The gut microbiota shapes intestinal immune responses during health and disease

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Abstract | Immunological dysregulation is the cause of many non-infectious human diseases such as autoimmunity, allergy and cancer. The gastrointestinal tract is the primary site of interaction between the host immune system and microorganisms, both symbiotic and pathogenic. In this Review we discuss findings indicating that developmental aspects of the adaptive immune system are influenced by bacterial colonization of the gut. We also highlight the molecular pathways that mediate host–symbiont interactions that regulate proper immune function. Finally, we present recent evidence to support that disturbances in the bacterial microbiota result in dysregulation of adaptive immune cells, and this may underlie disorders such as inflammatory bowel disease. This raises the possibility that the mammalian immune system, which seems to be designed to control microorganisms, is in fact controlled by microorganisms.

Mutualism
A symbiotic association in which both members benefit from the relationship.

Pathogen
An opportunistic organism that rarely comes into contact with the host, but causes acute or chronic disease following infection. Derived from the Greek word 'pathos', which means suffering.

Microbiome
The collective genomes of a microbiota.

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Humans represent a scaffold on which diverse microbial ecosystems are established. Immediately after birth, all mammals are initiated into a life-long process of colonization by foreign microorganisms that inhabit most environmentally exposed surfaces (such as the skin, mouth, gut and vagina)[1,2]. Shaped by millennia of evolution, some host–bacterial associations have developed into beneficial relationships, creating an environment for mutualism. A key example of such an environment is provided by the vast numbers and diversity of bacteria that are found in the lower gastrointestinal tract of mammals[1,3–5]. By young adulthood, both humans and other mammals support one of the most complex microbial ecosystems on the planet, with over 100 trillion bacteria in the distal gut[6,7]. Symbiotic bacteria of the mammalian gut have long been appreciated for the benefits they provide to the host: they supply essential nutrients, metabolize indigestible compounds, defend against colonization by opportunistic pathogens and even contribute to the development of the intestinal architecture[8]. Moreover, it seems that certain basic developmental features and functions of the mammalian immune system depend on interactions with the human microbiome[9]. Unlike opportunistic pathogens, which elicit immune responses that result in tissue damage during infection, some symbiotic bacterial species have been shown to prevent inflammatory disease during colonization. Surprisingly, the 'normal' microbiota also contains microorganisms that have been shown to induce inflammation under particular conditions. Therefore, the microbiota has the potential to exert both pro- and anti-inflammatory responses, and the composition of the bacterial communities in the gut may be intimately linked to the proper functioning of the immune system.

The immune system is responsible for recognizing, responding and adapting to countless foreign and self molecules and is therefore important during conditions of both health and disease. Although the immune system is classically thought to have evolved to protect from infection by microbial pathogens, animals peacefully coexist with a vast and complex microbiota, which extensively interacts with the immune system. In this Review, we discuss recent evidence suggesting that a beneficial partnership has evolved between symbiotic bacteria and the immune system. The molecular interactions seem to direct the development of immune responses, and in turn the immune system shapes the composition of the microbiota. We highlight seminal examples of microorganisms that have a role in preventing inflammatory bowel disease (IBD) and discuss the beneficial immune responses they elicit during protection. Furthermore, technological advances now allow a more detailed understanding of the alterations of the

Microbiota
The amalgam of microorganisms that make up a complex and diverse community living within a given anatomical niche (usually an environmentally exposed surface of the body).

Inflammatory bowel disease
A chronic condition of the intestine that is characterized by severe inflammation and mucosal destruction. The most common forms in humans are ulcerative colitis and Crohn's disease.

Gut-associated lymphoid tissue
Lymphoid structures and aggregates associated with the intestinal mucosa, specifically the tonsils, Peyer's patches, lymphoid follicles, appendix or caecal patch and mesenteric lymph nodes. They are enriched in conventional and unconventional lymphocytes and specialized dendritic cell and macrophage subsets. They provide the first line of defence against entry of pathogens across the mucosal barrier.

microbial population of the gut during IBD. If some bacteria are actively shaping a healthy immune system, does the absence of these organisms lead to disease? It has recently been proposed that the total information encoded by the mammalian genome is not sufficient to carry out all functions that are required to maintain health and that products of our microbiome are crucial for protection from various diseases[10]. It is possible that alterations in the development or composition of the microbiota (known as dysbiosis) disturb the partnership between the microbiota and the human immune system, ultimately leading to altered immune responses that may underlie various human inflammatory disorders.

Insights gained from germ-free mice

Developmental defects in germ-free mice. Several important effects of the microbiota on the host immune system have been determined by studies of gnotobiology, which is the selective colonization of germ-free (sterile) animals, the immune responses of which have not been influenced by interactions with molecules of pathogenic and beneficial microorganisms. Germ-free animals show extensive defects in the development of gut-associated lymphoid tissues[9,11] and in antibody production (BOX 1), and have fewer and smaller Peyer's patches and mesenteric lymph nodes (MLNs) compared with animals housed under specific pathogen free (SPF) conditions (TABLE 1). A recent report has also shown that germ-free animals show impaired development and maturation of isolated lymphoid follicles (ILFs)[12]. These inducible structures seem to form normally following the introduction of gut bacteria, suggesting a dynamic relationship between the immune system and the microbiota.

Together with various morphological tissue defects that are observed in the intestines of germ-free animals, it seems that the entire ultrastructural development of the gut is intimately connected to intestinal bacteria. For example, intestinal epithelial cells (IECs), which line the gut and form a physical barrier between lumenal contents (including the microbiota) and the underlying cells of the immune system, have altered patterns of microvilli formation and decreased rates of cell turnover in germ-free animals compared with wild-type animals[13]. Furthermore, gut bacteria have been shown to direct the glycosylation of lumenally exposed surface proteins of IECs[14].

IECs have many immunological functions (TABLE 1): they secrete and respond to various cytokines and express molecules that directly interact with lymphocytes (such as MHC molecules). Expression and localization of pattern recognition receptors (such as Toll-like receptors; TLRs) by the epithelium is influenced by bacterial colonization of the gut, and the expression of defensins and other antimicrobial proteins is defective in germ-free animals (TABLE 1). Consistent with this notion, the Gram-negative commensal organism *Bacteroides thetaiotaomicron,* but not the Gram-positive microorganism *Bifidobacterium longum*, induces the expression of the antimicrobial peptide REG3γ (regenerating islet-derived 3γ) by specialized IECs known as Paneth cells[15,16]. Intriguingly, the specificity of REG3γ is directed towards certain Gram-positive bacteria. It is therefore tempting to speculate that symbiotic bacteria direct innate immune responses of the gut in an effort to protect their environment. Collectively, the observations of developmental defects in germ-free mice at the tissue, cellular and molecular levels suggest that normal immune function may be impaired in the absence of the gut microbiota.

Defects in immune responses in germ-free mice. Germ-free animals are more susceptible to infection by certain bacteria, viruses and parasites. When challenged with the Gram-negative enteric pathogen *Shigella flexneri*, germ-free animals showed decreased immune resistance to infection and increased mortality compared with conventionally colonized animals[17]. Prior colonization with specific commensal bacteria antagonized *S. flexneri* infection, whereas colonization with control species such as *Escherichia coli* did not, implying that some members of the microbiota provide protection against intestinal bacterial pathogens[18]. Infection by the Gram-positive intracellular pathogen *Listeria monocytogenes* results in decreased bacterial clearance in germ-free animals compared with colonized animals[19]. The mechanism for this increased susceptibility has been attributed to a T cell trafficking defect to the site of *L. monocytogenes* infection in germ-free animals. Specifically, germ-free mice that have been infected with *L. monocytogenes* have decreased accumulation of $CD44^+CD62L^+$ T cells (CD44 and CD62L (also known as L-selectin) are known to be involved in the homing of lymphocytes to sites of inflammation), resulting in increased bacterial burden compared with SPF animals[20]. Finally, *Salmonella enterica* subspecies *enterica*

Box 1 | **Antibody responses in germ-free animals**

One of the first immunological defects observed in germ-free mice was a marked reduction in the levels of secretory IgA found in the intestine[94]. Association of mice with a specific bacterial species leads to increased IgA expression. As numerous studies have shown that secretory IgA coats commensal (and pathogenic) bacteria, some have speculated that IgA is involved in limiting the penetration of bacteria into host tissues. Studies from activation-induced cytosine deaminase (AID)-deficient animals (which cannot undergo class switching to IgA), have shown that these mice display lymphoid hyperplasia of the gut and an altered microbiota, thereby favouring the outgrowth of specific classes of bacteria[95]. Although some studies have shown that IgA is involved in protection from infection by some enteric bacteria and viruses[96], other studies suggest that IgA deficiency does not cause increased prevalence of disease in animals and that IgA-deficient individuals are generally healthy. However, a role for symbiotic bacteria in actively shaping the production of secretory IgA is now emerging. Dendritic cells (DCs) that have acquired gut commensal bacteria migrate to mesenteric lymph nodes, where they induce the production of IgA from naive B cells[97]. This process is required to control the penetration of commensal bacteria through the gut epithelial cell barrier[98]. Recently, it has been shown that sensing of symbiotic bacterial products by Toll-like receptors on intestinal epithelial cells results in localized class switching to IgA2 (REF. 99), a process that potentially affects interactions between the immune system and the microbiota[100]. The discovery that symbiotic bacteria direct the function of a specialized mucosal DC population that induces IgA class switching also implicates a role for the microbiota in shaping intestinal immune responses[101]. Furthermore, IgA responses were recently shown to be involved in maintaining host–bacterial mutualism by limiting innate immune responses to a specific gut symbiont[102]. The renewed interest in the biological functions of intestinal secretory IgA promises to provide important clues on the molecular communication between the immune system and the microbiota.

Table 1 | **Intestinal immunological defects in germ-free mice**

Immunological defect	Site	Phenotype in germ-free mice compared with conventionally housed mice
Development of small intestine	Peyer's patches	Fewer and less cellular
	Lamina propria	Thinner and less cellular
	Germinal centres	Fewer plasma cells
	Isolated lymphoid follicles	Smaller and less cellular
Development of mesenteric lymph nodes	Germinal centres	Smaller, less cellular and with fewer plasma cells
$CD8^+$ T cells	Intestinal epithelial lymphocytes	Fewer cells and with reduced cytotoxicity
$CD4^+$ T cells	Lamina propria	Fewer cells; decreased T_H17 cells in the small intestine but increased T_H17 cells in the colon
$CD4^+CD25^+$ T cells	Mesenteric lymph nodes	Reduced expression of FOXP3 and reduced suppressive capacity
Expression of angiogenin 4	Paneth cells	Reduced
Expression of REG3γ	Paneth cells	Reduced
Production of secretory IgA	B cells	Reduced
Levels of ATP	Intestine	Reduced
Expression of MHC class II molecules	Intestinal epithelial cells	Reduced
Expression of TLR9	Intestinal epithelial cells	Reduced
Levels of IL-25	Intestinal epithelial cells	Reduced

FOXP3, forkhead box P3; IL-25, interleukin 25; REG3γ; regenerating islet-derived 3γ; T_H17, T helper 17; TLR9, Toll-like receptor 9.

Peyer's patches
Groups of lymphoid nodules that are present in the small intestine (usually the ileum). They occur massed together on the intestinal wall, opposite the line of attachment of the mesentery. Peyer's patches consist of a dome area, B cell follicles and interfollicular T cell areas. High endothelial venules are present mainly in the interfollicular areas.

Mesenteric lymph node
(MLN). A lymph node that is located at the base of the mesentery. MLNs collect lymph (including cells and antigens) draining from the intestinal mucosa.

Specific pathogen free
Conditions in which animals are reared and maintained in an environment with an unknown complex microbiota that is free from specific known pathogens.

Isolated lymphoid follicles
Small lymphoid aggregates located in the anti-mesenteric wall of the small intestine, which contain B cells, dendritic cells, stromal cells and some T cells. They may contain germinal centres. They are thought to have a role in maintaining equilibrium between the immune system and the microbiota.

Pattern recognition receptor
A host receptor (such as Toll-like receptors) that can sense pathogen-associated molecular patterns and initiate signalling cascades (which involve the activation of nuclear factor-κB) that lead to an innate immune response.

Commensal
A microorganism that benefits from an association with no known effects on the host. Derived from the Latin phrase 'com mensa', meaning to share a table.

serovar Typhimurium is known to cause a more severe acute gastroenteritis in germ-free animals[21]; however, the reasons for this remain unclear.

Establishing an infection requires the initial task of colonizing the host. For intestinal pathogens, this can be a challenge as all mammals are stably colonized by communities of bacteria that can act as a barrier to infection (known as colonization resistance). Recent studies suggest that inflammation induced in response to *S.* Typhimurium infection changes the composition of the microbiota and suppresses its regrowth. *S.* Typhimurium exploits this deficiency in colonization resistance to establish infection and cause disease[22].

In addition to maintaining a barrier to the colonization of potentially pathogenic organisms, the microbiota might also provide immunological benefits to the host. In support of this idea is the finding that germ-free animals show reduced antigen-specific systemic immune responses to *S.* Typhimurium[23]. This suggests that enteric pathogens such as *S.* Typhimurium might have developed strategies to counter both the immune system and the microbiota during the infectious process.

Although a lot of work is still required to determine the beneficial immune responses that are induced by the microbiota, it is exciting to consider the teleological notion that indigenous bacteria actively prevent enteric disease by infectious microorganisms to fortify their niche. If this is true, then an evolutionary alliance has been forged between mammals and beneficial bacteria that is crucial for maintaining the long-term survival of both. In other words, is our well-being dependent on the microorganisms we harbour?

The microbiota and IBD

Immune system regulation during IBD. The impact of the microbiota on human health is best exemplified by studies on IBD, such as Crohn's disease and ulcerative colitis[24–26]. Both are serious medical disorders that are characterized by aberrant inflammation in the gastrointestinal tract, resulting in severe clinical outcomes in affected patients. The causes of these diseases are complex and include contributions from genetic, geographic and habitual factors[27]. IBD (particularly Crohn's disease) is generally believed to be driven by T cells and has classically been thought to be associated with increases in pro-inflammatory cytokines such as tumour necrosis factor (TNF) and interferon-γ (IFNγ). However, recently a new population of inflammatory T cells, termed T helper 17 (T_H17) cells, which produce the pro-inflammatory cytokine interleukin-17 (IL-17) and require IL-23 for their maintenance and function, have been implicated in the pathogenesis of human and experimental colitis[28–33]. These pro-inflammatory responses are counterbalanced by specialized T cells known as regulatory T (T_{Reg}) cells. The development and function of T_{Reg} cells is thought to be controlled by the transcription factor forkhead box P3 (FOXP3), the absence of which results in massive multiorgan lymphoproliferative disease[34]. The mechanisms by which T_{Reg} cells suppress inflammation are diverse and include the following: expression of inhibitory cytokines such as IL-10, transforming growth factor-β (TGFβ) and IL-35; disruption of cellular metabolism through the expression of IL-2 receptor (IL-2R; which comprises IL-2R α-chain (CD25), IL-2R β-chain and common

cytokine receptor γ-chain); cytolysis; and targeting the maturation of dendritic cells (DCs) through cell surface expression of molecules such as cytotoxic T lymphocyte antigen 4 and lymphocyte activation gene 3 (REF. 35). A population of intestinal DCs expressing the cell surface antigen CD103 has recently been shown to be instrumental in the development and function of intestinal FOXP3$^+$ T_{Reg} cells. CD103$^+$ DCs, but not CD103$^-$ DCs, can promote the conversion of CD4$^+$FOXP3$^-$ T cells into CD4$^+$FOXP3$^+$ T_{Reg} cells in a TGFβ- and retinoic acid-dependent manner[36], which indicates that specialized mechanisms exist in the intestine to promote the induction and maintenance of T_{Reg} cells.

The importance of T_{Reg} cells in the regulation of intestinal homeostasis is best illustrated by the finding that these cells can prevent the induction of experimental colitis following transfer into diseased hosts[37]. The ability of T_{Reg} cells to secrete IL-10 and IL-35 has been reported to be important during protection. Indeed, T_{Reg} cells that are deficient in either of the two subunits of IL-35 (Epstein–Barr virus-induced protein 3 and IL-12α) cannot provide protection from induction of experimental colitis[38]. In addition, animals in which IL-10 has been specifically ablated from CD4$^+$FOXP3$^+$ T cells succumb to inflammatory disease of the intestine (as well as of the skin and lungs), but show no signs of autoimmunity. So, it seems that cytokine production by T_{Reg} cells might be an important protective mechanism that limits uncontrolled immune responses at environmentally exposed surfaces such as the gut.

Recent studies have begun to reveal the mechanisms of intestinal immune modulation by the microbiota. A recent study[39] has shown that germ-free animals have defective T_H17 cell development in the small intestine and that the reduction in IL-17 production is associated with a reciprocal increase in the number of CD4$^+$FOXP3$^+$ T_{Reg} cells in the colon of these mice. Reconstitution of these animals with a complex and diverse microbiota that does not contain the prominent phyla Bacteroidetes does not restore proper immune balance, suggesting that distinct organisms might have the capacity to modulate pro- and anti-inflammatory responses in the gut. The identity of specific bacterial species and bacterial molecules that are involved in regulating the balance between T_H cell and T_{Reg} cell subsets in the gut remain unknown. However, several common bacterial products are known to have immunomodulatory effects. For example, ATP generated by intestinal bacteria specifically increases the production of IL-17 (but not IFNγ) in the colon[40]; consistent with this, germ-free animals have reduced IL-17 and ATP levels in the colon. In addition, DNA from commensal bacteria triggers TLR9 signalling and confers resistance to the enteric parasite *Encephalitozoon cuniculi*[41]. Accordingly, antibiotic treatment of animals to eliminate gut bacteria results in increased susceptibility to infection by this parasite. Treatment of parasite-infected mice with DNA isolated from the intestinal microbiota led to the upregulation of T_H1 and T_H17 cell responses, which coincided with suppression of T_{Reg} cell activity; this resulted in decreased parasite burden. Previous work has suggested that TLR signalling is important for maintaining gut homeostasis[42], and these recent findings extend this observation to suggest that a molecular dialogue between immune receptors and microbial molecules confers resistance to enteric infection.

The contribution of the microbiota to the development of T_{Reg} cells remains unclear, as conflicting observations have been reported. An initial study[43] observed a reduction in the percentage of FOXP3$^+$ cells within the CD4$^+$CD25$^+$ T cell subset in the MLNs of germ-free mice compared with conventionally colonized animals. *Foxp3* mRNA levels were also lower in CD4$^+$CD25$^+$ cells isolated from the lymph nodes of germ-free mice. Consistent with these findings, another study[45] observed lower levels of *Foxp3* mRNA in CD4$^+$CD62L$^-$ T cells from germ-free mice. In addition, it has been reported that T_{Reg} cells from germ-free animals are not as effective as T_{Reg} cells from conventionally colonized animals at suppressing CD4$^+$ T cell proliferation *in vitro*[44]. Indeed, populations of T_{Reg} cells from germ-free animals produced less IL-10 and could not prevent disease in a transfer model of experimental colitis[45]. In contrast to these observations, recent studies have reported no change in the percentage of CD4$^+$FOXP3$^+$ T cells in the colon lamina propria of germ-free mice[46], and in fact another study[39] reported increased percentages of CD4$^+$FOXP3$^+$ in the small intestine[39].

These conflicting observations might be due to the particular subsets of T_{Reg} cells that were analysed, differences in experimental methodologies and/or the tissues from which T_{Reg} cells were harvested. Alternatively, the particular diet given to the animal might influence T_{Reg} cell subsets in the intestine, as most animal food might have varying amounts of microbial molecules (such as TLR ligands), even if autoclaved. However, these data collectively suggest that intestinal bacteria interact with the mammalian immune system to direct the differentiation of both pro- and anti-inflammatory T cell populations. Therefore, induction of effector T cell responses and modulation of T_{Reg} cell function by the microbiota may be a crucial component of diseases such as IBD. It is possible that different classes (or even species) of bacteria induce distinct immunological functions. Therefore, the equilibrium between inflammation and homeostasis in the gut could be due to the composition of the microbiota.

IBD and a breakdown in tolerance to gut bacteria. IBD involves a shift from a regulated intestinal immune response to one that is driven by unrestrained immune cell activation and pro-inflammatory cytokine production[47–49]. The cause of this increase in immune stimulation is of great interest, and several lines of evidence indicate a fundamental role for commensal bacteria in the progression of disease[50]. Patients with IBD respond favourably to antibiotic treatment and faecal diversion and have higher antibody titres against indigenous bacteria than unaffected individuals[51–53]. In addition, inflammatory lesions are more pronounced in areas of the intestine that contain the highest number of bacteria.

Crohn's disease
A form of chronic inflammatory bowel disease that can affect the entire gastrointestinal tract, but is most common in the colon and terminal ileum. It is characterized by transmural inflammation, strictures and granuloma formation, and is believed to result from an abnormal T cell-mediated immune response to commensal bacteria.

Ulcerative colitis
A chronic disease that is characterized by inflammation of the mucosa and sub-mucosa of the large intestine.

Regulatory T (T_{Reg}) cell
A specialized type of CD4$^+$ T cell that can suppress the responses of other T cells. These cells provide a crucial mechanism for the maintenance of peripheral self tolerance and are characterized by expression of CD25 (the α-chain of the interleukin-2 receptor) and the transcription factor forkhead box P3 (FOXP3).

Parasite
An opportunistic organism that maintains a prolonged and close association with the host, which benefits the parasite at the expense of the host.

Further evidence for the involvement of gut bacteria in IBD is provided by studies of animal models. Pre-treatment of mice with antibiotics has been shown to alleviate subsequent intestinal inflammation in several animal models[54,55]. HLA-B27-transgenic rats, IL-10- and IL-2-deficient mice raised in conventional conditions spontaneously develop chronic colitis, whereas they do not develop intestinal inflammation if raised in germ-free conditions[56–58]. In a model of colitis induced by the adoptive transfer of pathogenic T cells into immunodeficient (*Scid*$^{-/-}$ (severe combined immunodeficient) or *Rag*$^{-/-}$ (recombination-activating gene)) recipient mice, colonization of animals with intestinal pathogens such as *Helicobacter hepaticus* was found to exacerbate inflammation[59]. Moreover, colitis can be induced in healthy animals through the adoptive transfer of T cells that are reactive against specific commensal organisms[50,60].

The only microorganism reported to be strongly associated with Crohn's disease is adherent-invasive *E. coli*[61]. However, it seems that inflammatory responses in human and experimental IBD are directed towards certain subsets of commensal organisms that have pathogenic potential, such as *Helicobacter*, *Clostridium* and *Enterococcus* species. Curiously, these organisms are abundant in the microbiota and are not typically pathogenic. As the microbiota of all mammals contains these potentially harmful species, known as pathobionts (FIG. 1), it is not entirely clear why inflammation ensues only in subjects affected by IBD.

It is well known that genetic factors have an important role in the pathogenesis of IBD, as shown by the familial aggregation of IBD and the increased concordance for IBD in monozygotic twins. Genome-wide association studies have identified genetic variants that are highly linked to disease. These include disease-associated mutations in genes that are involved in bacterial sensing (such as *NOD2* (nucleotide-binding oligomerization domain 2; also known as *CARD15*))[62] and T cell immunity (such as *IL23R*)[30], which highlights the connection between microorganisms and inflammation in IBD. In addition, studies in animal models provide further evidence to support the importance of host genetics in susceptibility to IBD. Indeed, microorganisms that do not initiate disease in immunocompetent mice, such as *E. coli* and *Enterococcus faecalis*, cause disease when introduced into genetically susceptible mouse strains[63].

In addition to the crucial role of genetic factors in determining susceptibility to IBD, some investigators have suggested that IBD results, at least in part, from dysbiosis of the microbiota and not because of the acquisition of an infectious agent[64]. It remains unclear whether dysbiosis directly causes disease or is a result of the altered intestinal environment. Future studies using animal models in which the microbiota can be selectively manipulated during the course of experimental disease may begin to address this important issue.

Recently, the notion that dysbiosis influences intestinal disease has gained increasing attention[5,65,66], with studies showing evidence that the composition of the microbiota alone (and not genetics or environment) may be important for the induction of disease. T-bet (encoded by *Tbx21*) is a T-box family transcription factor that controls type 1 pro-inflammatory responses[67]. Loss of T-bet in mice lacking an adaptive immune system (such as *Rag*$^{-/-}$ mice) resulted in the development of spontaneous intestinal inflammation that resembled ulcerative colitis[68]. Treatment of these mice with broad spectrum antibiotics cured the intestinal disease, which indicates that inflammation was driven by the microbiota. Furthermore, when wild-type animals were co-housed with *Tbx21*$^{-/-}$*Rag*$^{-/-}$ mice that had colitis, they developed a comparable colitis-like disease[68], suggesting that the transfer of colitogenic microorganisms alone (the identity of which is still unclear) was sufficient to induce experimental ulcerative colitis.

A second study[69] addressing the involvement of dysbiosis in disease provided metagenomic analysis (culture-independent analysis of microbial community structure) of microbiota in a mouse model of obesity using leptin-deficient (*ob/ob*) mice. Remarkably, transfer of the microbiota from *ob/ob* mice into germ-free wild-type mice resulted in an increase in the mean body fat of the recipient animals[69,70]. Consistent with this observation, the proportion of *Bacteroidetes* spp. in the microbiota of

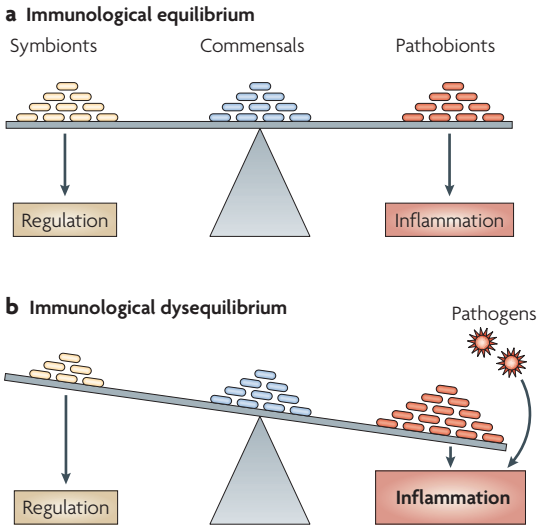

Figure 1 | **Immunological dysregulation associated with dysbiosis of the microbiota. a** | A healthy microbiota contains a balanced composition of many classes of bacteria. Symbionts are organisms with known health-promoting functions. Commensals are permanent residents of this complex ecosystem and provide no benefit or detriment to the host (at least to our knowledge). Pathobionts are also permanent residents of the microbiota and have the potential to induce pathology. **b** | In conditions of dysbiosis there is an unnatural shift in the composition of the microbiota, which results in either a reduction in the numbers of symbionts and/or an increase in the numbers of pathobionts. The causes for this are not entirely clear, but are likely to include recent societal advances in developed countries. The result is non-specific inflammation, which may predispose certain genetically susceptible people to inflammatory disease and may be caused by pathogens, which are opportunistic organisms that cause acute inflammation.

Pathobiont
A symbiont that does not normally elicit an inflammatory response but under particular conditions (environmentally induced) has the potential to cause dysregulated inflammation and lead to disease.

VSL#3
A mixture of bacteria consisting of four strains of *Lactobacillus* (*L. casei*, *L. plantarum*, *L. acidophilus* and *L. delbrueckii* subspecies *bulgaricus*), three strains of *Bifidobacterium* (*B. longum*, *B. breve* and *B. infantis*) and *Streptococcus salivarius* subspecies *thermophilus*.

obese people is lower than that of lean people, suggesting that alterations in the normal human microbiota may affect disease. Similarly to IBD, obesity seems to have a strong genetic component[71]. The complex interplay between host genotype and its effects on the microbiota is an area worthy of investigation, and such studies may provide further support for an important role for dysbiosis in metabolic disorders.

Similar to the findings in animal studies, dysbiosis has been implicated in IBD in humans, with several studies showing a significant alteration in the microbiota of patients with IBD[72–74]. A recent metagenomic study[74] compared the microbiota of patients with IBD with that of non-IBD controls and revealed a statistically significant difference in their composition. Specifically, the microbiota of IBD patients showed abnormal microbial composition that was characterized by depletion of two phyla of bacteria, the Firmicutes and Bacteriodetes, which are both prominently represented in non-IBD controls. Longitudinal studies are required to determine whether this particular profile (that is, the loss of certain classes of bacteria) can be used as a diagnostic tool to identify people with a greater likelihood of developing IBD. Although our understanding of how dysbiosis might affect IBD is still at its infancy, new sequencing technologies provide the means to analyse the microbiomes of numerous healthy and diseased individuals[75]. With increased knowledge of species-specific alterations during disease, the molecular mechanisms that link dysbiosis of the microbiota to intestinal inflammation can systematically be explored in both animal and human studies.

Beneficial gut bacteria promote homeostasis

The evidence described above implicates the microbiota in shaping immune responses during health and disease, but it is still not known which particular organisms are mediating these beneficial responses and, more importantly, how this is achieved. Here we review the mechanisms by which the microbiota affects the mammalian immune system and the implications for the prevention or treatment of IBD.

In the early 1900s, Ilya Mechnikov was the first to propose the use of live microorganisms to maintain bowel health and prolong life. Now, the term probiotic is used to describe dietary microorganisms that are beneficial to the health of the host[76]. As shown in TABLE 2, many individual or combinations of bacterial species have been shown to ameliorate the symptoms of IBD in humans and mouse models. Although many of these probiotic strains decrease toxic microbial metabolic activities, more recent evidence shows that these organisms can modulate intestinal immune responses. The common feature of almost all bacterial species that are used as probiotics is their ability to control inflammation. Bacterial species can act on several cell types (epithelial cells, DCs and T cells), but recent evidence suggests that the induction of T_{Reg} cells by these microorganisms is crucial to their ability to limit inflammation and disease. Treatment of mice with colitis with the probiotic cocktail VSL#3 increased the production of IL-10 and the percentage of TGFβ-expressing T cells[77]. More importantly, transfer of lamina propria mononuclear cells from VSL#3-treated

Table 2 | **Bacteria shown to be protective in inflammatory bowel disease**

Bacterial strain	Model system	Disease type or model	Mechanism of disease suppression
VSL#3*	Human and mouse	Pouchitis, ulcerative colitis and TNBS-induced colitis	Induction of IL-10- and TGFβ-expressing T cells
Bifidobacteria lactis	Rat	TNBS-induced colitis	Decreased levels of colonic TNF and iNOS
Bifidobacteria infantis	Mouse	*Salmonella enterica*-induced enteritis	Induction of T_{Reg} cells and inhibition of NF-κB activation
Escherichia coli Nissle 1917	Human and mouse	Ulcerative colitis and DSS-induced colitis	Decreased colonic inflammation induced by TLR2 and TLR4 activation
Lactobacillus rhamnosus GG	Mouse and rat	TNBS-induced colitis and HLA-B27-associated colitis	Induction of T_{Reg} cells
Lactobacillus salivarius	Mouse	TNBS-induced colitis	Decreased colonic inflammation
Lactobacillus reuteri	Mouse	IL-10-deficient mice	Upregulation of NGF and decreased levels of IL-8 and TNF in cell lines
Lactobacillus plantarum 299v	Mouse	IL-10-deficient mice	Decreased levels of IFNγ and IL-12p40
Lactobacillus fermentum	Rat	TNBS-induced colitis	Decreased levels of colonic TNF and iNOS
Lactobacillus casei	Rat	TNBS-induced colitis	Decreased levels of colonic cyclooxygenase 2
Bacteriodes thetaiotaomicron	Rat	*S. enterica*-induced enteritis	Decreased levels of IL-8 and TNF in colorectal adenocarcinoma cell line
Bacteriodes fragilis	Mouse	T cell transfer and TNBS-induced colitis	Production of $CD4^+$ T cell-derived IL-10
YO-MIX Y109 FRO 1000‡	Mouse	TNBS-induced colitis	ND
Faecalibacterium prausnitzii	Mouse	TNBS-induced colitis	Decreased levels of NF-κB, IL-8 and TNF and increased IL-10 production

*A mixture of *Lactobacillus* spp. (*Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus acidophilus* and *Lactobacillus delbrueckii* subspecies *bulgaricus*), *Bifidobacterium* spp. (*Bifidobacterium longum*, *Bifidobacterium breve* and *Bifidobacterium infantis*) and *Streptococcus salivarius* subspecies *thermophilus*. ‡A mixture of *S. thermophilus*, *L. acidophilus* and *B. longum*. DSS, dextran sulphate sodium; IFNγ, interferon-γ; IL, interleukin; iNOS, inducible nitric oxide synthase; ND, not determined; NF-κB, nuclear factor-κB; NGF, nerve growth factor; TGFβ, transforming growth factor-β; TLR, Toll-like receptor; TNBS, trinitrobenzene sulphonic acid; TNF, tumour necrosis factor; T_{Reg}, regulatory T.

Symbiont
An organism that lives in association with a host (usually for a lifetime) without obvious benefit or harm to either member.

mice prevented colitis in recipient mice, indicating that the VSL#3 cocktail can initiate the generation of a protective population of cells. Depletion of TGFβ-bearing $CD4^+$ T cells from mice treated with probiotic bacteria before the transfer of lamina propria cells abolished the protective capacity of these cells[77]. More recently, in a model of pathogen-induced inflammation, treatment of mice with *Bifidobacteria infantis* led to a reduction in intestinal inflammation and an increase in the number of $CD4^+CD25^+$ T_{Reg} cells[78]. Adoptive transfer of the $CD4^+CD25^+$ T_{Reg} cell population from mice fed with *B. infantis* inhibited inflammation-induced activation of nuclear factor-κB (NF-κB) in recipient mice.

Naive $CD4^+$ T cells can adopt a regulatory phenotype by interacting with intestinal DCs[36]. Foligne *et al.*[79] show that bone-marrow-derived DCs (BMDCs) internalize *Lactobacillus rhamnosus*, but maintain an immature phenotype. Transfer of BMDCs incubated with *L. rhamnosus* could protect against the induction of intestinal disease[79]. Moreover, depletion of the $CD4^+CD25^+$ T cell subset abolished the ability of the treated DCs to protect against disease, suggesting that *L. rhamnosus*-treated DCs can initiate T_{Reg} cell activity[79]. It has recently been shown that some patients with Crohn's disease have a specific reduction in a prominent gut microorganism, *Faecalibacterium prausnitzii*[80]. Intriguingly, oral administration of *F. prausnitzii* or supernatant from *F. prausnitzii* cultures increased the production of IL-10 by peripheral blood mononuclear cells, reduced the production of TNF in the colon and ameliorated intestinal disease in mice[80]. This seminal study therefore provides evidence for a direct link between a decrease in a particular species from the human microbiota and the development of intestinal disease, suggesting that symbiotic microorganisms might have a direct role in maintaining a healthy gut. The specific molecules produced by these bacterial species remain unknown.

The first demonstration that a single molecule made by a commensal microorganism could promote beneficial immune responses was provided by the identification of polysaccharide A (PSA), which is produced by the human symbiont *Bacteroides fragilis* (FIG. 2). Colonization of germ-free mice with *B. fragilis* or treatment with purified PSA directs the development of the immune system, including the expansion and differentiation of splenic $CD4^+$ T cells[81]. PSA has several immunomodulatory activities in germ-free mice that are colonized by *B. fragilis,* and these include correcting systemic T cell deficiencies, restoring balance between T_H cell subsets and directing lymphoid organogenesis. The importance of *B. fragilis* in maintaining a healthy immune response was recently illustrated by the finding that colonization of germ-free mice by *B. fragilis* or treatment with purified PSA can protect against the induction of experimental IBD[66]. Moreover, mice that are colonized by a mutant form of this microorganism that lacks expression of PSA (*B. fragilis* ΔPSA) are no longer protected from the disease. Furthermore, oral treatment of mice with purified PSA protects recipient animals from weight loss, decreases levels of the pro-inflammatory cytokines TNF, IL-17 and IL-23, and inhibits epithelial cell hyperplasia and neutrophil infiltration to the gut, which are associated with disease induction in these models[66].

To provide insight into the mechanistic basis for PSA-mediated protection, it was shown that increases in the local production of IL-10 were required for the anti-inflammatory properties of PSA. Accordingly, PSA does not protect against the induction of colitis in IL-10-deficient mice, indicating that PSA functions by inducing the production of IL-10. Indeed, $CD4^+$ T cells purified from MLNs during PSA-mediated protection of colitis produced increased levels of IL-10 compared with control mice. In addition, transfer of IL-10-deficient $CD4^+$ T cells into $Rag^{-/-}$ recipient mice abolished the ability of PSA to protect against experimental colitis[66]. These findings indicate that a single bacterial product can stimulate $CD4^+$ T cells to produce IL-10 and lead to the suppression of the inflammatory process during colitis. This suggests that other beneficial bacteria may also produce factors that can positively shape the host immune response in IBD.

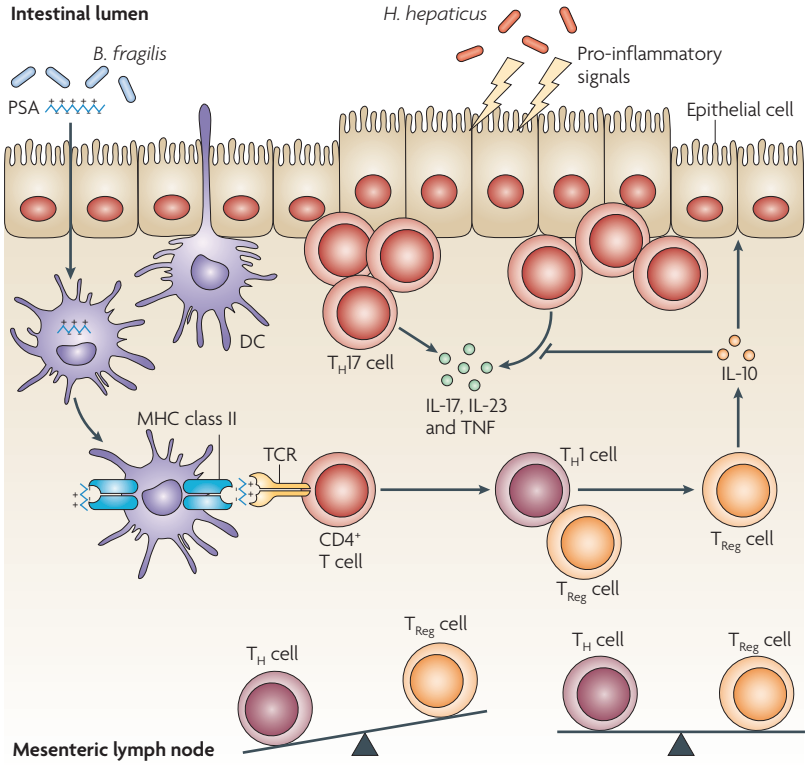

Figure 2 | **Model for *Bacteroides fragilis*-mediated protection from disease induced by *Helicobacter hepaticus*.** *Bacteroides fragilis* produces polysaccharide A (PSA), which induces an immunoregulatory programme that provides protection from inflammation induced by *Helicobacter hepaticus*. PSA is taken up by intestinal dendritic cells (DCs), which presumably migrate to the local mesenteric lymph nodes, where they initiate T cell responses by presenting PSA on MHC class II molecules to $CD4^+$ T cells. Recognition of PSA by naive $CD4^+$ T helper (T_H) cells promotes the induction of anti-inflammatory or regulatory characteristics that include the production of interleukin-10 (IL-10). IL-10 then suppresses the production of pro-inflammatory cytokines (such as IL-17, IL-23 and tumour necrosis factor (TNF)) induced by *H. hepaticus* and protects against the induction of experimental colitis. The balance between the pro-inflammatory T_H17 cell responses to *H. hepaticus* and the regulatory T (T_{Reg}) cell responses to *B. fragilis* supports the control of intestinal inflammation. TCR, T cell receptor.

Symbiosis
A constant and intimate relationship that occurs between dissimilar species, which was originally defined as 'living together'. Although it is often used to describe a beneficial relationship, symbiosis does not necessarily imply that either partner gains an advantage.

For many years, IL-10-producing regulatory T cells (known as T_R1 cells) were considered to be distinct from naturally occurring, thymic-derived $CD4^+CD25^+FOXP3^+$ T_{Reg} cells[82]. It is now apparent that there is overlap between these two populations and that IL-10-producing T_R1 cells can be found in the $FOXP3^+$ T_{Reg} cell subset and are crucial for the control of experimental colitis. Although T_R1 cell clones that are specific for caecal bacterial contents have been generated[83], the ability of a molecule from symbiotic bacteria to regulate $FOXP3^+$ T_{Reg} cell differentiation and function awaits further validation. Nevertheless, current evidence supports that idea that certain beneficial bacteria have evolved molecules (known as symbiosis factors) that induce protective intestinal immune responses. Knowledge of which beneficial species of bacteria can prevent or cure disease, and harnessing the potent immunosuppressive potential of symbiosis factors will be important steps towards designing new and natural therapeutics for IBD.

The microbiota hypothesis and human disease

Does harbouring certain strains of bacteria predispose an individual to disease or protect from it? *B. fragilis* has been shown to protect its host from inflammatory disease caused by subsequent infection with *H. hepaticus* in an animal model of experimental colitis[66]. As symbiotic bacteria seem to have evolved mechanisms to provide protection from colonization by pathobionts that are present in the microbiota, does disease result from the absence of these symbiotic organisms and their beneficial molecules? In other words, if symbiosis factors actively maintain health, can dysbiosis compromise bacterium-mediated immune regulation and lead to inflammation? Recent epidemiological and clinical reports have described marked increases in the incidence of several immune-mediated disorders, such as IBD, asthma, atopy (affecting the skin, respiratory tract and gut), rheumatoid arthritis, type 1 diabetes and multiple sclerosis, in 'Western' populations, including those of western European nations, the United States and Japan. The rapidity of the increase in disease rates does not support a solely genetic basis for these observations[84]. Instead, this could be due to changes in host–microorganism interactions caused by the implementation of antimicrobial strategies (including vaccination, sanitation, Western diets and antibacterial therapeutics) that do not discriminate between infectious and non-infectious microorganisms (FIG. 3).

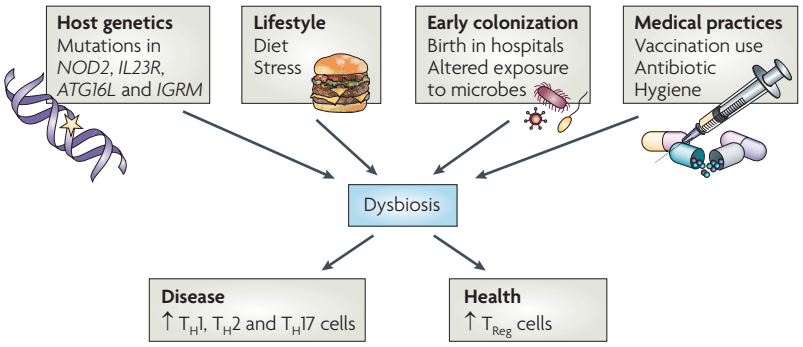

Figure 3 | **Proposed causes of dysbiosis of the microbiota.** We propose that the composition of the microbiota can shape a healthy immune response or predispose to disease. Many factors can contribute to dysbiosis, including host genetics, lifestyle, exposure to microorganisms and medical practices. Host genetics can potentially influence dysbiosis in many ways. An individual with mutations in genes involved in immune regulatory mechanisms or pro-inflammatory pathways could lead to unrestrained inflammation in the intestine. It is possible that inflammation alone influences the composition of the microbiota, skewing it in favour of pathobionts. Alternatively, a host could 'select' or exclude the colonization of particular organisms. This selection can be either active (as would be the case of an organism recognizing a particular receptor on the host) or passive (the host environment is more conducive to fostering the growth of select organisms). Selection of pathobionts by the host could tip the balance in favour of inflammation. Diet and stress also have the potential to influence the microbiota[103]. Birth in the sterile environment of hospitals can protect from exposure to dangerous pathogens, but can also prevent early exposure to health-promoting bacteria. Overuse of vaccination and antibiotics, which do not distinguish between pathogenic or symbiotic microorganisms, could adversely alter the microbiota. *ATG16L*, autophagy-related gene 16-like; *IGRM*, immunity-related GTPase family, M; *IL23R*, interleukin-23 receptor; *NOD2*, nucleotide-binding oligomerization domain 2; T_H, T helper; T_{Reg}, regulatory T.

If improvements in hygiene and health care have altered the process by which a healthy microbiota is assembled and maintained, then patients with these diseases in developed countries should display signs of dysbiosis. This indeed seems to be the case, at least according to a growing number of studies that are now linking these diseases (which are mostly prevalent in Western populations) to alterations in the microbiota. The bacterial composition of the intestines of patients with IBD is known to differ from that of healthy controls[74]. However, studies that aimed to identify the specific pathogenic organism (or organisms) that triggers inflammation have repeatedly identified reactions only to intestinal bacteria that are shared by all humans — healthy and ill. So far, no infectious organisms have been conclusively shown to be the causative agents of IBD. This raises the possibility that the targets of inflammation in IBD are not pathogens and instead are pathobionts that are overrepresented during dysbiosis (FIG. 1). Indeed, in 1999 an investigation of the role of intestinal bacteria in the development of asthma concluded that allergic children from Sweden and Estonia had lower levels of colonization by *Bacteroides* spp. and higher levels of colonization by aerobic microorganisms than non-allergic children from either region[85]. Epidemiological studies have provided evidence for a link between altered intestinal microbiota to other allergic disorders, such as atopic eczema and rheumatoid arthritis[86–88]. Although it is not clear whether dysbiosis is the cause or an effect of disease, it seems that deviations in the composition of the gut microbiota may be one factor underlying the development of disease in genetically predisposed individuals.

On these basis of these recent studies, investigators are now turning their attention to understanding how (and, more importantly, why) mammals harbour multitudes of symbiotic bacteria. As discussed above, the effects of the microbiota on the immune system are becoming increasingly evident. It is therefore of great interest that the immune-mediated disorders, the incidence of which has increased in Western countries, seem to involve reduced T_{Reg} cell activity. Indeed,

studies in animal models and humans indicate that deficiencies in T_{Reg} cell populations or function underlie asthma, IBD, rheumatoid arthritis, type 1 diabetes and multiple sclerosis[89] and that $CD4^+CD25^+FOXP3^+$ T_{Reg} cells can prevent, and in some cases treat, these disorders in laboratory animals. In addition, the observed reduction in the numbers and function of certain T_{Reg} cell populations[45,43], together with numerous other immunological defects that may precipitate disease in germ-free animals, implicates a role for the microbiota in actively supporting health. After millions of years of co-evolution, have societal advances paradoxically and adversely affected human health by reducing our exposure to health-promoting bacteria?

Concluding remarks

Although it has been known for decades that we harbour millions of commensal bacteria, recent studies have only just begun to reveal the extraordinary complexity and diversity of the human microbiota. This consortium of bacteria contains tenfold more cells than the human body, 100 times the number of genes than the human genome and has the metabolic capacity of the human liver[90,91]. How is such a complex microbial network assembled after birth? A recent study[92] on development of the intestinal microbiota in infants revealed that in the first few days to weeks of life, the microbiota of newborns is highly variable and subject to waves of temporal fluctuations (possibly representing a time of sampling or 'trial and error') to coordinately assemble a stable microbiota. The first years of life are also a time of great post-natal development of the immune system. As the microbiota has marked influences on the immune system, deviations from the normal development of the microbiota (through modern strategies such a caesarean section, formula-based diet, hygiene, vaccination and use of antimicrobials in infants) may alter the outcome of immune development and potentially predispose individuals to various inflammatory diseases later in life (FIG. 3).

On the basis of clinical, epidemiological and immunological evidence, it seems possible that changes in the intestinal microbiota may be an essential factor in the incidence of numerous inflammatory disorders. It is conceivable that the absence of beneficial microorganisms (owing to dysbiosis) that promote the appropriate development of the immune system leads to the induction of inflammatory responses and immune-mediated disease. Recent studies have shown that at least for experimental IBD, spontaneous disease occurs when immune suppression is defective; thus, inflammation seems to be a default immunological state in the absence of regulation[93]. Pathogenic bacteria clearly induce local inflammation during acute infections, but have symbiotic bacteria evolved to regulate the inflammatory processes that are harmful to the host (and therefore, harmful to the existence of the symbiont)? Research has implicated innate and adaptive immune suppression during the control of disorders such as IBD, autoimmunity, asthma and allergy, cancer and infectious diseases. According to numerous recent studies, we propose that there is a vast, intricate and unexpected level of interdependence between beneficial bacteria and the immune system. It is possible that genetic and habitual factors shape the composition of the microbiota, which in turn shapes the immune system of individuals that are predisposed to inflammatory disease (FIG. 3). The recent identification of symbiotic bacteria with potent anti-inflammatory properties, and their correlative absence during disease, suggests that certain aspects of human health may depend on the status of the microbiota. The medical and social reconsideration of the microbial world may have profound consequences for the health of our future generations.

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DATABASES

Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
FOXP3 | IFNγ | IL-10 | IL-17 | *IL23R* | IL-35 | *NOD2* | *Tbx21* | TGFβ | TLR9 | TNF

FURTHER INFORMATION

Sarkis K. Mazmanian's homepage:
http://biology.caltech.edu/Members/Mazmanian

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