with inflammatory bowel diseases, suggesting that each detection system is key for regulating mucosal homeostasis[7,55–57]. As noted above, NOD2 was identified as IBD1, providing the most compelling evidence for the importance of microbial–mucosal interaction in the pathogenesis of IBD. NOD2 protein is expressed in macrophages, dendritic cells, intestinal epithelial cells and Paneth cells, and may have cell-specific functions. Individuals who are either homozygotes or compound heterozygotes for any one of the three 'common' identified germline variations of *NOD2* have as much as a 40-fold increased likelihood of developing ileal Crohn's disease.

Muramyl dipeptide (MDP), found in bacterial peptidoglycan, is recognized by the leucine rich repeat (LRR) domain of NOD2 and leads to the activation of NF-κB through a receptor-interacting serine-threonine kinase-2 (RIPK2)-dependent signalling pathway (Fig. 3a)[58,59]. Recent studies have demonstrated that the signalling pathway is positively and negatively regulated by interacting partners[60–64]. A number of observations point to multiple regulatory steps in NOD2 signal transduction. Analogous to plant R proteins, NOD1 and NOD2 interact with SUGT1 and HSP90, thereby determining the specific transmission of activating and desensitizing signalling outputs that are evolutionarily conserved[62,65]. MDP is also recognized by the NLRP1 inflammasome[66]. Of note, duration of MDP stimulation may regulate tolerance in these pathways. Tissue-specific splice variants could also contribute to signalling outcome.

The mechanism by which NOD2-mediated functions contribute to intestinal immune homeostasis and how dysregulation of these functions in individuals with disease-associated NOD2 polymorphisms contribute to the increased propensity to develop Crohn's disease remain incompletely understood. Antibacterial defensins are expressed in a NOD2-dependent manner and patients with Crohn's disease seem to have diminished defensin production[36,37]. *NOD2*$^{-/-}$ mice do not develop spontaneous intestinal inflammation but are more susceptible than control mice to intra-oral *Listeria* infection[67]. The most common Crohn's disease-associated variant has been found to have reduced ability to control intracellular *Salmonella* survival in epithelial cells compared with those expressing wild-type NOD2 (ref. 30). Primary-monocyte-derived macrophages from patients with Crohn's disease who are homozygous for the truncating mutation in the LRR sensor domain (Leu1007fsinsC) have a globally blunted transcriptional response to MDP[68]. In contrast, *NOD2* frameshift-mutation knock-in mice have an enhanced response to MDP and are susceptible to dextran sodium sulphate colitis[69]. Comparative analysis of the colonic flora in *NOD2* knock-in and deficient mice may reconcile the disparate loss-of-function and gain-of-function phenotypes found when studying the Crohn's disease-associated NOD2. Collectively, studies suggest that NOD2 primarily functions in antibacterial immunity and that persistent bacterial survival might be a driver of persistent inflammatory responses in IBD. However, a more comprehensive understanding of the relationship between NOD2-dependent pathways and cellular processes for handling internalized bacteria is needed.

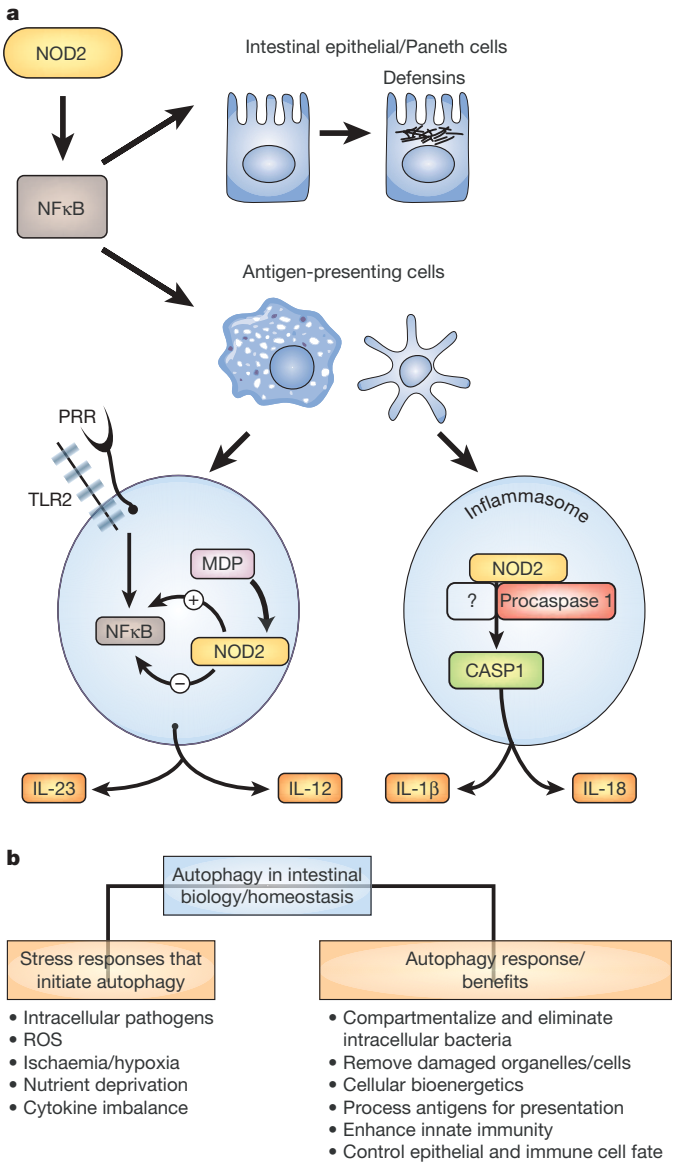
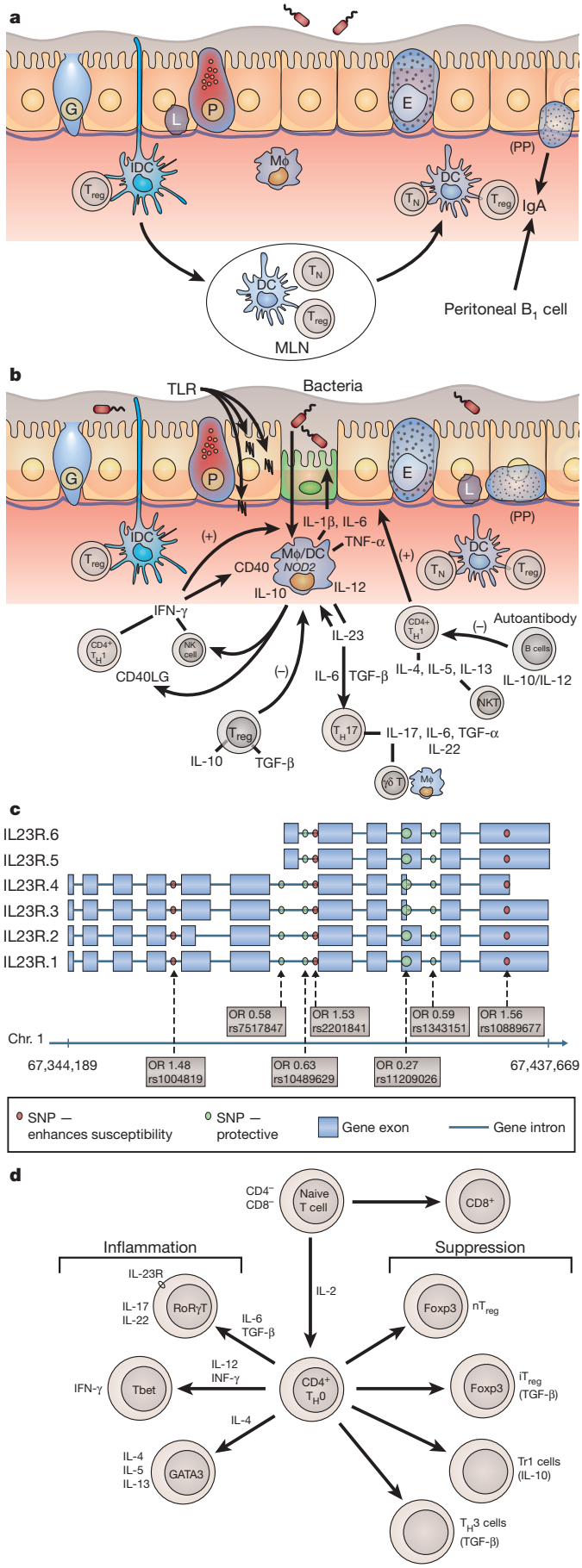


Figure 3 | Several IBD susceptibility gene products modulate host-cell functional response to microbial flora. **a**, Schematic representation of cell-specific NOD2/CARD15 signalling pathways. In intestinal epithelial cells/Paneth cells and antigen-presenting cells, muramyl dipeptide (MDP) found in bacterial proteoglycans is recognized by the leucine rich repeats (LRR) domains of NOD2 and leads to the activation of NF-κB. In Paneth cells, NOD2-mediated NF-κB activation leads to the induction of defensins. Mutations in NOD2 attenuate selective α-defensin production and protect epithelial cells from bacterial infection. In antigen-presenting cells, NOD2 signalling is modulated by TLR signalling inputs and, via interaction with procaspase 1, regulates pro-inflammatory cytokine production. **b**, Potential roles for autophagy in IBD. Autophagy is essential for cellular homeostasis, providing a mechanism of response among all cell types to limit the harmful effects of diverse exogenous and endogenous stresses. The schematic flow diagram depicts the multiple stages at which autophagy may have a role in intestinal physiology, acute stages of inflammatory injury and the resolution phase of IBD. PRR, pattern recognition receptor; ROS, reactive oxygen species.



The intracellular responses of autophagy and IBD

As noted above, a working model of IBD pathogenesis has evolved from a central focus on adaptive immunity to innate immunity. A constellation of findings, particularly in the last year, suggest a more focused orientation of that model on intracellular responses to low-level invasive bacteria. These include recent findings implicating alterations in autophagy and phagosomal function.

The autophagy pathway plays a part in protecting mammalian cells against various bacterial pathogens and the cytotoxic effect of bacterial toxins[70–72]. Following low-level stimuli or pathogen infection, autophagy could represent a primary attempt to re-establish homeostasis and when autophagic capacity is overwhelmed apoptosis could be triggered. The relevance to IBD is highlighted by the recent discovery that a synonymous SNP in the auto-phagocytic gene *ATG16L1* is associated with increased risk for Crohn's disease[8,12] (Fig. 3b). *ATG16L1* is broadly expressed in the intestinal epithelium, APCs, CD4/8 T cells, B1 cells and memory B cells. Preliminary data have implicated *ATG16L1* in host responses to intracellular bacteria[12]. Recent studies have also implicated a second autophagy gene, *IRGM* in Crohn's disease risk[73]. Short interfering (si)RNA studies have demonstrated that *IRGM* is required for mycobacterial immunity and may have an analogous role in the granulomatous response often observed in Crohn's disease[74,75]. Future studies will need to systematically determine the functional implications of disease variants in a cell- and tissue-specific context with the goal of gaining insight into the pleiotropic mechanisms behind human IBD.

NADPH oxidases and associated accessory proteins are also essential components of cellular response in microbial invasion[76–78]. An SNP within the first intron of *NCF4* ($p40^{Phox}$) is associated with enhanced susceptibility to IBD[12]. The presence of mutations within *NCF4* also prompts the hypothesis that altered phagosome function affecting handling of commensal flora may contribute to disease. Functionally altered phagosomes may kill microbes less effectively, resulting in prolonged immune activation or incomplete pathogen clearance.

Innate signals meet adaptive immunity

Although innate immune responses seem to be a prerequisite for the excessive activation of adaptive immunity, the latter is the more proximate driver of tissue damage that is manifest in IBD patients (Fig. 4a, b). Adaptive responses are effected by a combination of resident and recruited cell populations. These comprise mucosal B cells producing secretary immunoglubulin A and immunoglobulin G, a complex mixture of T cells that are dominated by a T_H1, T_H17

Figure 4 | Mucosal immune responses to luminal flora are multi-dimensional. a, Mucosal immune responses to dietary and microbial antigens. DC, dendritic cell; IgA, immunoglobulin A; E, enterocyte; G, goblet cell; L, lymphocyte; IDC, immature dendritic cell; T_N, T cell (naive); MLN, mesenteric lymph node; Mϕ, macrophage; P, Paneth cells; PP, Peyer's patches. **b**, Mucosal immune response initiated by microbial sensing systems activates adaptive immune responses. Pathogenic bacteria or commensal microbes in genetically susceptible hosts disrupt epithelial barrier function, triggering the recruitment and activation of innate immune responses and colitogenic $CD4^+$ T cells. Depicted cells and cytokines imply that multiple components are involved in controlling mucosal immune responses in physiological and pathological states of inflammation. NK, natural killer. **c**, Schematic representation of IL23R isoforms and susceptibility loci implicated in Crohn's disease. The SNPs depicted are not all independent associations (the differences in the ORs presented are not necessarily biologically relevant differences). Additional genetics and functional studies will be essential to identify truly functional SNPs and to determine mechanisms by which some confer risk and others protection. Chr., chromosome; OR, odds ratio. **d**, Differentiation of CD4 T cells. The relative balance between effector T cells and regulatory T cells determines intestinal immunity and inflammation. T_H1, T_H2, T_H17 and T_{reg} cells are present in the intestinal mucosa and their differentiation is determined by cytokines, chemokines, self-ligands and microbial products present in the local and systemic milieu. nT_{reg}, natural T_{reg}; iT_{reg}, induced T_{reg}; Tbet, TBX21.

or T_H2 phenotype, and the coincident presence of regulatory T/B cells. T_H1 development is triggered by microbes that stimulate production of interferon-γ and IL-12p40, which signal through STAT1, TBX21 and STAT4. STAT6 is essential for enhancement of T_H2 cell differentiation and GATA3 for the induction of IL-4, IL-5 and IL-13 (refs 79–81).

In simplistic terms, ulcerative colitis seems to exhibit a T_H2-type-like cytokine profile, and Crohn's disease, a T_H1 profile. Recent studies suggest a more complex and significant overlap between the two major forms of IBD. Another CD4 T-cell lineage (T_H17) has been recognized that is characterized by the production of the eponymic cytokine IL-17 and the development of which is promoted by IL-23 and suppressed by transcription factors required for T_H1 and T_H2 cells[81,82]. Although the precise mechanism by which IL-23 maintains T_H17 responses *in vivo* is still not well understood, recent studies have shown that T_H17 cell lineage commitment is driven by TGF-β in the presence of proinflammatory cytokines, whereas IL-23 seems to expand or maintain T_H17 cell populations[83]. Furthermore, studies have demonstrated that IL-2 signalling using STAT5 inhibits T_H17 differentiation, which by its positive effects on regulatory T (T_{reg}) cells (see below) may constrain T_H17-mediated inflammation[84]. Compelling evidence indicates that RORγt (retinoic acid-related orphan nuclear hormone receptor gamma-t) is necessary for T_H17 commitment and differentiation[85].

Recent studies have demonstrated that an IL23R coding variant is associated with reduced risk of IBD[6] (Fig. 4c). Further analysis suggests that there are multiple variants in the region independently associated with risk of IBD and establish that IL23R signalling is central to IBD. IL-23 is predominantly produced by activated dendritic cells and phagocytic cells, further suggesting that there may be distinct populations of dendritic cells expressing IL-12 and IL-23. IL-23 signals via the constitutive association of JAK2 in a STAT3/STAT4-dependent pathway, and IL-23-stimulated cells express IL-17A, IL-17F, IL-6, IL-22, TNF and CXCL1. Many microbial signals, CD40 and PGE2 (also known as PTGES2) receptors enhance the expression of the IL-23 subunits[82,86].

Mechanistic studies also suggest the potential importance of the IL-23 axis in IBD pathogenesis. Anti-IL-12p40 (common to both IL-12 and IL-23) or interferon-γ-specific antibodies antagonize the development of spontaneous IBD in the IL-10-deficient mouse, but only neutralization of IL-12p40 ameliorated the disease. The spontaneous development of IBD in IL-10-deficient mice is prevented by a cross with IL-23p19 (also known as IL23a)-deficient mice, also suggesting an essential role for IL-23 in the induction of colitis[48,87–89]. Future studies will need to determine if IL-17-deficiency alters onset of murine colitis in these models.

In addition to its ability to support the development of T_H17 cells, IL-23 induces the secretion of IL-17 by non-T-cells in an inflammatory environment, and both T cells and monocytes serve as sources of increased expression in the mucosa of IBD patients. Recent data suggest that IL-17 induces antimicrobial peptides and may regulate tight junction barrier formation[90]. Taken together, data suggest that IL-23–IL-23R signalling may be subject to compartment-specific regulation at multiple levels and seems to function as a key conductor of innate and adaptive inflammatory responses in the intestinal mucosa.

Compelling evidence in human as well as murine models also suggest a role for T_{reg} cells in maintaining intestinal homeostasis[91]. In addition to thymic-derived T_{reg} cells, induced T_{reg} cells may be generated in the periphery from activated effector memory $CD4^+CD25^-$ cells[92]. As outlined in (Fig. 4d), in the absence of inflammatory mediators, TGF-β promotes the development of $Foxp3^+$ T_{reg} cells associated with suppression of the inflammatory response[93]. In contrast, in the presence of proinflammatory cytokines such as IL-6, TGF-β induces the differentiation of T_H17 cells. The complex interactions between pleiotropic factors such as IL-17 and TGF-β influence intestinal homeostasis and may affect initiation, persistence and relapses in human IBD.

In the lamina propria, T_{reg} cells may have evolved to suppress immune responses to resident commensal microbes. T_{reg} cells could have a critical role in modulating the clinical spectrum of the disease. T_{reg} cells are expanded in both inflamed and non-inflamed ulcerative colitis[94]. Additional regulatory cells within the CD8 compartment ($CD8^+CD28^-$) have been described in IBD[95]. These cells are reduced or absent in lamina propria of patients with IBD. In addition to T cells, B cells termed B_{reg} also have a regulatory role in intestinal inflammation, on the basis of observations in a number of murine models[96]. Unlike T_{reg} cells, these cells are seen only in states of inflammation and suppress progression rather than initiation of murine colitis. Additional evidence points to a key role for CD1d-restricted natural killer T (NKT) cells in the induction and amplification of T_H2-cytokine-driven intestinal inflammation in ulcerative colitis[97]. Future studies will need to identify additional phenotypic markers for these cells in the lamina propria and address the role of these location-specific regulatory cells in the prevention and the resolution of intestinal inflammation in human IBD.

Future perspectives

The number of potential IBD genes continues to increase[9,12,14,73]. Additional novel loci map to chromosome 16q24.1, *TNFSF15*, *NKX2-3* and an intergenic region on chromosome 10q21.1. Future biological studies will be required to identify the functional or gene regulatory implications of these non-coding variants in human IBD, and how qualitative and quantitative differences in expression contribute to disease pathogenesis. Modelling the available data predicts that the IBD-prone genotype results from multiple genetic variants that each exert a small effect on overall risk. Genetic variants may permit improved definition of disease phenotype, help monitor clinical progression and more importantly provide insight for the development of targeted preventive therapies. Furthermore alternative splicing of messenger RNA precursors, such as those that occur within *NOD2* and *ATG16L1*, is a common mechanism of gene control and future studies will need to address the functional implications of splicing within genetic variants that contribute to immunity.

An important conceptual development in the understanding of IBD pathogenesis has been the more focused appreciation of the nature of the microbial–innate-immune-response interaction during the transition from physiological to pathological intestinal inflammation. As highlighted above, achieving a thorough understanding may depend as much on more rigorous analysis of the complex dynamics of luminal microbial communities as on understanding of the host mucosal defence and response mechanisms.

Explaining the geographic restriction of inflammation to the intestine is a major cognitive challenge when disease-associated gene variants are usually expressed broadly. This paradox might be explained by the combined effect of a relatively subtle alteration of gene function and an exceptional challenge provided by the dense microbial flora unique to the gut. When innate immune function is only slightly attenuated, a phenotype may be manifest only where there is a very dense bacterial antigenic challenge, resulting in gut-restricted disease. Host defence defects in ulcerative colitis patients may be sufficiently subtle that inflammation occurs only in the context of the intense stimulus of the colonic flora. This supposition may explain why inflammation frequently occurs in the pouch created from the terminal ileum in ulcerative colitis patients undergoing colectomy when the 'ileal flora' becomes more colonic-like, despite the absence of ileal inflammation in ulcerative colitis patients before surgery.

Recent studies indicate that the outcome of microbe–host-cell interaction may depend on the competence of the host response rather than the intrinsic invasiveness of the bacteria *per se*. If the mechanisms for intracellular inhibition and killing are diminished, persistent survival may stimulate progressive inflammation. A

better definition of disease, coupled with host gene expression profiles, metabolomic profiling of microbes and metagenomic approaches may help narrow the microbial factors central to disease pathogenesis[98].

Finally, functional dissection of genetic variants in IBD susceptibility is still in its infancy. Searching for more complete genotype–phenotype correlations and for independent evidence of the functional consequences of sequence alteration will be an important step in placing disease variants in functional biology. Systematic pathway analysis will help guide future in-depth functional studies and provide a common framework for understanding how these genetic variants influence biological pathways in ways that lead to IBD.

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