

Interrelatedness between dysbiosis in the gut microbiota due to immunodeficiency and disease penetrance of colitis

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Summary

The composition of the microbiome in health and disease has only recently become a major research focus. Although it is clear that an imbalance or dysbiosis in the microbiota is associated with disease, its interrelatedness to disease penetrance is largely unknown. Inflammatory bowel disease (IBD) is an excellent disease in which to explore these questions because of the extensive genetic studies identifying disease susceptibility loci and the ability to easily sample the intestinal microbiota in IBD patients due to the accessibility of stool samples. In addition, mouse models of IBD have contributed to our understanding of the interrelatedness of the gut microbiota and genes associated with IBD. The power of the mouse studies is that multiple colitis models exist that can be used in combination with genetically modified mice that harbour deficiencies in IBD susceptibility genes. Collectively, these studies revealed that bacterial dysbiosis does occur in human IBD and in mouse colitis models. In addition, with an emphasis on immune genes, the mouse studies provided evidence that specific immune regulatory proteins associated with IBD influence the gut microbiota in a manner consistent with disease penetrance. In this review, we will discuss studies in both humans and mice that demonstrate the impact of immunodeficiencies in interleukin-10, interleukin-17, nucleotide-binding oligomerization domain (NOD) 2, NOD-like receptor proteins 3 and 6, Toll-like receptor or IgA have on the interrelatedness between the composition of the gut microbiota and disease penetrance of IBD and its mouse models.

Keywords: colitis; dysbiosis; immunodeficiency; inflammatory bowel disease; microbiota

Introduction

It has long been appreciated that the host microbiota can influence disease with an emphasis on infectious disease due to either the outgrowth of a pathogenic commensal or the introduction of a new bacterial species. More recently, science has embraced the concept that the host and its microbiota are in balance, existing in a symbiotic relationship. Although all mucosal surfaces and the skin harbour commensals, the largest bacterial load exists in the intestinal tract. Indeed, the intestinal tract harbours up to 10^{14} total bacteria, with the total human microbiota making up at least 90% of the total cellular content of the human body.^{1,2} Imbalance or dysbiosis in the microbiota between the abundance of beneficial and harmful organisms can either contribute to disease or are a conse-

quence of disease. The cause and effect of dysbiosis in disease pathogenesis has been difficult to discern. This question has perhaps been best studied in inflammatory bowel disease (IBD) in humans, which is manifested in two forms: Crohn's disease (CD) and ulcerative colitis (UC).³ The environment, through its impact on the gut microbiota, is thought to be a major driver of IBD in genetically susceptible individuals.^{3,4} The analysis of alterations in the microbiota in IBD has largely been accomplished by measuring the bacterial load and composition in faeces using high throughput 16S rRNA sequencing. 16S rRNA is exclusively expressed in prokaryotes and is evolutionarily conserved allowing for PCR expansion of all species of bacterial DNA without background from the mammalian genome.^{5,6} In IBD, increases in the Gram-negative anaerobes *Enterobacteriaceae* and *Bacteroides*

among others have been observed.^{4,7–10} In addition, IBD has been associated with decreased abundance of the presumably beneficial bacteria *Bifidobacteria*.^{9,11} Hence, it is not clear whether the gain of potentially pathogenic bacteria or the loss of protective species contributes more to susceptibility and penetrance of IBD.

It is clear that the environment influences the gut microbiota, but genetic susceptibility is an important contributor determining whether gut inflammation driven by bacteria will lead to IBD. More than 160 IBD susceptibility loci have been identified, with pathways associated with microbial sensing and immune regulatory function being highly represented.^{3,12} IBD is a highly variable disease that can affect any area of the intestinal tract from the mouth to the anus.³ This difference in disease penetrance is evident from studies in monozygotic twins, whereby concordance is only 10–20% for UC and 50–75% for CD.^{3,13–15} In this review, we discuss the impact of immunodeficiencies in genes associated with IBD on the composition of the microbiome and their collective impact on colitis disease penetrance in both humans and mice.

Interleukin-10 is essential for gut homeostasis and the prevention of colitis

Interleukin-10 (IL-10) is a potent anti-inflammatory cytokine that is essential for intestinal homeostasis.¹⁶ This is evidenced by the development of spontaneous enterocolitis in IL-10-deficient mice.¹⁷ In the gut, IL-10 is produced by a number of cells including B cells, T cells and macrophages as well as intestinal epithelial cells (IEC).¹⁶ The cellular sources deemed of most importance in suppression of intestinal inflammation by IL-10 are CD4⁺ T regulatory (Treg) cells and IEC (Fig. 1).^{18–23} In the gut, two distinct populations of IL-10-producing Treg cells exist that accumulate at sites of inflammation.¹⁶ The first expresses Foxp3 and conditional deletion of IL-10 in Foxp3⁺ cells resulted in spontaneous enterocolitis.²⁴ In inflammation associated with colitis IL-10 plays an additional role whereby IL-10 receptor (IL-10R) signalling in Treg cells is essential for the maintenance of Foxp3 expression.²⁵ The second population are termed Tr1 cells, which do not express Foxp3 and are induced via chronic T-cell activation at sites of inflammation in the presence of IL-27 and IL-10.^{26–28} However, unlike Foxp3⁺ Treg cells, a specific role for Tr1 cells in either preventing or treating colitis has not been firmly established using conditional ablation.

The IL-10R is a heterodimer composed of the IL-10R1 and IL-10R2 subunits and downstream signalling occurs via activation of the Janus kinase-signal transducer and activator of transcription (STAT) signalling pathway, with STAT3 being of importance in anti-inflammatory signalling in macrophages.²⁹ Deletion of either IL-10R1 or

STAT3 in myeloid cells rendered mice susceptible to spontaneous enterocolitis.^{30–33} In humans, although immune deficiencies in IL-10 or its receptor are rare, genome-wide association studies revealed a linkage between susceptibility to IBD and polymorphisms in the *IL10* gene (Fig. 1).^{34,35} In addition, loss of function mutations in *IL10RA* and *IL10RB* led to early-onset colitis in humans.^{36–38}

In mice immunodeficient in IL-10, a link between the microbiome and colitis disease penetrance is clear. This is evident by the finding that spontaneous enterocolitis occurs in a microbiome-dependent manner and is colony dependent.^{17,39–41} In particular, the Gram-negative opportunistic pathogen *Helicobacter hepaticus* has been associated with colitis. Although *Helicobacter hepaticus*-free *Il10*^{-/-} mice do not succumb to spontaneous enterocolitis, its introduction is sufficient to drive disease.^{39,42,43} However, the penetrance of *H. hepaticus*-induced colitis is dependent upon the genetic background of the mice and the composition of the enteric microbiome.^{39,41–44} The finding that monoassociation of germ-free *Il10*^{-/-} mice with *H. hepaticus* did not lead to colitis indicates that co-colonization with multiple bacteria is required to drive pathogenic inflammatory responses.^{45,46} Why *H. hepaticus* monoassociation is not sufficient to drive colitis is not completely understood, but it has been hypothesized to be the result of an immune system defect in the germ-free mice that cannot mount a full inflammatory response because of underdeveloped lymphoid tissues.^{16,47–51} The immune system defects in germ-free mice are reversed upon colonization with commensal bacteria introduced by co-housing with specific pathogen-free mice.⁵⁰ In *Il10*^{-/-} germ-free mice, co-colonization with *H. hepaticus* and *Lactobacillus reuteri* resulted in severe typhlocolitis.⁴⁵ *Bifidobacterium wadsworthia*, a Gram-negative anaerobic bacteria, has also been associated with colitis induction in *Il10*^{-/-} mice when fed a diet high in saturated fat due to induction of taurocholic acid.⁵² Interestingly, monoassociation with *Bifidobacterium wadsworthia* in *Il10*^{-/-} germ-free mice was sufficient to induce colitis.⁵²

Susceptibility to colitis is clearly dependent upon the composition of the microbiome in *Il10*^{-/-} mice, but specific bacterial species also provide protection by altering the composition of specific immune cells in the gut mucosa. Of particular interest are IL-10-producing Foxp3⁺ Treg cells (Fig. 1). In this regard, the daily administration of a probiotic mixture of *Bifidobacteria*, *Lactobacilli* and *Streptococcus salivarius* to mice in the remission period of colitis induced by 2,4,6-trinitrobenzene sulphonic acid (TNBS) resulted in less severe disease following the second course of treatment and was associated with an increase in IL-10 production by lamina propria mononuclear cells.⁵³ However, the cellular source of the IL-10 was not investigated. In a later study, with the capacity to study Foxp3⁺ cells, it was shown that

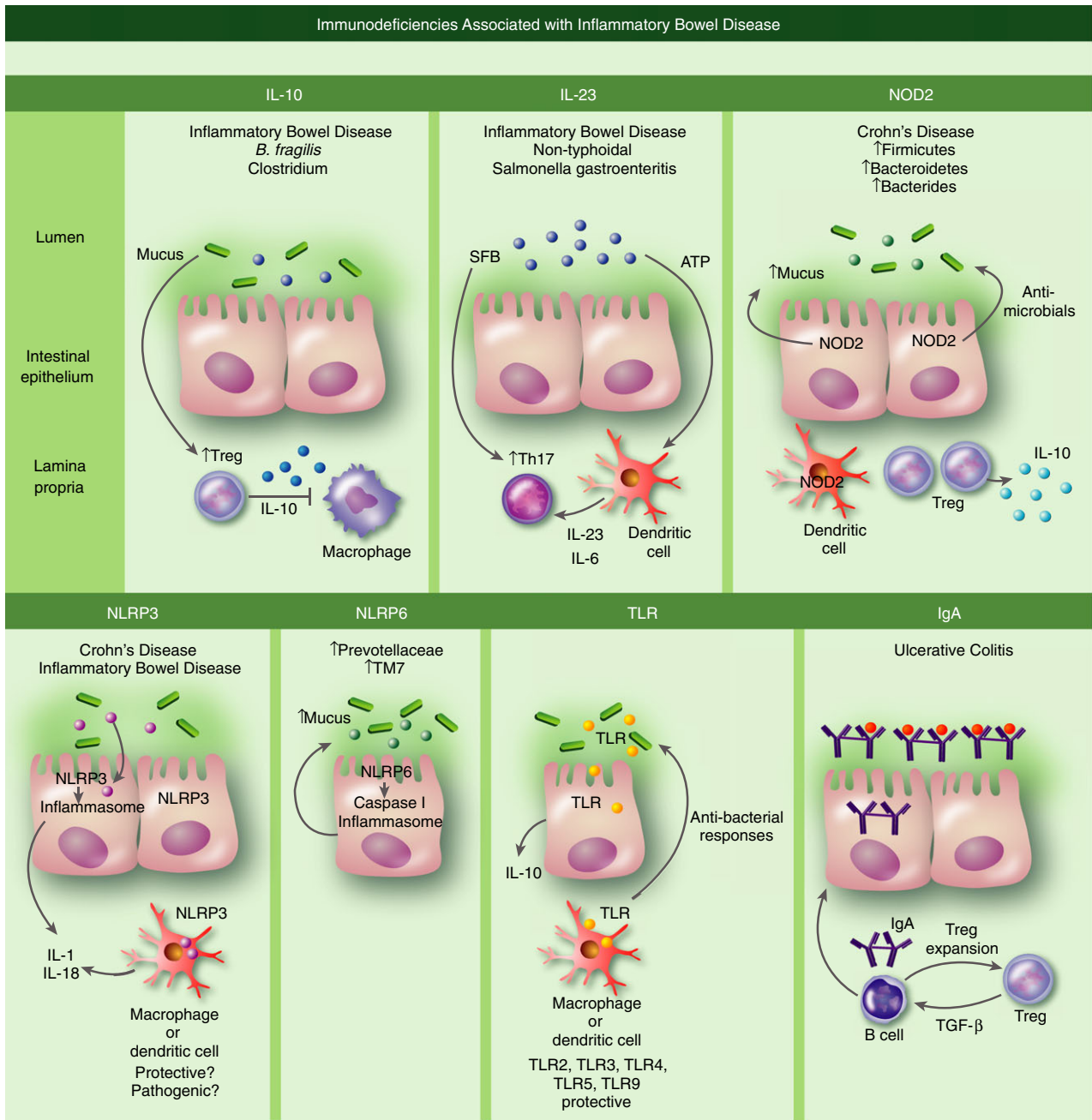


Figure 1. Immune mechanisms controlling gut homeostasis. Interleukin-10 (IL-10): Humans with immune deficiencies in IL-10 signalling have increased susceptibility to inflammatory bowel disease (IBD). Gut bacteria can promote the homeostasis of regulatory T (Treg) cells that generate anti-inflammatory IL-10 important in dampening macrophage activation. IL-23: Humans with immune deficiencies in IL-23 signalling have increased susceptibility to IBD. IL-23 and IL-6 produced by dendritic cells in response to ATP generated by gut bacteria are important cytokines in maintaining the stability of T helper type 17 (Th17) cells. Th17 cells can be induced by segmented filamentous bacterium (SFB) present in the gut microbiota. Nucleotide-binding oligomerization domain 2 (NOD2): Loss of function mutations in *NOD2* increase susceptibility to Crohn's disease in humans. NOD2 present in intestinal epithelial cells promotes their production of mucus and anti-microbials. NOD2 is also associated with the expansion of dendritic cells and Treg cells that presumably generate protective IL-10. NOD-like receptor protein 3 (NLRP3): Polymorphisms in the regulatory region of the NLRP3 gene and in genes encoding the IL-10R and IL-18R are linked to increased susceptibility to IBD. Microbial signalling activates the NLRP3 inflammasome that cleaves pro-IL-1 and IL-18. Experimental data in mouse models of IBD are conflicting regarding whether NLRP3 deficiency is pathogenic or protective. NLRP6: NLRP6 activates the caspase 1 inflammasome and is thought to regulate mucus production. Toll-like receptor (TLR): TLR signalling can lead to the generation of IL-10. Studies in mice deficient in TLR2, TLR3, TLR4, TLR5 or TLR9 indicate that they are protective in mouse models of IBD. IgA: Humans immunodeficient in IgA production can have increased risk for ulcerative colitis (UC). IgA is produced by B cells in the lamina propria in response to transforming growth factor- β (TGF- β) production by Treg cells. B cells in turn promote Treg expansion. Secretory IgA crosses the endothelial cell layer where it binds bacteria in the lumen.

monoassociation of *Bacteroides fragilis* in germ-free mice increased the numbers of IL-10-producing CD4⁺ Foxp3⁺ Treg cells in the colon, but not in the mesenteric lymph nodes (Fig. 1).⁵⁴ The increase in Treg cells was shown to be dependent upon polysaccharide A expression by *B. fragilis* and Toll-like receptor (TLR) 2 signalling.⁵⁴ It was also shown that polysaccharide A was sufficient to increase both Treg numbers and IL-10 and to protect from TNBS-induced colitis.⁵⁴ The accumulation of Treg cells in the colon is consistent with a study showing that of the various gut-associated lymphoid tissues, Treg numbers were only decreased in the colonic lamina propria in germ-free mice.⁵⁵ This study also showed that colonization of germ-free mice with *Clostridium* clusters IV and XIVa led to an accumulation of colonic IL-10-producing CD4⁺ Foxp3⁺ Treg cells (Fig. 1).⁵⁵ However, no increase in Treg cells was noted after monoassociation with *Lactobacillus* or *B. fragilis*.⁵⁵ *Clostridium*-abundant mice also exhibited attenuated disease severity in dextran sodium sulphate (DSS)-induced colitis.⁵⁵ CD4⁺ Foxp3⁺ Treg cells are composed of natural Treg cells that develop in the thymus and inducible Treg cells that differentiate at sites of inflammation in a transforming growth factor(TGF)- β -dependent manner.^{56–58} The above studies concluded that the increase in Treg cells following colonization with either *B. fragilis* or *Clostridium* was due to inducible Treg differentiation.^{54,55} However, it should be noted that both natural and inducible Treg populations are required to suppress colitis in the CD45RB^{high} CD4⁺ T-cell transfer model.⁵⁹

The above cumulative studies in *Il10*^{-/-} and germ-free mice clearly demonstrate the essential role for Treg cells and IL-10 in the maintenance of tolerance in the intestinal tract keeping immune responses in check and thereby preventing colitis. They also highlight the potential for probiotic use of specific bacterial strains via induction of IL-10-producing Treg cells for the suppression of gut inflammation and attenuation of IBD in humans.

IL-17-producing T cells are induced by the microbiota

Interleukin-17 is a pro-inflammatory cytokine important for protection against extracellular pathogens and is produced by T helper type 17 (Th17) CD4⁺ cells and by additional immune cell types.⁶⁰ Interleukin-17 immunodeficiencies in humans are associated with recurrent or persistent infection associated with chronic mucocutaneous candidiasis and staphylococcal dermatitis.⁶¹ Individuals who are immunodeficient for STAT3 also succumb to similar infections, which is thought to be due to its induction of IL-17 production.⁶² The Th17 cells are plastic and a key cytokine in their maintenance is IL-23.^{63–65} In models of colitis, IL-23 has emerged as a pathogenic factor, including in *H. hepaticus*-induced

T-cell-dependent colitis.^{66–68} Interleukin-23 has been linked to human IBD in genome-wide association studies that identified polymorphisms in the IL-23R gene locus associated with disease susceptibility (Fig. 1).⁶⁹ Humans with primary immunodeficiencies in IL-23-dependent pathways are susceptible to non-typhoidal *Salmonella* gastroenteritis (Fig. 1).⁷⁰ Complicating the investigation into the exact role of Th17 cells in colitis is their production of multiple cytokines in addition to IL-17 (IL-17A) including IL-17F, IL-21 and IL-22 and granulocyte-macrophage colony-stimulating factor.^{71,72} In mouse models of colitis, Th17-associated cytokines play both pathogenic and protective roles.⁷¹ Given that IL-23 has multiple effects on immune function, it is likely that it also contributes to intestinal inflammation in an IL-17-independent manner.

Although the exact role of Th17 cells in IBD is not clear, their development in the gut is regulated by the commensal segmented filamentous bacterium (SFB) (Fig. 1).⁷³ Experimental evidence suggested that the increase in Th17 cells was due to SFB induction of serum amyloid A production in the terminal ileum epithelial cells, which was probably through IL-23 production by dendritic cells promoting Th17 generation (Fig. 1).⁷³ In addition, the inclusion of SFB in a bacterial cocktail used to colonize germ-free mice that were subsequently infected with the pathogen *Citrobacter rodentium*, attenuated colonic inflammation.⁷³ Similarly, another study reported that ATP derived from commensal bacteria promoted the differentiation of Th17 cells through the induction of Th17-promoting cytokines, such as IL-6 and IL-23, by a subset of lamina propria CD11c⁺ cells (Fig. 1).⁷⁴ Of importance to colitis is the observation that ATP administration exacerbated T-cell-mediated colitis in SCID mice that was associated with an increase in IL-17-producing CD4⁺ T cells (Fig. 1).⁷⁴ Interestingly, we recently showed that CD11c⁺ dendritic cells are also implicated in the homeostasis of CD4⁺ Foxp3⁺ Treg cells.⁷⁵ Hence dendritic cells are an important link between Treg and Th17 cells. In addition, IL-17 is very important in preventing skin infections and a similar mechanism whereby the local microbiota promotes IL-17 production has been speculated to promote anti-bacterial functions of keratinocytes.⁷⁶ This is consistent with skin infections in humans with altered IL-17 immunity.

NLR deficiency alters the composition of the intestinal microbiota

Nucleotide-binding oligomerization domain (NOD)-like receptors (NLR) play an important role in mediating host-microbe interactions in the intestine. NLR are expressed by IEC and immune cells and are important for the recognition of pathogen-associated molecular patterns expressed by microorganisms. Deficiencies in NLR

are associated with intestinal inflammation in humans and mice.⁷⁷ Interestingly, some NLR proteins have been shown to contribute to the homeostatic maintenance of the gut microbiota.

Nucleotide-binding oligomerization domain containing 2 (NOD2), a member of the NLR family, plays an important role in controlling intestinal inflammation. Loss of function of *NOD2* results in uncontrolled inflammatory responses and is associated with CD in humans (Fig. 1).^{78–80} In particular, gene variants leading to the inability of NOD2 to sense the bacterial peptidoglycan structure muramyl dipeptide have been implicated in susceptibility to CD.^{81–83} Interestingly, CD patients with *NOD2* mutations were shown to have an altered microbiota composition in the ileum.⁸⁴ A higher prevalence of *Bacteroides*, *Bacteroidetes* and *Firmicutes* was observed compared with healthy controls (Fig. 1).⁸⁴ In addition, altered frequencies of *Faecalibacterium* and *Escherichia* have also been associated with *NOD2* risk alleles in CD patients with *NOD2* mutations.⁸⁵

Given the association of *NOD2* with CD it was surprising that *Nod2*^{-/-} mice did not develop spontaneous intestinal inflammation nor exhibit enhanced susceptibility to DSS-colitis^{86,87}. However, in a subsequent study *Nod2*^{-/-} mice were shown to exhibit exacerbated disease in an antigen-specific model of colitis, which was linked to TLR2 responsiveness.^{87,88} In addition, *Nod2* deficiency resulted in microbial dysbiosis consisting of increased bacterial load in the faeces and ileum along with a significant increase in the abundance of *Bacteroidetes* and *Firmicutes* compared with wild-type (WT) mice (Fig. 1).^{84,89,90} The absence of *Helicobacter* in the mouse colony examined in the Petnicki-Ocwieja study afforded the opportunity to determine that NOD2 plays a role in the clearance of this opportunistic pathogen after colonization by oral gavage.⁸⁹ Consistent with that finding is the increased susceptibility to *H. hepaticus* induction of granulomatous inflammation in the ileum of *Nod2*^{-/-} mice.⁹¹ Following a similar theme as with other immunodeficiencies associated with colitis, NOD2 was associated with the production of protective IL-10 and the expansion of dendritic cells and Treg cells (Fig. 1).⁹²

As NOD1 shares similar structure and downstream signalling molecules as NOD2,⁹³ and is crucial for controlling *Helicobacter* infection,⁹⁴ a compensatory gain of function or increased expression of NOD1 in the absence of NOD2 can be speculated. Other than *Helicobacter*, NOD1 is also required for the clearance of *Clostridium* and prevention of bacterial translocation.⁹⁵ Hence, NOD1-mediated regulation of the microbiota is a possibility. Indeed, the total bacterial burden was increased 100-fold in *Nod1*^{-/-} mice with significant over-representation of *Bacteroides*, *Clostridiales* and *Enterobacteriaceae*.⁵¹ However, it should be noted that NOD1 has not been linked to increased susceptibility to IBD.⁷⁷

NLRP3 is a member of the NLR family of proteins that contains a pyrin domain and functions to assemble the inflammasome, which is a multimeric complex composed of caspase 1, caspase-5, Pycard and the adaptor protein NALP1/Apoptosis-associated speck-like protein containing CARD adaptor (ASC).^{96–98} Inflammasomes act as sensors of damage as well as microbe-associated molecular patterns and are responsible for the proteolytic cleavage and maturation of pro-IL-1 and pro-IL-18 (Fig. 1).^{97,98} A single nucleotide polymorphism analysis revealed a set of single nucleotide polymorphisms in the regulatory region of the *NLRP3* gene in patients with CD (Fig. 1).⁹⁹ In addition, genetic studies linked polymorphisms in genes encoding both the IL-10R and IL-18R with susceptibility to IBD (Fig. 1).⁷⁷ The role that NLRP3 plays in colitis susceptibility has been examined in mice with contradictory results (Fig. 1). In one study, *Nlrp3*^{-/-} mice were shown to exhibit more severe DSS-induced and TNBS-induced colitis.¹⁰⁰ This study also demonstrated that NLRP3 plays a role in controlling commensal overgrowth and bacteraemia as evidenced by an increase in bacterial numbers in the stool, colon, mesenteric lymph node and liver in *Nlrp3*^{-/-} mice.¹⁰⁰ However, the extent to which NLRP3 regulates the microbial composition during the steady state is not clear. This issue was addressed comparing *NLrp3*^{+/+} and *NLrp3*^{-/-} littermates with *Nlrp3*^{-/-} mice harbouring a unique bacterial composition with members of the family *Enterobacteriaceae* including the species *Citrobacter*, *Proteus* and *Shigella*.¹⁰¹ In addition, increases in the genera *Mycobacterium*, *Collinsella*, *Subdoligranulum* and *Clostridium* were detected.¹⁰¹ With the large difference in composition of the intestinal microbiota it was not surprising that *Nlrp3*^{-/-} mice also exhibited increased susceptibility to DSS-induced and TNBS-induced colitis.¹⁰¹ Although the above reports indicate an anti-inflammatory protective role for NLRP3 in colitis, the opposite result was also obtained whereby *Nlrp3*^{-/-} mice exhibited less severe disease using a similar model of DSS-induced colitis.¹⁰² The reason for the difference in disease penetrance in *Nlrp3*^{-/-} mice is not clear, but could be due to differences in percentage of DSS used in the colitis induction protocol or to differences in the composition of the intestinal microbiota. In particular, the presence of *Citrobacter*, as noted in the Hirota *et al.*,¹⁰¹ study, is of interest given that *Citrobacter rodentium*-induced colitis was more severe in mice lacking NLRP3.¹⁰³

Similarly to NLRP3, NLRP6 uses the ASC to activate a Caspase-1 inflammasome and *Nlrp6*^{-/-} mice exhibited more severe DSS-induced colitis.^{104–108} To determine whether the microbiota influenced disease penetrance in the *Nlrp6*^{-/-} mice they were co-housed with WT mice, which was sufficient to transfer the severe colitis phenotype.¹⁰⁶ To evaluate the potential colitogenic microbiota present in *Nlrp6*^{-/-} mice, 16S rRNA analysis was

performed, demonstrating an increase in abundance of *Prevotellaceae* and TM7 (Fig. 1).¹⁰⁶ This same study evaluated *Nlrp3*^{-/-} mice that upon co-housing with WT mice exhibited attenuated colitis.¹⁰⁶ Hence NLRP3- and NLRP6-deficient mice serve as a clear example of how immunodeficiency influences the composition of the microbiota that in turn alters disease penetrance.

Altered microbiota in NLR-deficient mice could potentially be attributed to the anti-bacterial functions of these sentinel proteins that play an important role in host-pathogen interactions. NOD2 signalling, leads to the generation of anti-microbials like β -defensin-2, Human neutrophil peptide 1 (HNP-1) and malignant brain tumors 1 (DMBT1) (Fig. 1).^{109–111} It is thought that NOD2 regulates mucus production, an important anti-microbial mechanism in the gut, via its interaction with Polypeptide N-Acetylgalactosaminyltransferase 2 (GALNT2) (Fig. 1).¹¹² Similarly, a recent study reported a role for NLRP6 in regulating mucus production (Fig. 1).¹¹³ Goblet cell autophagy was impaired in *Nlrp6*^{-/-} mice, resulting in abrogated mucus secretion.¹¹³ Hence, impaired host defence in the absence of NLR proteins might lead to an expansion of pathogenic bacteria and their systemic dissemination that could trigger an inflammatory response via signalling through TLR.

TLR are protective in colitis

TLR are a family of pattern recognition receptors each recognizing distinct pathogen-associated molecular patterns.¹¹⁴ Genome-wide association studies have not specifically identified polymorphisms in TLR genes as susceptibility loci for IBD.¹¹⁵ However, the TLR2-R753Q variant alters disease penetrance, conferring increased risk to develop pancolitis.¹¹⁶ A dominant-negative *TLR5* variant was shown to confer resistance to development of CD.¹¹⁷ In addition, a single nucleotide polymorphism in *TLR9* has been linked to CD-associated variants in other genes.¹¹⁸ Although the *Tlr4* gene is localized to a CD susceptibility locus, strong evidence does not exist for its role in IBD penetrance.¹¹⁹ When the expression of TLR was assessed in IEC it was shown that non-IBD samples expressed high levels of TLR3 and TLR5 and low levels of TLR2 and TLR4.^{119,120} In IBD patients, TLR2 and TLR5 levels were unchanged and TLR3 either remained the same or was down-regulated depending on the IBD disease state, whereas TLR4 was greatly increased.¹²⁰ Hence, although TLR are important in sensing of microorganisms in the gut the extent to which they modulate disease penetrance in IBD remains unresolved.

TLR2, TLR4 and TLR5 all signal through the adaptor protein Myd88, whereas TLR3 does not and TLR4 also signals in a Myd88-independent manner.¹¹⁹ To determine whether Myd88 is required for colitis induction, DSS-colitis was induced in *Myd88*^{-/-} mice with the finding that

they exhibited more severe disease.¹²¹ These data suggest that TLR are protective. When individual TLR were examined for DSS-colitis penetrance; mice deficient in TLR2, TLR3, TLR4, TLR5 or TLR9 all exhibited more severe disease (Fig. 1).^{122–127} A role for multiple TLR in protection against DSS-colitis is consistent with each TLR sensing different bacterial pathogen-associated molecular patterns.

Although it is not clear how individual TLR confer protection, bacterial up-regulation of IL-10 production via TLR signalling in IEC as well as in innate and adaptive immune cells is a likely mechanism (Fig. 1).^{16,128,129} Another possible mechanism is that immunodeficiencies in specific TLR that alter sensing of particular bacterial species could lead to an alteration in the composition of the intestinal microbiota. Indeed, *Tlr5*^{-/-} mice were shown to have a phylum-level shift in which 116 bacterial phylotypes were either enriched or reduced, with no amplification or loss of a particular species.¹³⁰ In addition, *Tlr5*^{-/-} mice with transiently unstable microbiotas containing increases in proteobacteria exhibited colitis as compared to their non-colitic siblings, which stabilized their microbiotas resembling those of WT mice.¹³¹ In particular, increased levels of the enterobacteria species *Escherichia coli* in close proximity to the gut epithelium was a feature of colitis.¹³¹ Extensive studies have linked adherent-invasive *E. coli* (AIEC) with CD, but not in the context of a human immunodeficiency.¹³² In germ-free *Tlr5*^{-/-} mice, monoassociation with the CD-associated AIEC LF82 strain resulted in moderate colitis.¹³¹ To determine whether commensal bacteria contribute to the virulence of AIEC LF82 germ-free mice were moved to specific pathogen-free housing before introduction of AIEC LF82 to the gut microbiome, after which they developed spontaneous colitis with weight loss and diarrhoea as early as 3 days and was associated with decreased clearance of the bacteria.¹³¹ Spontaneous disease did not occur in WT mice or following introduction of the commensal flagellate *E. coli* strain F-18 to *Tlr5*^{-/-} mice.¹³¹ Overall, the above study demonstrated that one likely protective mechanism of TLR5 in Crohn's disease is through its recognition of AIEC flagellin promoting its clearance.¹³¹

IgA deficiency alters the gut microbiota

An important adaptive immune mechanism in gut homeostasis at the mucosal surface is B-cell secretion of IgA that functions to neutralize pathogens and microbial toxins and by confining commensals within the intestinal lumen.^{133,134} Secretory IgA produced in the lamina propria can cross the epithelial layer and be deposited in the gut lumen where it either diffuses or binds to the IEC mucus layer (Fig. 1).¹³³ IgA deficiency is the most prevalent primary immunodeficiency and is characterized by

low or absent serum IgA.¹³³ While up to 85–90% of IgA-deficient individuals remain asymptomatic, a number of gastrointestinal infections and disorders, including UC, have been observed (Fig. 1).¹³³ In mice, IgA deficiency resulted in more severe DSS-induced colitis.¹³⁵ IgA production is dependent upon TGF- β , which we and others showed could be provided by CD4⁺ Foxp3⁺ Treg cells (Fig. 1).^{136–138} In addition, we recently showed that mice deficient in B cells exhibit more severe DSS-induced colitis.¹³⁸ In this same study, consistent with our previous observations, we found that B cells confer protection by inducing proliferation of Treg cells that in turn promote IgA isotype class switching likely through their production of TGF- β (Fig. 1).^{138–141} In addition, we found no role for B-cell production of IL-10 in our study, consistent with Treg cells as the primary source of IL-10.¹³⁸

Although IgA is crucial for regulating microbes in the gut, conversely, the microbiota promotes IgA production.^{142,143} Interestingly, IgA deficiency led to an aberrant microbial composition in the gut. In activation-induced cytidine deaminase (*Aid*)-deficient mice, B cells failed to undergo class switch recombination, resulting in a complete loss of IgA production.¹⁴⁴ *Aid*^{-/-} mice harboured 100-fold more anaerobic flora in the small intestine compared with WT mice.¹⁴⁵ A later study further evaluated dysbiosis in *Aid*^{-/-} mice and found increased prevalence of SFB and *Clostridium* species in the small intestine, which was reversed with IgA.¹⁴⁶ Although dysbiosis in the absence of IgA is evident from these studies, one outstanding question is whether IgA preferentially binds to and limits certain microbes in the gut (Fig. 1). Recently, specificity of IgA towards *Enterobacteriaceae* in the microbiota of mice was reported.¹³⁵ It was shown that the microbiota in newborn mice was dominated by *Enterobacteriaceae*, which was replaced by *Bacteroidetes* and *Firmicutes* in the adult.¹³⁵ In the absence of IgA, the microbiota transition from the immature to mature stage did not occur, resulting in a higher prevalence of *Enterobacteriaceae*, which may contribute to more severe DSS-induced colitis.¹³⁵ Interestingly, changes in the IgA repertoire due to deficiency in PD-1 also resulted in an increase in *Enterobacteriaceae*.¹⁴¹ Similarly, a reduction in high-affinity IgA production due to a deficiency in Myd88 specifically in T cells led to an altered microbial composition in the gut.¹⁴⁷ From these studies it is apparent that IgA plays an important role in shaping the microbiota by restraining certain bacteria. It is tempting to speculate that IgA specifically neutralizes potential host–microbe equilibrium-altering and inflammation-promoting bacteria. Indeed, in a recent study it was found that colitogenic intestinal bacteria were highly coated with IgA in mice (Fig. 1).¹⁴⁸ Furthermore, faecal bacteria with high IgA coating isolated from IBD patients upon transfer to germ-free mice conferred susceptibility to DSS-induced colitis.¹⁴⁸

Concluding remarks

The above collective studies support the concept of co-evolution of the host immune system and the microbiota for maintaining homeostasis at the mucosal surface. In addition, it is clear that the immunological state of the intestinal tract is influenced by both host immune factors and the composition of the intestinal microbiota. Alterations in a variety of different immune regulatory loops are able to alter the gut homeostasis balance leading to dysbiosis and colitis-associated inflammation. Further insight into how and which species of the microbiota are affected by immunodeficiencies associated with IBD are still needed to determine which bacteria are pathogenic and which are protective. Although probiotics are considered a viable therapy for IBD, it still not clear how or whether they can reset the correct bacterial balance thereby alleviating inflammation in ongoing disease. This is particularly challenging because of differences in genetic susceptibility in each IBD patient that have the potential to uniquely alter the gut microbiota.

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Disclosures

The authors have no conflicts of interest.

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