Immunopathogenesis of IBD: current state of the art

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Abstract | IBD is a chronic inflammatory condition of the gastrointestinal tract encompassing two main clinical entities: Crohn's disease and ulcerative colitis. Although Crohn's disease and ulcerative colitis have historically been studied together because they share common features (such as symptoms, structural damage and therapy), it is now clear that they represent two distinct pathophysiological entities. Both Crohn's disease and ulcerative colitis are associated with multiple pathogenic factors including environmental changes, an array of susceptibility gene variants, a qualitatively and quantitatively abnormal gut microbiota and a broadly dysregulated immune response. In spite of this realization and the identification of seemingly pertinent environmental, genetic, microbial and immune factors, a full understanding of IBD pathogenesis is still out of reach and, consequently, treatment is far from optimal. An important reason for this unsatisfactory situation is the currently limited comprehension of what are the truly relevant components of IBD immunopathogenesis. This article will comprehensively review current knowledge of the classic immune components and will expand the concept of IBD immunopathogenesis to include various cells, mediators and pathways that have not been traditionally associated with disease mechanisms, but that profoundly affect the overall intestinal inflammatory process.

During the past few decades, the main components believed to be responsible for Crohn's disease and ulcerative colitis have been identified: the environment, the genetic make-up, the gut microbiota and the immune response. Chronic inflammation is, ultimately, a dysregulated immune response, and therefore much of the investigation of IBD pathogenesis has been focused on immune abnormalities. The study of such abnormalities was initially concentrated on adaptive immunity and lately on innate immunity. With the realization that many of the relevant antigens were of microbial origin and that most nonimmune cells display active immunoregulatory functions, the investigation of IBD immunopathogenesis has widened to include these components. The discovery of the inflammasome, regulatory RNAs and damage-associated molecular patterns (DAMPs) has further expanded the number of factors involved in mediating IBD. Thus, immune events must be integrated with and interpreted in the context of a larger scenario in which the environment, genetics and the microbiota have equal, or perhaps even more important, roles in the overall pathogenesis of IBD. This topic will be comprehensively discussed in this Review by primarily emphasizing studies relevant to human IBD and referring to experimental and animal models as necessary for supportive evidence.

Evolutionary steps and epidemiology
Profound changes associated with human behaviour have been empirically blamed for the increased incidence of IBD[1]. Among various components of modern lifestyle[1,2], several have emerged as modifiers of systemic and intestinal immunity, such as alterations of the microbiota, antibiotics, diet, smoking and vitamin D. Risk of IBD markedly increases in children repeatedly exposed to antibiotics in early life[3] and in adults after an episode of acute gastroenteritis[4], events probably secondary to changes in the gut microbiota. Western-like diets also modify the composition and function of the microbiota, as do smoking and ubiquitous food additives[5,6]. Availability of vitamin D, an important regulator of mucosal immunity, depends not only on ingestion, but also on sunlight, and low sunlight exposure is a risk factor for Crohn's disease[7]. Another factor is lipolysaccharide, a ubiquitous bacterial product with potent immunoregulatory actions, and lipopolysaccharide levels are lower in house dust samples from children with IBD than from healthy controls[8].

Epidemiological evidence shows a clear correlation between the decrease in infectious diseases, lack of parasites, use of antibiotics, vaccinations and a general improvement in food, water and housing sanitary

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conditions with an increase in the incidence of auto-immune and chronic inflammatory disorders[9]. This finding forms the basis of the so-called hygiene hypothesis, which is supported by the fact that the microbiota is fundamental to the 'education' of the immune system after birth. Strong epidemiological evidence shows that childhood asthma is inversely associated with *Helicobacter pylori* colonization[10], indicating that the loss of microbes commonly found in humans impairs immune education and predisposes to immune-mediated diseases. On the basis of this clinical observation and related experimental studies, a mechanistic paradigm of asthma pathogenesis has been proposed[11]: a well-balanced gut microbiota promotes the differentiation of naive gut dendritic cells (DCs) into tolerogenic DCs followed by generation of regulatory T (T_{REG}) cells and establishment of immune homeostasis; by contrast, if naive DCs are exposed to a less-diversified microbiota, allergens, pathogens or xenobiotics (xenobiotic being defined as a foreign substance that is not naturally produced), they would differentiate into immunogenic DCs which, in turn, would lead to the generation of effector T cells and subsequent inflammation. This paradigm fits nicely with the epidemiology of Crohn's disease and ulcerative colitis, and the well-established link between IBD immunopathogenesis and dysbiosis of the gut microbiota[12] (FIG. 1).

Pathogenic factors and mechanisms
Genetics

The known association of fibrostenosing Crohn's disease with *NOD2* (encoding nucleotide-binding oligomerization domain-containing protein 2) gene variants is found primarily in patients of European or Jewish ancestry, but not in patients of Japanese or Chinese ancestry[13,14]; an important observation demonstrating that different genetic factors predispose to IBD through diverse inflammatory pathways. The first IBD-based genome-wide association study (GWAS) revealed that *IL23R*, a gene encoding the receptor of the proinflammatory cytokine IL-23, was associated with both Crohn's disease and ulcerative colitis[15]. Using pooled next-generation

deep resequencing of GWAS loci, additional rare variants associated with IBD were subsequently identified[16]. Follow-up GWAS revealed that many of the Crohn's-disease-associated and ulcerative-colitis-associated variants were shared, although some were exclusive for one form of IBD. At present, 163 genetic loci have been recognized: 110 are associated with both forms of IBD, 30 are specific to Crohn's disease and 23 are specific to ulcerative colitis[17]. These loci are enriched for genes involved in primary immunodeficiencies, T-cell function, modulation of cytokine production and mycobacterial diseases.

NOD2. A member of the NOD-like receptor (NLR) family, NOD2 encodes the primary receptor for muramyl dipeptide (MDP) found in all Gram-negative and Gram-positive bacteria. NOD2 signalling is essential for bacterial recognition, making it a key player in innate immune responses and regulation of the commensal microbiota[18]. Most investigators currently agree that a loss of NOD2 function is a key pathogenic event in Crohn's disease, as defective NOD2 would lead to increased inflammation due to impaired bacterial clearance. In addition, NOD2 variants have been reported to suppress transcription of IL-10, a potent anti-inflammatory cytokine[19]. A complete understanding of the role of NOD2 in Crohn's disease is confounded by other NOD2-dependent activities, including induction of autophagy, alternate activation pathways and modulation of adaptive immunity[18].

ATG16L1 and IRGM. GWAS have identified an association of Crohn's disease with variants in *ATG16L1* and *IRGM*, two genes involved in autophagy[20,21]. Interestingly, a genetic interaction between reference single nucleotide polymorphisms rs2241880 of *ATG16L1* and rs10065172 of *IRGM* has been reported in Crohn's disease, pointing to a functional integration of autophagy with microbial sensing and endoplasmic reticulum (ER) stress in this condition[22]. Defects in autophagy fit nicely in the pathogenesis of Crohn's disease, as autophagy gene products regulate a vast number of immune activities, including innate and adaptive immune responses and effector functions mediating responses to and processing of microorganisms[23]. MDP-mediated activation of NOD2 in epithelial cells activates autophagy and increases bacterial killing in an ATG16L1-dependent and NOD2-dependent fashion[24], a response that is impaired by Crohn's-disease-associated *NOD2* variants. Thus, multiple interactions among gene variants are probably involved in defective microbial processing in IBD[25].

Other genetic associations. In addition to the associations confirmed by GWAS ([Supplementary Tables 1, 2 and 3 online](#))[17], other genes have potential pathogenetic relevance for Crohn's disease or ulcerative colitis. The gene encoding multidrug resistance 1 (*ABCB1*, also known as *MDR1*) encodes a protein highly expressed in epithelial cells that protects the intestinal barrier against xenobiotics, and an association of the 3435T allele with
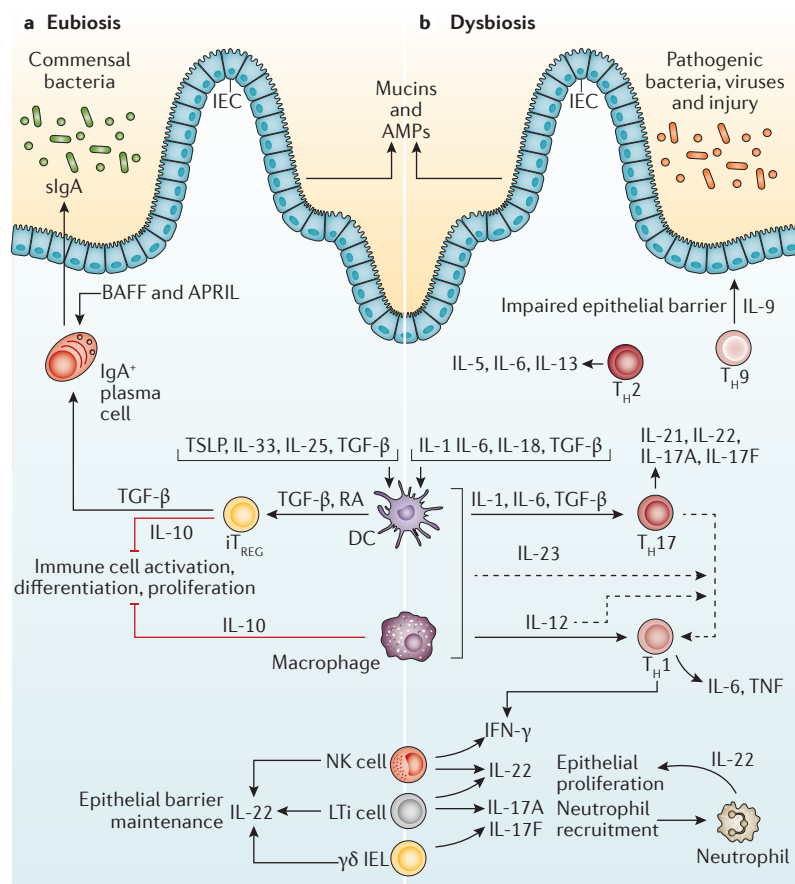
Figure 1 | **Intestinal immune homeostasis and inflammation. a |** In the presence of a balanced commensal microbiota (eubiosis), intestinal epithelial cells secrete mucins and AMPs, and microbe-associated molecular patterns (MAMPs) induce the secretion of epithelial cytokines (TSLP, IL-33, IL-25 and TGF-β) that promote development of tolerogenic DCs and macrophages. DCs, in turn, induce the development of iT$_{REG}$ cells through processes dependent on TGF-β and RA. In addition, T$_{REG}$–cell-derived TGF-β and epithelial-derived BAFF and APRIL promote generation of protective IgA plasma cells. Through additional mechanisms, including the secretion of TGF-β and IL-10 by iT$_{REG}$ cells and the secretion of IL-10 by macrophages, a physiological state of intestinal inflammation (tolerance) is induced and maintained. **b |** In the presence of pathogenic bacteria and viruses (dysbiosis), injury or xenobiotics, MAMPs stimulate the production of proinflammatory cytokines (IL-1, IL-6 and IL-18 from epithelial cells and IL-6, IL-12 and IL-23 from DCs and macrophages) that induce development of the effector CD4$^+$ T cells T$_H$1 and T$_H$17. Depending on the cytokine milieu, T$_H$2 or T$_H$9 cells are generated that mediate T$_H$2-type responses or impair epithelial barrier function, respectively. Intestinal innate lymphoid cells, including NK-like cells, LTi cells, and γδ IELs, respond to proinflammatory cytokines upregulating IL-22 (which protects the epithelial barrier) and IL-17A and IL-17F (which recruit neutrophils to promote inflammation and IL-22-mediated epithelial proliferation). AMP, antimicrobial peptide; APRIL, a proliferation-inducing ligand; BAFF, B-cell-activating factor of the TNF family; DC, dendritic cell; IEC, intraepithelial cell; IEL, intraepithelial lymphocyte; iT$_{REG}$ cell, induced regulatory T cell; LTi, lymphoid tissue inducer; NK cell, natural killer cell; RA, retinoic acid; sIgA, secretory IgA; TGF-β, transforming growth factor β; T$_H$, T helper cell; TSLP, thymic stromal lymphopoietin. Adapted with permission from Nature Publishing Group © Maynard, C. et al. Nature **480**, 231–241 (2012).

ulcerative colitis has been described[26]. A meta-analysis contested this association, as no statistically significant association between *MDR1* 3435C>T and the risk of ulcerative colitis or Crohn's disease was observed[27]. An association with the *TLR4* polymorphism encoding the Asp299Gly substitution with Crohn's disease

and ulcerative colitis[28], and of XBP1 (which encodes a transcription factor mediating the ER stress response) with both forms of IBD[29], have also been reported. Another study suggested that the inflammasome NLRP3 region contributes to Crohn's disease susceptibility[30], an observation not confirmed by a subsequent study.

Microbiota

Gut microbiota and immunity. The gastrointestinal tract harbours the largest microbial community of the body, and metagenomic studies have revealed an impressive number of microbial genes that powerfully influence host gene expression[31]. Early environmental exposures, including delivery mode, milk, food, hygiene and several other factors exert a fundamental effect on shaping the intestinal microbiota in childhood[32], whilst in adulthood the gut microbiota is more stable.

Early gut microbial colonization is essential to the development and maturation of the immune system[33], with the goal of establishing a symbiotic relationship of tolerance and protective immunity. Microbe-associated molecular patterns (MAMPs; including pathogen-associated molecular patterns, PAMPs) are sensed by innate immune receptors, such as Toll-like receptors (TLRs) and NLRs, a process essential to intestinal homeostasis[34]. Maintenance of this homeostasis is under the reciprocal control of equally important partners: the microbiota is controlled by products of epithelial and immune cells, such as the mucus, defensins, IgA, and RegIIIγ; mucosal immunity is regulated by the microbiota, with certain microbes favouring the growth of distinct T-cell subsets, such as segmentous filamentous bacteria, Clostridia and *Bacteroides fragilis* promoting the induction of type 17 T helper (T$_H$17), T$_{REG}$ cells and type 1 T helper (T$_H$1) cells, respectively[35].

Gut microbiota in IBD. Enhanced immune reactivity against microbial antigens has long been recognized in IBD. Patients with Crohn's disease have circulating serum antibodies against microbial antigens, including *Saccharomyces cerevisiae* (baker's yeast), *Escherichia coli* outer membrane protein C, anti-Cbir1 flagellin (the Crohn's-disease-related bacterial sequence I2) and anti-*Pseudomonas fluorescens*[36–39]. The same occurs for antibodies against glycans, common surface components of microorganisms and other human and animal cells[40]. Otherwise, evidence of increased cell-mediated immunity against microbial antigens is rather limited[41].

At the clinical level, relapses of Crohn's disease occur upon postoperative mucosal exposure to luminal contents[42]. At the microbiological level, abnormalities of the gut microbiota (or dysbiosis) are present in both forms of IBD, either quantitatively or qualitatively[43]. In patients with Crohn's disease, there is an increased abundance in Bacteroidetes and Proteobacteria and a decrease in abundance of Firmicutes[44], as well as a decreased bacterial diversity[45]. Evidence of abnormal gut microbiota in patients with ulcerative colitis has also been documented, but to a somewhat lesser degree than for Crohn's disease[46]. The potential pathogenic role of some specific microorganisms, such as

Mycobacterium avium paratuberculosis, *Campylobacter* and *Helicobacter* species, and adherent-invasive *E. coli*, remains to be determined[44].

A less-scrutinized issue is whether a decrease in numbers of protective microorganisms might be present in IBD. For instance, polysaccharide A, a product of the human symbiont *Bacteroides fragilis*, can suppress IL-17 production and improve experimental colitis[47], and a reduced number of *Faecalibacterium prausnitzii*, which have anti-inflammatory properties, is found in patients with Crohn's disease with an increased risk of postoperative recurrence after resection for ileal disease[48].

In new-onset paediatric patients with Crohn's disease, an increase in the abundance of Enterobacteriaceae, Pasteurellaceae, Vellonellaceae and Fusobacteriaceae and a decrease in the abundance of Erysipelotrichales, Bacteroidales and Clostridiales has been reported, which strongly correlated with levels of inflammation[49]. Antibiotic exposure amplifies this dysbiosis, again reinforcing the fundamental importance of environmental factors in shaping gut microbial communities.

Gut microbiota and IBD immunopathogenesis. A defective acute inflammatory response to intestinal MAMPs could result in impaired clearance of antigenic material, trigger a compensatory adaptive immune response, and eventually establish chronic inflammation[50]. This scenario could apply to Crohn's disease, in which impaired clearance of bacterial components occurs due to defective autophagy caused by *NOD2* and *ATG16L1* variants[51]. Similar events might occur with the unfolded protein response, which is induced by ER stress[52]. ER stress has been associated with human IBD through a candidate gene study of *XBP1*[29], and through the *ORMDL3* locus (identified via GWAS)[53]. In addition, the role of the enteric mycobiota and enteric virome in predisposing to, mediating or aggravating IBD has just begun to be explored[54,55].

An obvious and vital question is whether IBD-associated dysbiosis is a primary or secondary phenomenon. In support of the first possibility, evidence exists that the microbiota is influenced (at least in part) by the host's genotype[56]; in support of the second possibility, abundant clinical and experimental evidence suggests that infections, antibiotics, drugs and diet can induce dysbiosis[1]. Even inflammation *per se* is enough to disrupt the gut microbiota and promote preferential bacterial growth, such as Enterobacteriaceae[57]. Even more fundamental is the question of whether dysbiosis alone is sufficient to induce IBD, or whether disturbances of the gut microbiota are relevant only if combined with environmental or immune abnormalities. Mode of delivery, known to critically shape the gut microbiota[32], seems to not substantially affect the risk of Crohn's disease[58], suggesting that microbial alterations alone are insufficient to cause IBD.

Antimicrobial peptide production

NOD2 sensing of MDP leads to secretion of antimicrobial peptides such as α-defensins[59]. A defective expression of antibacterial peptides, with reduced levels of β-defensins HBD2, HBD3 and HBD4, has been reported in colonic Crohn's disease compared with ulcerative colitis[60]. Patients with ileal Crohn's disease also present diminished expression of Paneth-cell-derived α-defensins (HD5 and HD6)[61], this being more pronounced in patients with *NOD2* mutations. Defective antimicrobial peptide production in IBD might be compounded by defects in autophagy, as variants in *NOD2*, *ATG16L1* and *IRGM* might experimentally result in inappropriate responses to intracellular pathogens[62]. This theory is compatible with studies showing that defects in *ATG16L1* or *IRGM* contribute to replication and survival of adherent-invasive *E. coli in vitro*[63].

Innate immunity

Innate immune responses offer the first line of defence against any aggression and are mediated by a variety of different cell types, including classic immune cells such as neutrophils, monocytes, macrophages and DCs, as well as nonimmune cells, such as epithelial, endothelial and mesenchymal cells. One of the earliest signs of intestinal inflammation is the infiltration of the gut mucosa and epithelium by polymorphonuclear leukocytes (neutrophils), which persist throughout the course of IBD as long as active inflammation is present. Neutrophils contribute to IBD pathogenesis through a variety of mechanisms that include impairment of epithelial barrier function, tissue destruction through oxidative and proteolytic damage, and the perpetuation of inflammation through the release of multiple inflammatory mediators[64].

Macrophages.

Macrophages have been classified as classically activated macrophages (M1) or alternatively activated macrophages (M2) based on their cytokine secretory patterns and proinflammatory versus immunoregulatory activity[65]. However, this classification might simply translate as different but interchangeable functional states depending on the microenvironment the macrophages encounter[66], an intriguing notion relevant to IBD pathogenesis.

In the healthy gut mucosa, human macrophages display an anergic signature, fail to produce proinflammatory cytokines, but retain phagocytic and bactericidal activity *in vitro*, all activities compatible with scavenger function[67]. In patients with Crohn's disease, CD14+ macrophages have been reported that display both macrophage (CD14, CD33, CD68) and DC (CD205, CD209) markers, produce abundant IL-6, IL-23 and TNF, and contribute to IFN-γ production by local mononuclear cells[68]. By contrast, another study found impaired secretion of proinflammatory cytokines in response to *E. coli* and TLR ligation, and low levels of intracellular TNF in macrophages derived from peripheral blood monocytes from patients with Crohn's disease[69]. These findings would underlie an impaired acute inflammatory response by macrophages in Crohn's disease that would then lead to defective bacterial clearance and granuloma formation. Although compatible with the existence of an immunodeficient state in Crohn's disease, it is important to note these results are based on macrophages derived from *in vitro* peripheral blood mononuclear cells, and not the gut mucosa[69].

Dendritic cells. DCs are heterogeneous and include myeloid, plasmocytoid, tissue-resident and blood-monocyte-derived types[70]. Their primary function is to monitor the surrounding microenvironment, sample antigens and set up subsequent immune events, that is, induce tolerance or incite a host defence pro-inflammatory response. This dual function puts DCs in the unique position of controlling the interaction between innate and adaptive immunity[70]. Mucosal DCs display unique properties that enable them to interact with T cells and B cells, the intestinal epithelium and the stroma, and thus contribute to the maintenance of mucosal homeostasis or induce inflammation[71].

Under normal circumstances, the crosstalk between epithelial cells and DCs fosters homeostasis as epithelial cell-derived thymic stromal lymphopoietin conditions DCs to a noninflammatory T_H2-like phenotype[72]. In Crohn's disease, low to undetectable levels of thymic stromal lymphopoietin mRNA are expressed in intestinal epithelial cells, a finding implying an improper conditioning of DCs that could promote inflammation[72]. Human mucosal DCs express markedly increased TLR2 and TLR4 levels in the context of Crohn's disease and ulcerative colitis compared with DCs from normal control mucosa; DCs in Crohn's disease also express higher levels of CD40 and produce more IL-12 and IL-6 than normal mucosal DCs, all findings compatible with an activated state[73]. DCs in Crohn's disease express the chemokine receptor CCR7, which binds CCL19 and CCL21, chemokines that could contribute to the attraction and retention of DCs in the inflamed mucosa and promote inflammation[74].

Adaptive immunity

Humoral immunity. A general activation of the humoral immune response is found in both Crohn's disease and ulcerative colitis, manifested by various alterations in immunoglobulin subclass production[75,76]. A human epithelial colonic autoantigen recognized by tissue-bound IgG antibodies in ulcerative colitis, but not Crohn's disease mucosa, has been described[77]. Intriguingly, this autoantigen is also present in the skin, bile ducts, eyes and joints[78], typical sites of the extraintestinal manifestations of ulcerative colitis, suggesting that an antibody-mediated immune response could be responsible for both intestinal as well as extraintestinal pathology in patients with ulcerative colitis[79]. The putative autoantigen was tentatively identified as tropomyosin, and patients with ulcerative colitis, but not Crohn's disease, exhibit a mucosal antibody response against the tropomyosin 5 and 1 isoforms[80]. The importance of these observations is still unclear, but evidence that classic autoimmune phenomena are involved in IBD pathogenesis is missing.

Anti-neutrophil cytoplasmic antibodies are present in sera of patients with ulcerative colitis, with a prevalence varying from 50–90%[81]; they can also be found in sera of some patients with Crohn's disease, but their frequency and titres are consistently lower than in patients with ulcerative colitis. Patients with Crohn's disease have a distinct propensity to develop antibodies against a variety of microbial antigens. Such serum antibodies are directed against *Saccharomyces cerevisiae*[82], the bacterial antigen flagellin (CBir1), and the *E. coli* outer membrane protein C[37,38]. Antibodies against glycans, components of many cells as well as microorganisms, have also been reported in association with Crohn's disease[40]. So far, none of the antibodies described in Crohn's disease or ulcerative colitis have been shown to have pathogenic potential, but studies of the humoral immune response have yielded two precious pieces of information: the first is to show that the more numerous antibacterial antibodies and the higher their titres are, the more severe the clinical course is[37]; the second is to further support the notion that microbial antigens are involved in IBD pathogenesis.

Effector T cells. T_H cells are intrinsically plastic and adaptable to stimuli from the surrounding environment[83], a characteristic extremely relevant to IBD pathogenesis. In fact, adaptation to the host's need under changing circumstances — such as age, diet, xenobiotics or microorganisms — makes biological sense for immune homeostasis and health, whereas biological 'stiffness' and lack of adaptation prevents restorative changes and might maintain an immune response that develops into chronic inflammation[84].

Until less than a decade ago, Crohn's disease was designated as a T_H1 condition based on an elevated production of IL-12 and IFN-γ, whereas ulcerative colitis was characterized as an atypical T_H2 condition based on enhanced production of IL-5 and IL-13, but low levels of IL-4. This T_H1/T_H2 paradigm was revised with the discovery of IL-17-producing T_H17 cells and the interplay among T_H1, T_H2, T_H17 and T_{REG} cells[85].

In what was previously believed to be typical T_H1 models of experimental IBD, intestinal pathology was actually dependent on IL-23-driven, IL-17-producing lymphocytes[86]. Furthermore, using knockin and knock-out mice, a crossregulation of IL-12 by IL-23 could be demonstrated in the T-cell-mediated model of trinitro-benzene sulphonic acid (TNBS) colitis, adding another layer of functional complexity to IBD-associated T_H1 and T_H17 immune responses[87]. This complexity is present in patients with Crohn's disease, for whom mucosal lymphocytes produce both IL-17 and IFN-γ, redefining this form of IBD as a mix T_H1 and T_H1/T_H17 condition[88]. Increased IL-17 expression is found in the mucosa and serum of most patients with IBD, but is consistently higher in those with Crohn's disease than those with ulcerative colitis[89].

T_H17 cells produce multiple cytokines, including IL-21 and IL-22[84]. Intestinal tissue levels of IL-21 are higher in samples from patients with Crohn's disease than those with ulcerative colitis and normal control tissue and they promote T_H1 signalling and IFN-γ production[90]. In the dextran sodium sulphate (DSS) and TNBS models of colitis, IL-21 production is elevated and *Il21*^{−/−} mice are protected from colitis[91]. IL-22 expression is increased in active Crohn's disease lesions and in mice with DSS[92], but is decreased in actively inflamed ulcerative colitis tissue[93]. IL-22 might exert protective functions in

intestinal inflammation, at least in colitis in mice[94]. Not all T_H17-derived products foster intestinal inflammation and they might actually be protective. Bacteria-primed human DCs promote IL-17 production and inflammation to eliminate pathogenic microbes which, when regulated, represents a defence mechanism[95]. This scenario might not occur if *NOD2* function is defective, such as in Crohn's-disease-associated *NOD2* variants, and chronic IL-17-dependent gut inflammation could ensue[95].

A study published in 2014 shows an elevated number of IL-9-producing T cells in the mucosa of patients with ulcerative colitis and in mice with oxazolone-induced colitis[96], suggesting the existence of a T_H9 subset involved in the pathogenesis of human ulcerative colitis and in mice with experimental colitis. Additional subsets of T_H cells that are relevant to specific IBD subtypes probably exist, which could explain the variations of the immune response in patients with IBD.

Regulatory T cells. Regulatory cells are essential to develop tolerance to self and nonself antigens[97]. Among various regulatory cell types are CD4+ T cells that express the forkhead box protein P3 transcription factor (FoxP3+) and the IL-2 receptor α chain (CD25+), termed T_{REG} cells. Given their importance in both innate and adaptive immunity, defects in T_{REG}-cell function underlie infectious, autoimmune and chronic inflammatory conditions, including IBD[98].

Typical CD4+FoxP3+CD25+ T_{REG} cells have been detected in the normal mucosa of mice; their number expands during inflammation and can suppress T_H1-mediated colitis[99]. T_{REG} cells are sensitive to changes in the gut microbiota in regard to number and activation state[98], an observation particularly relevant to mucosal immunity and inflammation. The role of T_{REG} cells in human IBD is poorly defined[98]. Although the number of CD4+CD25+FoxP3+ T_{REG} cells in the peripheral blood decreases during active disease[100], they are numerically expanded in the lamina propria and display immunosuppressive activity *in vitro*[100,101]. This finding suggests that their regulatory capacity is insufficient to control IBD, prompting the notion that IBD immunopathogenesis is due, at least in part, to insufficient suppressor function[102]. Boosting T_{REG}-cell function would have beneficial effects, a possibility supported by finding that TNF blockade restores the suppressive function of T_{REG} cells[103]. Accordingly, anti-TNF treatment increases the number of FoxP3+ T_{REG} cells in the mucosa of children with Crohn's disease[104], and reverses apoptosis of mucosal CD4+FoxP3+ T_{REG} cells in the mucosa of adults with active IBD[105].

Natural killer and innate lymphoid cells. The intestinal mucosa contains other types of immune cells relevant to IBD pathogenesis: natural killer (NK) cells, natural killer T (NKT) cells and innate lymphoid cells (ILCs). Although biologically distinct, they share with T cells and among themselves phenotypic markers, functions and products such as, for instance, the production of IL-17A and IL-22[106,107].

NK cells bearing the NKp44+NKp46− and NKp44−NKp46+ markers are present in the human gut mucosa,

but their balance is disrupted in Crohn's disease and is associated with IFN-γ production[108]. A subset of CD4+ T cells expressing the marker NKG2D (NKG2-D type II integral membrane protein) produce IL-17 and IL-22 in patients with Crohn's disease[109]. The ulcerative colitis mucosa is populated by type II NKT cells that respond to a sulphatide self-antigen by producing IL-13, suggesting that this form of IBD is an autoimmune condition in which a self-glycolipid activates lamina propria NKT cells mediating epithelial cell damage[110].

ILCs were initially described in mice with bacteria-induced innate colitis associated with high production of IL-23-induced IL-17[111], and a similar cell population was later identified in the mucosa of patients with IBD[112]. Various types of ILCs have been identified and in mice three distinct types of ILCs have been proposed (type 1, 2, 3), each group displaying selective transcription factors and discrete patterns of soluble mediators[113]; they exert broad regulatory activity on microbial communities, mediate resistance to microbes and helminths, promote inflammation and orchestrate tissues damage and repair[113]. ILCs are present in the gut of patients with IBD, but functional information on intestinal ILCs is essentially restricted to animal studies[114]. However, some genes associated with IBD are expressed or linked to ILC function, suggesting a possible role in IBD pathogenesis.

T-cell apoptosis

Antigen-activated T cells die by apoptosis to avoid an unnecessarily prolonged and potentially damaging immune response[115]. Normally only lamina propria T cells undergo enhanced apoptosis compared with T cells in blood[116], whereas Crohn's disease mucosal T cells are resistant to apoptosis, a phenomenon associated with abnormal ratios of Bcl-2 family proteins[117]. Another negative regulator of intestinal immunity is the tumour suppressor gene *TP53*, which inhibits T-cell cycling and consequently prevents T-cell expansion[118]. This control mechanism is lost in Crohn's disease, in which mucosal T cells cycle faster and expand more compared with normal control mucosal T cells[119]. Additional mechanisms contributing to T-cell resistance to apoptosis in IBD are mediated by IL-6 trans-signalling[120]. Survivin, a member of the inhibitors of apoptosis gene family, is markedly increased in mucosal T cells in Crohn's disease[121]. Survivin is protected from proteasome degradation by HSP90, resulting in augmented proliferation and decreased apoptosis. In Crohn's disease upregulation of HSP90 dramatically increases survivin expression in mucosal T cells, a response that is absent in T cells in ulcerative colitis and underscores fundamental differences of T-cell behaviour between the two types of IBD[121].

If inappropriate accumulation of activated T cells is relevant to IBD immunopathogenesis, promoting cell death could have therapeutic value, an assumption which has been postulated as the mechanism explaining the beneficial effect of the immunosuppressants[122] infliximab[123] and adalimumab[124]. As a corollary, the ineffectiveness of agents such as the anti-TNF antibody etanercept in patients with Crohn's disease is supposedly due to its failure to induce T-cell apoptosis[125]. However,

this distinction has been questioned after the successful management of Crohn's disease with certolizumab, an anti-TNF antibody seemingly incapable of inducing T-cell apoptosis *in vitro*[126].

Soluble mediators of immunity and inflammation

A large number of products are actively secreted by immune and nonimmune cells, and their role in initiating, mediating and perpetuating inflammation and gut tissue injury has been intensely investigated because of the potential therapeutic effect of their blockade. Excellent and extensive Reviews on this topic have been published elsewhere and the reader is referred to them for an in-depth discussion of this subject[127,128], although it will be briefly reviewed here.

Cytokines. Production of IL-2 reveals a differential pattern in IBD, with mucosal T cells in Crohn's disease producing greater amounts of IL-2, expressing higher levels of IL-2 receptor α and exhibiting greater IL-2-induced proliferation and cytotoxicity than these cells in ulcerative colitis[129]. This finding implies a more vigorous response and T-cell function in Crohn's disease compared with that of ulcerative colitis, a conclusion corroborated by observations in regard to apoptosis and stress response[121]. In the context of Crohn's disease, production of IFN-γ is increased, whereas in ulcerative colitis IL-5 is produced in substantially higher amounts[130]. The increased production of IFN-γ in Crohn's disease fits well with reports describing in Crohn's disease, but not ulcerative colitis, increased levels of IL-12 and IL-18, cytokines that strongly induce IFN-γ production[131,132]. Intriguingly, the production of IL-4 by mucosal T cells *in vitro* is decreased in both Crohn's disease and ulcerative colitis[133], whereas T cells in ulcerative colitis produce large amounts of IL-13 derived from nonclassic NKT cells *in vitro*[134]. IL-10, a potent immunoregulatory cytokine, has been reported to be produced in equal or increased amounts in the gut of patients with IBD compared with that of normal gut[135,136].

The production of proinflammatory cytokines (such as IL-1β, IL-6 and TNF) is predictably increased in both Crohn's disease and ulcerative colitis mucosa. Mucosal immune cells, but not intestinal epithelial cells, are an abundant source of IL-1β[137]. IL-6 is also abundantly produced in IBD mucosa and has broad implications in IBD pathogenesis through its trans-signalling effects via soluble IL-6 receptor, resulting in immune cell activation, inhibition of apoptosis and induction of T_H17-cell differentiation[138]. The first indication that TNF was involved in IBD came from studies of sera and stool samples from children with IBD[139], but the fundamental contribution of TNF to IBD pathogenesis came with the discovery of the therapeutic effect of its blockade in patients with Crohn's disease[140].

Transforming growth factor (TGF)-β occupies a special position among other cytokines due to its wide range of actions: it supports epithelial restitution and fibrosis, and displays powerful immunoregulatory activities essential to tolerance and homeostasis[141]. Despite its high levels in human IBD tissue, TGF-β cannot dampen the local inflammatory response apparently because of inhibition of TGF-β signalling. Under normal circumstances, binding of TGF-β1 induces the phosphorylation of SMAD2 and SMAD3, and the subsequent activation of SMAD4 leads to gene expression. In IBD, levels of SMAD7, a key intracellular inhibitor of SMAD signalling, are increased, preventing SMAD2 and SMAD3 phosphorylation and consequently inflammation is maintained[142]. Blocking SMAD7 activity could, therefore, have therapeutic effects, as suggested by a report published in 2015 showing that a SMAD7 antisense oligonucleotide might induce remission in patients with Crohn's disease[143].

Chemokines. All types of immune cells can be directed to sites of inflammation or injury by chemokines[144]. Their action is mediated by receptors present on essentially all immune and nonimmune cells, and belong to four families: CXCR1–5, CCR1–10, XCR1 and CX3CR1[145].

One of the first chemokines described in IBD was IL-8, primarily a neutrophil chemoattractant, whose levels were found to be elevated in tissue homogenates from patients with ulcerative colitis or Crohn's disease compared with tissues from normal control individuals[146]. Subsequently, but not surprisingly, essentially all chemokines investigated in IBD were found at higher levels in IBD than in control mucosa, including RANTES, CCL3 (also known as MIP-1α), CCL4 (also known as MIP-1β), CCL2 (also known as MCP-1), CCL7 (also known as MCP-3), CXCL10 (also known as IP-10), CXCL5 (also known as ENA-78) and fractalkine[147–149]. Thus, chemokines practically control the migration, distribution and homing of all immune cells in Crohn's disease and ulcerative colitis, and thus control composition of the inflammatory infiltrates in IBD lesions. A complementary level of control is exerted by the correspondent receptors. CCR9 is highly expressed by small, but not large, intestinal lamina propria lymphocytes, suggesting that this receptor and its ligand CCL25 might selectively control migration of T cells to the small bowel, making this system particularly relevant to ileal Crohn's disease[150]. In SAMP/YT mice that develop spontaneous ileal inflammation, the CCL5–CCR5 axis is upregulated in the inflamed intestine, causing migration of CCR5+ T cells to the bowel[151]. CCR5 is highly expressed by FoxP3+ T_{REG} cells and its blockade causes exacerbation of inflammation by preventing migration of T_{REG} cells to the inflamed bowel[151].

Other pathogenic components
Epithelial cells

The possibility that intestinal epithelial cells (IECs) could contribute to IBD pathogenesis has been considered for a long time[152], and because antigen presentation is fundamental to immunoregulation, the search for possible abnormalities of IEC antigen-presenting capacity in IBD was prompted. Primary human IECs from normal mucosa can process and present antigens to primed peripheral blood CD8+ T cells that function as nonspecific suppressor cells in various *in vitro* systems[153]. IBD-associated IECs are impaired in their capacity of inducing CD8+ T suppressor cells, suggesting a possible defect

in mucosal immunoregulation predisposing to IBD[154]. Another defect potentially relevant to IBD pathogenesis is the reduced expression of peroxisome proliferation-activated receptor (PPAR)-γ in the colonic epithelium of individuals with ulcerative colitis, but not in patients with Crohn's disease[155]. PPAR-γ is a nuclear receptor with the ability of suppressing the production of proinflammatory molecules and, therefore, its selective decreased expression in one form of IBD (that is, ulcerative colitis) not only points to a feature distinguishing ulcerative colitis from Crohn's disease, but also implies that defective PPAR-γ activity could eliminate an anti-inflammatory mechanism and contribute to inflammation.

Intestinal permeability

The first line of defence against harmful agents in the gut lumen is the mucous layer. Mucus originates from gel-forming mucins secreted by goblet cells, and is composed of an inner dense and sterile layer and an outer more permeable layer populated by commensal microbes[156]. The relationship between these microbes and mucus is complex and reciprocal, as experimental evidence shows that some properties of mucus, including penetrability, depend on the composition of the gut microbiota[157]. $Muc2^{-/-}$ mice develop colitis and colorectal cancer, illustrating the critical role of mucus in preventing bacterial penetration and intestinal inflammation[158]. In agreement with these observations, reduced expression of MUC1 mRNA has been observed in the inflamed ileum of patients with Crohn's disease and of MUC3, MUC4 and MUC5B mRNA in the noninflamed ileum[159]. Furthermore, colonic mucus in patients with active ulcerative colitis allows bacteria to reach the epithelium, an event with possible pathogenic consequences[160]. Immediately below the mucus layer is the intestinal epithelium consisting of enterocytes and specialized epithelial cells, such as goblet cells and Paneth cells, which produce antimicrobial peptides both constitutively and in response to bacterial stimulation[161].

A link between intestinal permeability and IBD immunopathogenesis is intuitive, as a properly regulated intestinal mucosal barrier is essential to health and disease[162]. Any disruption of the gut mucosal barrier could facilitate the absorption of dietary and microbial products as well as xenobiotics, and incite an altered response that might lead to inflammation. Increased intestinal permeability has long been described in patients with active Crohn's disease[163], and is positively correlated with an increased risk of relapse[164]. Thus, a barrier defect could be a primary causative step in IBD immunopathogenesis, as it is found not only in patients with Crohn's disease, but also in up to 10% of their first-degree relatives[165]. Although this possibility is still valid, the careful assessment of intestinal permeability in humans and animals has revealed that changes in small and large bowel permeability are quite common in general and can be induced by innumerous factors[166], which makes it difficult to embrace changes in gut permeability as a clinically useful indicator of subclinical Crohn's disease[164].

NOD2, in addition to serving as a bacterial sensor, can also act as a modulator of epithelial cell function. In fact, variants of the *NOD2* gene are associated with defective intestinal barrier function[167], as are *JAK2* variants and polymorphisms of *MUC1* and *MUC3*[168,169]. These associations could underlie not only an abnormal immune response to luminal antigens, but also a concomitant increase in gut permeability, establishing a link between two key components of IBD immunopathogenesis.

Mesenchymal cells, ECM and fibrosis

Intestinal fibrosis, due to the excessive deposition of extracellular matrix (ECM), constitutes a common complication of IBD leading to stricture formation and obstruction[170]. Fibrosis in Crohn's disease is more pronounced and usually transmural[171,172], but fibrosis also occurs in ulcerative colitis, in which ECM deposits in the superficial layers of the intestinal wall[173].

Intestinal injury and repair comprises multiple mechanisms that result in the recruitment of mesenchymal cells and the generation of activated myofibroblasts, the key functional unit responsible for excessive ECM deposition[174]. Functional differences have been demonstrated in human intestinal fibroblasts isolated from patients with IBD. These cells migrate less than the normal counterparts[175], and those from Crohn's disease fibrotic tissue express high levels of tissue inhibitor of metalloproteinase-1, which inhibits ECM degradation mediated by matrix metalloproteinases (MMPs). Multiple MMPs are highly expressed in IBD tissues[176], the breakdown of collagen fibres being mediated by interstitial collagenase (MMP-1) and MMP-2, whereas fistula formation in Crohn's disease has been associated with increased expression of MMP-3 and MMP-9[177]. In addition to a dysregulated production of these molecules, an imbalance exists between MMPs and tissue inhibitors of metalloproteinase, resulting in the excessive deposition of various ECM components[170]. All these abnormalities are probably the result of exposure to a number of inflammation-derived soluble mediators that control ECM deposition[178]. Ongoing investigation suggests that the enteric microbiota is also involved in the process of intestinal fibrogenesis[179]. This intriguing possibility implies that, in addition to contributing to inflammation, the gut microbiota might also contribute to IBD-associated fibrosis.

Angiogenesis and lymphangiogenesis

Angiogenesis. The growth of new vessels and the expansion of tissue-specific microvascular networks, angiogenesis, is a component of IBD pathogenesis[180]. Mucosal and plasma levels of vascular endothelial growth factor (VEGF)-A are upregulated in patients with IBD, as are levels of the receptor VEGF-R2[181]. Interestingly, the gut microbiota might promote angiogenesis in IBD: exposure of human intestinal microvascular cells to ligands for TLR2 or TLR6, TLR4, NOD1 and NOD2 induces proliferation, migration, transmigration as well as tube formation in human intestinal microvascular cells, as well as vessel-sprouting in the mouse aortic ring assay[182]. Moreover, ligands for TLR4 and NOD1 are as potent as VEGF in inducing *in vivo* vessel formation in collagen plugs in mice[182]. The pathogenic relevance of these findings is supported by evidence showing that
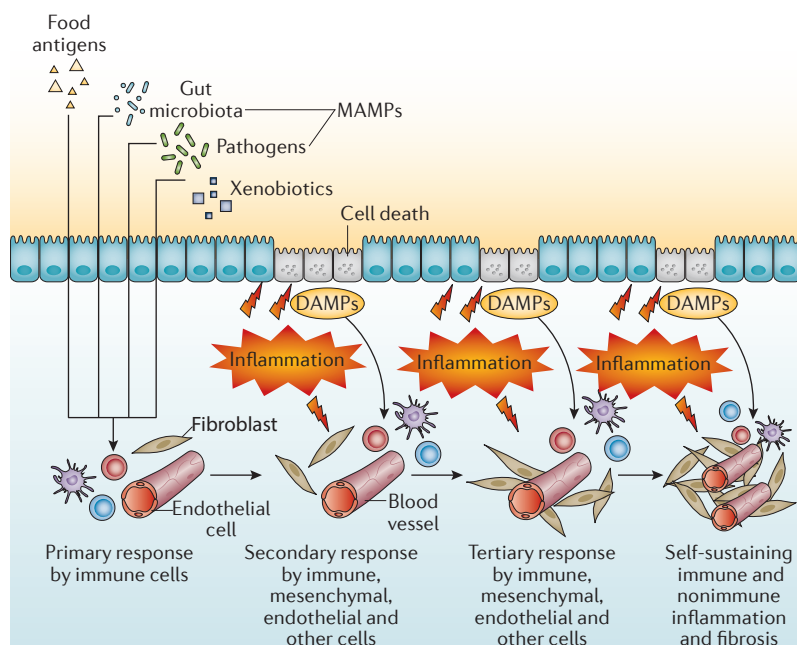
Figure 2 | Chronic intestinal inflammation induced by multiple exogenous and endogenous signals and mediated by multiple immune and nonimmune cells. The epithelial translocation of exogenous substances, including dietary antigens, gut microbiota-derived MAMPs, pathogens or xenobiotics, or a combination of them, can trigger an initial response mediated primarily by immune cells that initiates mucosal inflammation. This primary inflammatory response induces proliferation of endothelial and mesenchymal cells, tissue damage and cell death resulting in the release of endogenous DAMPs, which trigger a secondary inflammatory response mediated by immune and nonimmune cells (endothelial, mesenchymal and other cells) that amplifies inflammation and stimulates angiogenesis, fibrogenesis and structural changes; continuous inflammation induces further tissue damage, cell death, and additional release of DAMPs acting on both immune and nonimmune cells, eventually resulting in a self-sustaining cyclic of chronic inflammation associated with angiogenesis, lymphangiogenesis and fibrosis. DAMP, damage-associated molecular pattern; MAMP, microbe-associated molecular pattern.

the anti-angiogenic peptide ATN-161 decreases angiogenesis and severity of colitis in *Il10*−/− mice[183]. Also relevant is the observation that platelets circulate in an activated state in patients with IBD and promote microvascular inflammation in the mucosa[184]. Together, these *in vitro* and *in vivo* observations confirm the presence of angiogenesis in IBD, but also show how key components of IBD pathogenesis, such as microbial products, promote angiogenesis and that inhibiting angiogenesis has therapeutic potential in IBD.

Lymphangiogenesis. The lymphatic system has not been appreciated enough in IBD pathogenesis, even though prominent 'lymphangitis', manifested by grossly enlarged mesenteric lymphatics and lymph nodes, was one of the earliest anatomical hallmarks of Crohn's disease[185]. The lymphatic vasculature is essential to immune regulation as it is responsible for draining and removal of antigens and leukocytes from sites of inflammation[186]. Lymphatic obstruction and remodelling, as seen in chronic inflammation, impairs effective pumping and results in antigen and leukocyte retention which, in turn, promotes lymphangiogenesis and fosters inflammation[187].

A report published in 2014 provides evidence for a novel mechanism that controls lymphangiogenesis and lymphatic functions in the setting of experimental IBD[188]. Administration of the pro-lymphangiogenic factor VEGF-C protects mice against acute and chronic colitis by increasing inflammatory-cell mobilization and bacteria clearance from the inflamed tissue to the draining lymph nodes. Promoting lymphatic function could therefore represent a novel therapeutic strategy for IBD, particularly in Crohn's disease in which lymphatic hyperplasia is prominent.

The enteric nervous system

Morphological, histological and immunohistochemical abnormalities of the enteric nervous system have been long recognized in human IBD tissues[189], and a variety of neuropeptides with immunomodulatory functions has been proposed to have a role in IBD pathogenesis, such as substance P, corticotropin-releasing hormone, neurotensin, vasoactive intestinal peptide, μ-opioid receptor agonists and galanin[190]. Extensive and complex interactions of the central and peripheral nervous system with the gut microbiota, dietary products and inflammatory responses are now recognized and actively investigated[191–193]. However, although at the experimental level a link between the enteric nervous system and intestinal inflammation is well-established, the actual contribution of enteric nerves and their products to human IBD is still unclear.

Obesity, mesenteric fat and adipocytes

The dramatic increase in the prevalence of obesity during the past two decades has been defined as a form of 'epidemic inflammation' as obesity represents a state of chronic inflammation[194]. The proinflammatory effects of obesity are mediated by several factors: adipocyte-derived soluble mediators, also called adipokines, such as adiponectin, resistin, leptin and numerous others, the macrophages that routinely infiltrate adipose tissue[195], and the NLRP3 and NLRP6 inflammasomes[196]. Differentiation of adipocytes in the systemic subcutaneous tissue from those in the visceral adipose tissue, represented by the omentum and mesenteric fat, is critical: this distinction is important not only because of the anatomical location, but because the two types of adipose tissues display different biological properties[197].

Although patients with obesity and IBD still represent a minority, they tend to have more complications and a more severe clinical course[198]. Children with Crohn's disease have more visceral adipose tissue than children without IBD, and a higher volume of visceral adipose tissue at diagnosis is associated with higher disease activity scores, more hospitalizations, more complications and a shorter interval to surgery[199]. Creeping fat secretes more adiponectin, leptin, macrophage colony-stimulatory factor and migration inhibitory factor than fat tissue from non-IBD control patients[200]. Mesenteric fat in patients with Crohn's disease is a rich source of C-reactive protein whose levels correlate well with autologous plasma levels[201], and microarray analysis of visceral fat in patients with Crohn's disease shows a molecular profile

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characterized by upregulation of multiple inflammatory genes and downregulation of genes involved in lipid metabolism[202]. Neuropeptides also seem to be involved in the proinflammatory activity of visceral adipose tissue in Crohn's disease[203]. In summary, obesity and expansion of visceral adipose tissue must also be considered a component of IBD pathogenesis[203], another example of how the complex interaction of various, disparate and, until lately, unappreciated components come together to mediate IBD pathogenesis.

Novel pathogenic components

All chronic inflammatory diseases, including IBD, are not only complex, but also intrinsically accompanied by the inability to restore homeostasis[204]. Accordingly, investigation of IBD cannot be static and remain focused on factors generally accepted as relevant, and must dynamically explore new pathways that offer insights into novel anti-homeostatic and pro-homeostatic responses. Three such pathways are herein exemplified: the inflammasome, regulatory RNAs and DAMPs.

The inflammasome

Inflammasomes are cytosolic protein complexes that recognize exogenous, microbial, stress and endogenous danger signals and respond by activating caspase-1 and producing IL-1β and IL-18 (REF. 205). Inflammasomes belong to the NLR or the pyrin family, the best characterized being the members of the NOD-like receptor family, such as NLRP1a/b, NLRP3, NLRC4 and AIM2, which regulate immune responses, metabolism and disease pathogenesis[206]. What makes the inflammasome particularly attractive and biologically relevant to IBD immunopathogenesis is its importance in regulating the crosstalk between the mucosal immune system and the microbiota[207].

The role of the inflammasome in human and experimental IBD is still controversial. Some studies indicated that the inflammasome promotes experimental colitis, whereas others claimed that inflammasomes protect from colitis, opposing results that might depend on differences in the composition of the gut microbiota[208–210]. Studies of the inflammasome in healthy humans and patients with IBD lag far behind studies in animal models. Circumstantial evidence suggests that inflammasome activity is increased in Crohn's disease based on reports of increased caspase 1 activity and elevated levels of IL-1β and IL-18 in immune cells isolated from Crohn's disease tissues, and a positive correlation between IL-1 activity and Crohn's disease clinical severity[132,137,210]. No information is available on the inflammasome in patients with ulcerative colitis.
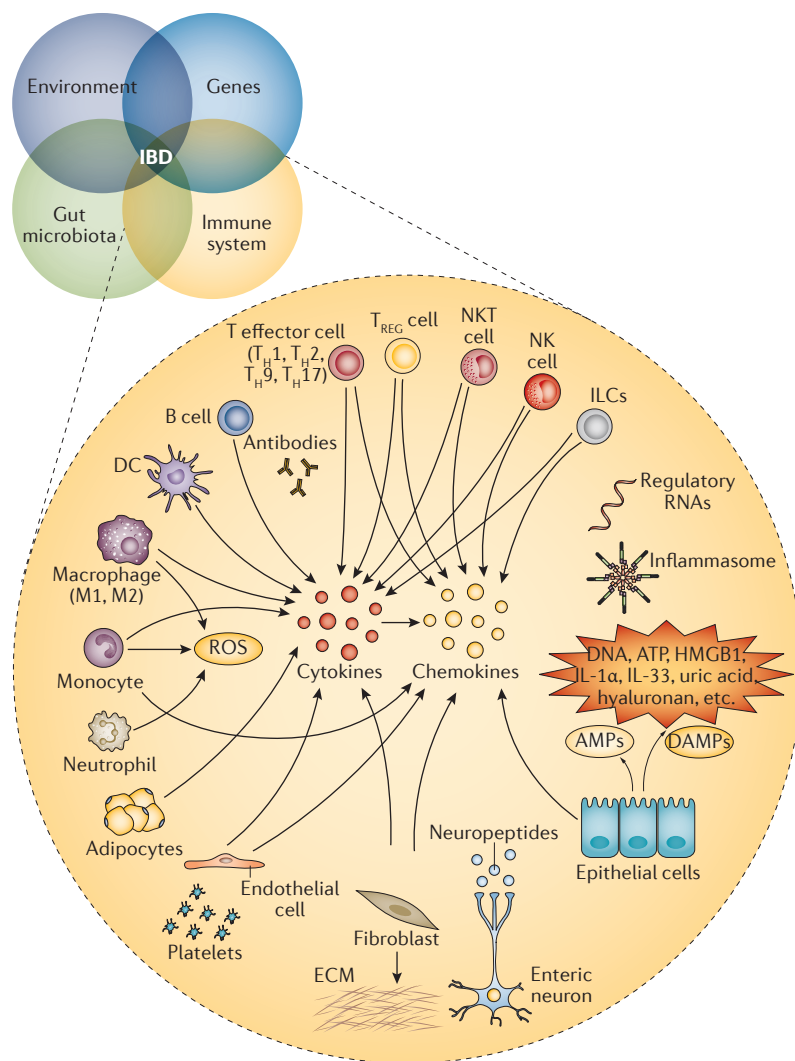
Regulatory RNAs

MicroRNAs (miRNAs) are short, single-stranded noncoding RNAs that exert important regulatory functions on gene expression primarily by repressing (silencing) genes through degradation of target RNAs or inhibition of translation[211]. Other types of noncoding RNAs have been identified, such as long noncoding RNAs and circular RNAs[212]. These noncoding RNAs also exert crucial regulatory functions, and how such functions might be involved in IBD pathogenesis is becoming the focus of considerable attention[213]. Dysregulation of or insufficient miRNA-mediated suppression could result in excessive immune reactivity and inflammation, both of which are relevant to IBD.

In one study, active ulcerative colitis has been associated with the differential expression of 11 miRNAs, three being decreased and eight increased[214]. In particular, miR-192 was localized to epithelial cells and was substantially decreased, a potentially important observation as this miRNA regulates the expression of the chemokine CCL3. miR-143 and miR-145 have also been reported to be downregulated in patients with ulcerative colitis[215], and region-specific alterations of miRNA expression have also been found in colonic Crohn's disease[216]. A study published in 2013 described reduced levels of miR-124 in the colon of children with ulcerative colitis and, because this miRNA seems to regulate the expression of STAT3, it could explain the excessive expression of this transcription factor and the promotion of inflammation in the mucosa[217]. miRNAs are also found in the peripheral circulation[218], and could become valuable biomarkers for IBD diagnosis or clinical activity. However, considerable difficulties lie ahead before these optimistic hopes become reality: miRNAs exist in the thousands and display an enormous range of regulatory functions, and dysregulated expression of tissue and blood miRNAs in IBD already numbers >100 (REF. 218).

DAMPs

Inflammation, especially when chronic and severe as seen in IBD, leads to tissue damage and various forms of cell death outcomes (apoptosis, necrosis, necroptosis and pyroptosis) with the release of multiple cytosolic and nuclear products with intrinsic proinflammatory properties[219]. These products are grouped under the denomination of DAMPs (also called alarmins or endogenous danger signals), and they bind to TLR1–9 as well as other receptors[219,220]. DAMPs cause inflammation in their own right and independently of microbial or infectious

low-density lipoproteins), ECM degradation products (for example, hyaluronan fragments) and nucleic acids (mRNA, single-stranded RNA, and so on)[220].

Interestingly, faecal calprotectin, the most frequently used and most sensitive marker of IBD clinical activity, is a complex of S100A8–S100A9, two prototypical DAMPs[222], and levels of faecal HMGB1 are also useful as markers of inflammation and mucosal healing[223]. Extracellular ATP released by stressed cells is also a DAMP, and can trigger intestinal inflammation through ligation with the purinoreceptor P2X7 receptor, whose expression is upregulated in active Crohn's disease mucosa[224]. HMGB1 has been implicated in the development of colitis and colitis-associated cancer in studies in mice[225,226], and in animal studies the alarmin IL-33 was shown to promote the generation of T_{REG} cells in the inflamed intestine, a protective action restrained by IL-23[227]. The alarmin IL-1α is quickly released by damaged epithelial cells in the intestinal lumen before immune-cell-derived IL-1β, reactivating inflammation in mice in clinical remission from DSS colitis[228]. Thus, any substance, drug or infection injuring the epithelium could induce a flare up of IBD and recurrence of clinical disease through a DAMP-mediated mechanism.

One aspect that could be particularly relevant to chronic diseases is the integration of DAMPs with MAMPs signals, resulting in amplification and maintenance of inflammation[229]. This concept has been emphasized by Nathan and Ding[230], who propose that "nonresolving inflammation" (a synonym for chronic inflammation) results from the concurrent action of PAMP and DAMP stimuli, particularly after the inflammatory response is ingrained in the affected organ. Thus, a self-perpetuating amplification cycle of events can be envisioned, in which an initial inflammatory insult caused by a single or multiple stimuli leads to proliferation of mesenchymal and endothelial cells and tissue damage with the release of DAMPs which, in turn, lead to further DAMP-mediated and/or PAMP-mediated inflammation and damage, eventually resulting in chronic inflammation, angiogenesis, lymphangiogenesis and fibrosis as seen in IBD (FIG. 2).

IBD immunopathogenesis in perspective

During the past few decades, the pathogenesis of IBD has been largely focused on its immune components and the investigation of how dysfunctional immune cells and their secreted products induce and maintain a state of chronic inflammation in the gut of patients with Crohn's disease and ulcerative colitis. This approach is justified and bolstered by the therapeutic success of immunosuppressive drugs, cytokines and receptor blockers, or apoptosis inducers developed as the result of an improved understanding of IBD immunopathogenesis. A full comprehension of the cause and mechanisms of IBD is still out of reach, we are nowhere near able to explain the various clinical phenotypes based on specific pathogenic pathways, and much remains to be done before IBD can be cured using pathophysiology-based strategies. Various reasons exist for this lack of fulfilment (BOX 1): the inability to distinguish primary from

Figure 3 | **The intricate universe of immune and nonimmune components involved in IBD immunopathogenesis.** When considering IBD comprehensively, the immune system is only one contributor and its role in disease pathogenesis must be considered in light of the other major components, that is, the environment, the genetic make-up and the gut microbiota. Together, all four components must be functionally integrated before they can trigger disease, as no single component alone can initiate or mediate IBD. A dysregulated immune response represents the effector arm of the inflammatory response, which includes a number of diverse cell types of immune and nonimmune (myeloid, lymphoid, epithelial, endothelial, mesenchymal, neurogenic) origin as well as their products (cytokines, chemokines, neuropeptides, ROS, AMPs, DAMPs, etc.), in addition to regulatory and mediator elements (regulatory RNAs, inflammasome, etc.). A large number of specific and nonspecific stimuli (diet, microbes, infectious agents, xenobiotics) can activate the mucosal immune system, but at present the temporal sequence of downstream events leading to chronic inflammation is still uncertain, making it impossible to distinguish primary from secondary abnormalities in IBD immunopathogenesis. AMP, antimicrobial peptide; DAMP, damage-associated molecular pattern; DC, dendritic cell; ECM, extracellular matrix; HMGB1, high mobility group box chromosomal protein 1; ILC, innate lymphoid cell; NK cell, natural killer cell; NKT cell, natural killer T cell; ROS, reactive oxygen species; T_H, T helper; T_{REG} cell, regulatory T cell.

agents (MAMPs) by inducing so-called sterile inflammation[221]. The numbers and types of DAMPs are large and include proteins and peptides (High mobility group box-1 protein [HMGB1], defensins, heat-shock proteins, S100 proteins, IL-1α, IL-33, and so on), lipoproteins and fatty acids (such as serum amyloid A and oxidized

secondary events and the uncertain sequence in which they occur; the use of simplistic clinical tools to classify patients with Crohn's disease or ulcerative colitis and the assumption that each form of IBD has a distinctive pattern of immune abnormalities; the still not fully appreciated fact that traditional and nontraditional immune responses are inextricably related and are jointly responsible for inflammation (FIG. 3); the reality that immunopathogenic mechanisms represent only one factor in disease pathogenesis and must be interpreted in the context of additional factors: drastic environmental changes caused by humans, the unique genetic make-up of any single patient with IBD, and a gut microbiota altered by modern human behaviour. Although the first three reasons are theoretically amenable to change and full comprehension and therefore intervention, the fourth reason is far less controllable: IBD susceptibility gene variants are inherited and epigenetically modified in unpredictable ways, and a health-conscious behaviour has yet to be promoted by the threat of disease-causing environmental changes brought about by humans.

Accepting the above as a realistic perspective of where we stand in regard to understanding and eventually curing IBD, a pragmatic option is to actively pursue the investigation of gut inflammatory mechanisms in an open-minded, dynamic and systems-biology-integrated way as additional cellular and molecular mechanisms mediating IBD will unquestionably be discovered in the near future.

Conclusions

In summary, substantial progress has been made in understanding IBD immunopathogenesis, including the interplay between the immune system and environmental, genetic and microbial factors. Immunopathogenic events in IBD now encompass classic immune cells and components and nonimmune cells (for example, mesenchymal cells), mediators and pathways that also profoundly affect intestinal inflammation. A full understanding and cure for IBD will only be possible once we understand and integrate all the underlying components and how they interact.

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Author contributions

Both authors contributed equally to all aspects of this manuscript.

Competing interests statement

H.S.P.d.S. and C.F. declare no competing interests.

Review criteria

Cited studies were searched through PubMed using specific key words representative of individual components of IBD pathogenesis; in addition, the personal files of the authors were also screened based on the same criteria. Selected information derived from both sources was included in each section of the review.

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