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# Gastrointestinal tract: the leading role of mucosal immunity

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## Summary

An understanding of mucosal immunity is essential for the comprehension of intestinal diseases that are often caused by a complex interplay between host factors, environmental influences and the intestinal microbiota. Not only improvements in endoscopic techniques, but also advances in high throughput sequencing technologies, have expanded knowledge of how intestinal diseases develop. This review discusses how the host interacts with intestinal microbiota by the direct contact of host receptors with highly conserved structural motifs or molecules of microbes and also by microbe-derived metabolites (produced by the microbe during adaptation to the gut environment), such as short-chain fatty acids, vitamins, bile acids and amino acids. These metabolites are recognised by metabolite-sensing receptors expressed by immune cells to influence functions of macrophages, dendritic cells and T cells, such as migration, conversion and maintenance of regulatory T cells and regulation of proinflammatory cytokine production, which is essential for the maintenance of intestinal homeostasis and the development of intestinal diseases, such as inflammatory bowel diseases. First interventions in these complex interactions between microbe-derived metabolites and the host immune system for the treatment of gastrointestinal diseases, such as modification of the diet, treatment with antibiotics, application of probiotics and faecal microbiota transplantation, have been introduced into the clinic. Specific targeting of metabolite sensing receptors for the treatment of gastrointestinal diseases is in development. In future, precision medicine approaches that consider individual variability in genes, the microbiota, the environment and lifestyle will become increasingly important for the care of patients with gastrointestinal diseases.

***Key words:*** *ulcerative colitis, Crohn's disease, macrophages, dendritic cells, immune system, metabolites, microbiome, metabolite recognition receptors*

**Abbreviations**

| | |
|---|---|
| AhR | aryl hydrocarbon receptor |
| APRIL | a proliferation-inducing ligand |
| cAMP | cyclic adenosine monophosphate |
| CD | cluster of differentiation |
| CYP7A1 | cholesterol 7α-hydroxylase |
| FACS | fluorescence-activated cell sorting |
| FFAR | free fatty acid receptor |
| FGF15 | fibroblast growth factor 15 |
| FMT | faecal microbiota transplantation |
| FODMAP | low fermentable oligosaccharides, disaccharides, monosaccharides, and polyols |
| FXR | farnesoid X receptor |
| GCN | general control nonderepressible |
| GPR | G-protein coupled receptor |
| IBD | inflammatory bowel disease |
| IBS | irritable bowel syndrome |
| IDO | indoleamine-2,3-dioxygenase |
| Ig | immunoglobulin |
| IL | interleukin |
| LPS | lipopolysaccharide |
| MACS | magnetic-activated cell sorting |
| MAIT | cell, mucosal-associated invariant T cell |
| mTOR | mammalian target of rapamycin |
| NcoR1 | nuclear receptor co-repressor |
| NF-κB | nuclear factor kappa-light-chain-enhancer of activated B-cells |
| NO | nitric oxide |
| PPAR | peroxisome proliferator-activated receptor |
| REG | regenerating islet-derived protein |
| RALDH | retinal dehydrogenases |
| rRNA | ribosomal ribonucleic acid |
| SCFA | short-chain fatty acids |
| TAT | T-type amino acid transporter |
| TGF | transforming growth factor |
| TGR | transmembrane G protein coupled receptor |
| Th | T helper cell |
| Th17 | IL-17 producing T helper cells |
| TLR | Toll-like receptor |
| TNF | tumour necrosis factor |
| $T_{reg}$ | regulatory T cell |

## Introduction

Every individual has to eat and drink and most people have experienced symptoms (such as nausea, abdominal pain, vomiting or diarrhoea) that originate from the digestive tract at least at one point during their life. To study, dia-

gnose and treat disorders of the digestive tract, gastroenterology is a medical specialty with a constant need for new technological devices. Advances in imaging technologies, especially the development of high-resolution endoscopes, have revolutionised the way in which gastrointestinal diseases are examined [1]. Nowadays endoscopes are not only used as diagnostic tools, but also for treating patients: to stop bleedings [2], remove polyps [3], dissect pre- or even cancerous lesions and [4] to drain extraintestinal cysts into the gastrointestinal tract [5]. The response to treatments in patients with inflammatory bowel diseases (IBD) is in part evaluated by endoscopy (mucosal healing) [6]. Confocal endomicroscopy can be used to predict relapses by determining cell shedding and barrier loss [7] and predict the response to antitumour necrosis factor (TNF) therapies by detecting *in vivo* fluorescein-labelled anti-TNF antibodies [8]. Even parts of the microflora (i.e., *Helicobacter pylori*) can be visualised with confocal endomicroscopy [9]. However, gastrointestinal diseases, such as IBD, are very complex. IBD is caused by the interplay of various genetic, environmental and immunological factors [10]. IBD manifests in different phenotypes and requires complicated therapies that have to be tailored individually for every patient in an effort to achieve precision medicine [11, 12]. There have been 163 gene loci identified that are associated with IBD [13]. However, only three main loci predict the clinical phenotype of IBD; for example, *NOD2* is associated with ileal Crohn's disease and *HLA* susceptibility is associated with colonic Crohn's disease. No variant is able to predict disease progression, which can so far only be predicted by epidemiological or environmental factors, such as age of disease onset or smoking [14]. Mucosal immunology together with genetics, microbiology and virology is essential to explain interindividual differences between patients [15]. The importance of immunological factors in gastrointestinal diseases is highlighted by the fact that most lymphocytes in the human body are located within the digestive tract [16, 17], where they have to deal with a high abundance of microbial consortia (up to $10^{14}$ organisms, exceeding our own cell number by a factor of 10) [18, 19], viruses and bacteriophages [20]. There is a growing tendency to consider humans as superorganisms consisting of cells and genes of eukaryotic and prokaryotic origin. Both prokaryotic and eukaryotic factors affect our behaviour and physiological processes (uptake and metabolism of food products, utilisation of vitamins, defence against pathogens). Throughout most of our life this superorganism is perfectly adapted to our needs [21]. In rare circumstances, however, the normal function of the superorganism is disturbed and diseases develop. The inability of the mucosal immune system to ignore or tolerate our own microbial flora leads to the development of not only gastrointestinal diseases, such as coeliac disease [22], IBD [23] or gastrointestinal cancers [24], but also to diseases at other body sites, such as rheumatoid arthritis [25], type I diabetes [26], multiple sclerosis [27], nonalcoholic steatosis hepatitis [28, 29], obesity [30] and neurodevelopmental disorders, including autism spectrum disorder [31]. In order to understand the disease course and to help our patients, a detailed analysis of the host mucosal immune system is essential. In this review we outline critical host-microbial interactions and discuss potential future lines of research in mucosal immunity.

## It all starts at or even before birth

As embryos we develop in a sterile intrauterine environment that prevents rejection of the foreign foetus by the mother [32]. The placenta forms a barrier that separates not only the mother's immune system from the foetus, but also prevents the passage of microorganisms into the foetus [33]. However, bacterial products, such as lipopolysaccharides (LPS) or bacterial metabolites are able to cross the placenta to programme the development of the foetus. For example, neutralisation of LPS in stressed animals prevents abortion of the foetus [34]. During birth the baby is exposed to multiple prokaryotic organisms located in the vaginal tract, an overwhelming environmental stressor. Several mechanisms enable the baby to survive the first contacts with microbes [35]: the expression levels of Toll-like receptor 3 (TLR3) [36], the antimicrobial peptides regenerating islet-derived proteins (REG) 3-β and -γ, the α-defensins, the cytokines interleukin (IL)-18 and IL-15 and the proliferation-inducing ligand APRIL are reduced as compared with adults [35]. Conversely, the expression of cathelicidin-related antimicrobial peptide, IL-27, transforming growth factor-β (TGF-β) and thymic stromal lymphopoietin is increased in the neonate [35]. Furthermore, monocyte-derived cells replace yolk sac derived macrophages at the time of birth [37]. CD44- and CD69-expressing T and B cells that populate the lamina propria prevent overwhelming immune responses to colonisation with the bacterial consortia [38]. Activation of CD69 leads to the production of the immuneregulatory cytokine TGF-β and downregulation of the production of proinflammatory cytokines [39]. It needs to be pointed out that the microbial consortia of newborns are not stable at the beginning of the colonisation period. The final composition of the flora depends on the mode of delivery, environmental hygiene status, diet and medication. At birth, facultative anaerobic bacteria, including *Escherichia coli*, *Firmicutes* species and *Staphylococcus* species, dominate in the first few days as shown by the analysis of the newborn's meconium [40]. When oxygen is deprived, the anaerobic bacteria, such as *Bacteroides*, *Clostridium* and *Bifidobacterium,* replace *E. coli*, *Firmicutes* species and *Staphylococcus* species [40]. At the time when the baby is weaned from the mother's milk and regular food is introduced around 1 year of age, the composition of the intestinal flora stabilises. In adults, the dominant bacterial genera are *Bacteroides, Clostridium, Fusobacterium, Eubacterium, Ruminococcus, Peptococcus, Peptostreptococcus* and *Bifidobacterium* with predominance of the *Bacteroides*, *Prevetolla* or the *Ruminococcus* enterotypes [41]. However, the intestinal microflora and the host mucosal immune system need to be seen as one entity, in which the microflora shapes the immune system and in which the immune system influences the composition of inhabitants in our gut. During the neonatal period, the immune system and the intestinal flora form one entity, influencing each other until equilibrium is reached. At this stage, the immune system is primed and the first weeks of life have been

considered as a "window of opportunities" that might impact the development of diseases in later life, such as allergies and asthma [42, 43]. The "foetal programming hypothesis" suggests that microbial metabolites of the mother influence the intrauterine development of the foetus with impact on diseases in later life [44]. The "hygiene hypothesis" proposed that a reduction of the microbial load during the neonatal period and childhood influences the development of autoimmune diseases [45]. Namely, the increased prevalence of IBD [46], atopic and allergic diseases [47] and other autoimmune diseases in industrialised countries have been attributed to the improved hygiene conditions during the neonatal period and vaccination programmes during childhood [45]. Children born in farming conditions rich with microbial antigen load are protected from the development of asthma [48]. These data strongly indicate that the composition of the bacterial flora and the priming of the immune system influence the development of diseases later in life.

## Host-microbe interactions drive intestinal diseases

The outcome of host-microbe interactions depends on (i) the genetic background of the host and (ii) the microbes that challenge us [49]. When a pathogen, such as *Salmonella* or *Yersinia,* is ingested, infectious gastroenteritis will likely develop. There have been increasing numbers of pathobionts described. A pathobiont is a potentially pathogenic organism that lives as a symbiont under normal conditions [50]. In mice, segmented filamentous bacteria are an example of pathobionts, as they drive the generation of IL-17 producing helper T cells (Th17 cells) in the gut, which protects the animals from infection with *Citrobacter rodentium* [51]. However, animals colonised with segmented filamentous bacteria may be prone to develop autoimmune diseases, such as rheumatoid arthritis [25]. In patients with Crohn's disease, increased numbers of adherent-invasive *E. coli* have been observed, which may play a role in driving IBD in genetically predisposed individuals [52]. Nevertheless, one single species has not been identified as the sole driver of IBD. Rather, changes of the intestinal microflora have been observed, such as reduced diversity and increased abundance of mucosal-associated aero-tolerant bacteria [53]. It needs to be pointed out that most studies have investigated microbial consortia in biopsies from the rectum. Although this method may allow prediction of the course of some diseases, it does not represent changes in the upper parts of the digestive tract that occur, for instance, in ileitis [23]. Preparation for colonoscopy disturbs the composition of microbial consortia in the gastrointestinal tract [54] and there is currently no technique available to take biopsies of the upper parts of the gastrointestinal tract in unprepared patients. To overcome these difficulties, researchers have hypothesised that, in contrast to regular commensal bacteria, IBD-driving bacteria induce high affinity IgA responses and become highly coated with IgA [55]. Identification of IgA-coated bacterial genera (fig. 1), together with the genetic characteristics of the host, may in the future provide fingerprints of the changes in individual patients with IBD.

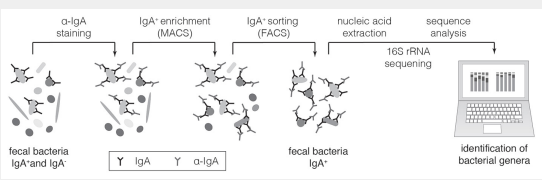


**Figure 1**

**Identification of disease-associated bacterial consortia in the gut.** The bacteria coated with IgA in faecal material can be labelled with an antibody directed against IgA. IgA-coated bacteria are then enriched by magnetic-activated cell sorting (MACS), purified by fluorescence-activated cell sorting (FACS) and sequenced by 16S ribosomal RNA sequencing to identify the IgA coated genera.

## Host responses to the intestinal flora

Conceptually, the host immune system either (i) ignores the microbiota or (ii) actively tolerates the microbiota [17]. This means that constituents of the microbiota are not disseminated into the host, to prevent sepsis. Lamina propria dendritic cells sample intestinal bacteria and transport bacterial-derived antigens to mesenteric lymph nodes, but not further [56]. When the intestinal barrier is breached, as in patients with intestinal inflammation, the liver serves as a second line of defence [57]. In certain circumstances, such as autoimmune uveitis, the intestinal microflora activates autoreactive T cells in a noncognate manner in the gut, inducing autoimmunity at distant sites such as the immune-privileged eye [58]. There is emerging data that microorganisms train innate and adaptive immune cells to deal better with microbial encounters [59]. For instance, exposure to microbial products trains innate immune cells, such as macrophages and intestinal epithelial cells, by epigenetic modifications to tolerate re-exposures [60]. This is an active process by which the innate immune system is able to deal with the microflora. For example, re-exposure of intestinal epithelial cells to LPS leads to diminished cell responses, meaning that "innate tolerance" to bacterial products has developed [61]. Adaptive immunity includes the clonal expansion of T and B cells that provide life-long antigen-specific immune responses. The innate and adaptive immune system adapts to the challenges provided by the intestinal flora in active processes to prevent potential damage to the host.

## Recognition of bacterial products and metabolites by the host

The recognition of bacterial products by the host is a key event, which leads to the initiation of mucosal immune responses [62, 63]. Pattern recognition receptors recognise pathogen-associated molecular patterns – highly conserved structural motifs or molecules that are common to a broad range of microbes. For example, LPS is a common part of the cell wall of Gram-negative bacteria and binds to the Toll-like receptor TLR4 [64]. Recent work indicated that not only the recognition of bacterial motifs but also of bacterial-derived metabolites, produced by the bacteria during adaptation to the local environment in the gut, can modulate immune responses of the host (fig. 2). For ex-

ample, bacteria-derived adenosine triphosphate is recognised by purinergic P2Y and P2X receptors that are expressed by $CD70^{high}CD11c^{low}$ myeloid cells to drive Th17 cell responses in the gut [65]. This can explain the reduced number of Th17 cells in the intestinal lamina propria of germ-free animals [66]. Gut bacteria metabolise dietary fibres in the distal colon to short-chain fatty acids (SCFA), such as butyrate. These SCFA are recognised by the G protein-coupled receptor (GPR) 41 (free fatty acid receptor [FFAR] 3) and GPR43 (FFAR2) [67]. The recognition of SCFA by GPR43 leads to the production of anti-inflammatory cytokines, promotion of regulatory T cells in the mucosa and maintenance of epithelial integrity [68]. In table 1**,** we have summarised receptors that recognise bacterial-derived metabolites.

**Effects of short-chain fatty acid recognition on the host**

SCFA with a carbon length of 2 to 6, such as butyrate, propionate, formate, isobutyrate, valerate, isovalerate and acetate are the result of the bacterial fermentation of fibres, in particular nondigestable polysaccharides [69]. The SCFA are beneficial for the host as intestinal epithelial cells utilise them as an energy resource in mitochondrial respiration [70]. Furthermore, intestinal epithelial cells bathed in SCFA exhibit reduced autophagy [70]. Mitochondrial respiration and regulation of autophagy both help to maintain the integrity of the intestinal barrier and to prevent intestinal barrier breach as, for example, observed in patients with IBD [70]. SCFA are recognised by GPR41 [71], GPR43 [71] and GPR109A [72]. Signalling through GPR43 inhibits inflammatory pathways as demonstrated in GPR43 knockout animals, which develop rheumatoid arthritis, asthma and colitis [68]. SCFA regulate the suppressive functions of regulatory T cells ($T_{reg}$ cells) through expression of GPR43 [68]. Butyrate alone is able to generate $T_{reg}$ cells by histone-3-acetylation of the intronic enhancer region conserved in the noncoding sequence 1 of the forkhead box protein 3 locus, the master transcription factor in $T_{reg}$ cells [73]. GPR109A serves as a receptor for bacterial-derived butyrate and niacin [74]. GPR109A signalling prevents production of inflammatory cytokines by intestinal macrophages, supports IL-10 production by $T_{reg}$ cells and inhibits IL-18 production by epithelial cells [75]. This means that SCFA play a critical role in maintaining host homeostasis and tolerance of the intestinal microflora. This is further highlighted by the fact that SCFA enemas can be used to treat diversion colitis, which is observed in a fraction of patients with diverted colon segments after surgery.

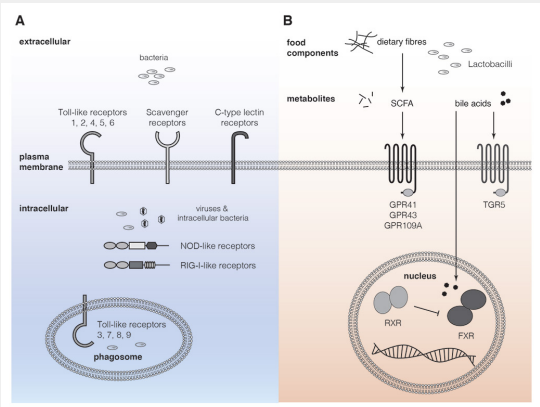


**Figure 2**

Not only conserved structural motifs of microorganisms, but also bacteria-derived metabolites are recognised by the host immune system. A. Pattern recognition receptors, such as Toll-like receptors, the scavenger receptors, the C type lectins, the RIG-I-like or the NOD-like receptors recognise pathogen-associated molecular patterns, which are highly conserved structural motifs or molecules common to microbes and viruses. B. Intestinal bacterial consortia metabolize food products and bile acids to short chain fatty acids (SCFA), secondary bile acids or vitamins. The G protein coupled receptor (GPR) recognizes SCFA, the intracellular farnesoid X receptor (FXR) and the transmembrane G coupled receptor 5 (TGR5) sense secondary bile acids and retinoic acid is recognized by the retinoid X receptor to modulate host responses.

**Recognition of long-chain fatty acids by the host**

Long-chain fatty acids with a carbon length of 13 to 21 can be divided into unsaturated fatty acids, essential and saturated fatty acids [76]. Polyunsaturated fatty acids are transformed to conjugated linoleic acids and trans fatty acids [76]. In germ-free rats, conjugated linoleic acids and trans fatty acids are significantly reduced in the small and large intestine [77]. Modified polyunsaturated fatty acids bind to the intracellular peroxisome proliferator-activated receptor (PPAR)-γ and -α. Binding of polyunsaturated fatty acids to PPAR-γ and -α inhibits nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-κB) activation and production of proinflammatory cytokines [78]. PPAR-γ and -α agonists are used for the treatment of metabolic syndromes, such as hyperlipidaemia and diabetes. Microbiota-dependent metabolism of long-chain fatty acids hence affects the immune system of the host.

**Bile acids and the immune system**

Primary bile acids are synthesised in the liver, stored in the gall bladder, secreted in the small intestine, transformed to secondary bile acids by constituents of the microbiota and reabsorbed in the ileum [79]. In the small intestine, bile acids form micelles, which are required for the digestion of lipids, absorption of vitamins and cholesterol metabolism [79]. Bile acids bind the nuclear farnesoid X receptor (FXR, also known as NR1H4) [80] and the transmembrane G protein-coupled receptor TGR5 [81]. Binding of bile acids to FXR leads to the production of fibroblast growth factor-15 (FGF15) in the ileum [82]. In turn, FGF15 inhibits the bile acid rate-limiting enzyme cholesterol 7α-hydroxylase (CYP7A1) in the liver [82]. Recognition of bile acids by FXR controls the synthesis of bile acids and regulates the available bile pool in the organism [82]. FXR-deficient animals are characterised by bacterial overgrowth, accumulation of neutrophils and disturbance of the mucosal barrier in the ileum [83]. This means that bile acids are an important factor in preventing dysbiosis. Bile acids also modulate the immune response of the host. Two ways in which bile acids modulate the function of macrophages have been described. After binding to the intracellular FXR receptor, the NF-κB-nuclear receptor co-repressor 1 (NcoR1) complex is stabilised and prevents the binding of the transcription factor NF-κB to responsive elements [84]. On the other hand, after ligation of bile acids to transmembrane TGR5, cyclic adenosine monophosphate

(cAMP) is activated and this promotes the interaction of cAMP response element-binding protein with the transcription factor p65 [85]. This interaction prevents the transcription of NF-κB-dependent inflammatory cytokine expression. By this mechanism, TGR5 prevents the development of colitis [86]. In this process, the intestinal microbiota is essential since primary bile acids are transformed to secondary bile acids in the small intestine through microorganisms. Sensing of bile acids is hence of importance for the maintenance of the integrity of the gut. This is further highlighted in the clinic, where bile acid malabsorption after ileocoecal resections or in Crohn`s disease patients with severe inflammation of the ileum is a common problem. This can lead to secondary malabsorption associated with diarrhoea and malnutrition. Bile acid binding agents, such as cholestyramine, or the FXR agonist obeticholic acid have been suggested for the treatment of this condition.

### Vitamins and host responses

Intestinal microorganisms can biosynthesise or modulate the biosynthesis of vitamins [87]. For example *Bifidobacterium* and *Lactobacillus* are able to synthesise vitamin B9 (folate) [88–90]. Vitamins are essential for the normal growth and development of our organism [87] and also influence the immune defence. A well-known example is the vitamin A metabolite retinoic acid [91]. Dietary retinol (vitamin A1) is metabolised by retinol dehydrogenases to retinal, which is further metabolised to retinoic acid by retinal dehydrogenases (RALDH) [91]. Retinoic acid binds to heterodimeric nuclear receptors, which bind to retinoic acid response elements in target genes. Dendritic cells located in the mesenteric lymph nodes and Peyer's patches express the genes *aldh1a2* and *aldh1a1*, which code for RALDH1 and RALDH2 [92]. Retinoic acid synthesised by dendritic cells imprints the expression of the chemokine receptor CCR9 and the integrin α4β7, gut homing receptors responsible for the migration of T cells to the gut [93, 94]. Furthermore, RALDH is predominantly expressed in dendritic cells at mucosal barriers [95]. Infections at barrier sites lead to increased RALDH activity [96]. Retinoic acid supports the TGF-β-dependent conversion of naïve T cells into $T_{reg}$ cells [97], promoting immune tolerance at mucosal sites. After stimulation of $T_{reg}$ cells with retinoic acid reduced expression of the receptor for IL-6 is observed, a cytokine required for the generation of IL-17-producing Th17 cells [98]. Retinoic acid also promotes Th1 differentiation [99], restrains Th1 plasticity (by inhibition of Th17 fate) [99] and is required for Th2 responses to parasite infections [96, 100].

Other dietary vitamins, such as vitamin B12, also modulate the immune system. Reduced lymphocyte numbers are observed in vitamin B12-deficient patients [101]. Natural killer cell activity is regulated by vitamin B12 [101]. Vitamin B2 (riboflavin) activates mucosal invariant T cells (MAIT cells) [102]. Vitamin D deficiency is associated with IBD susceptibility and colitis-associated cancer [103]. Animal studies have shown that the expression of the vitamin D receptor by intestinal epithelial cells protects the gut from inflammation and carcinogenesis [104].

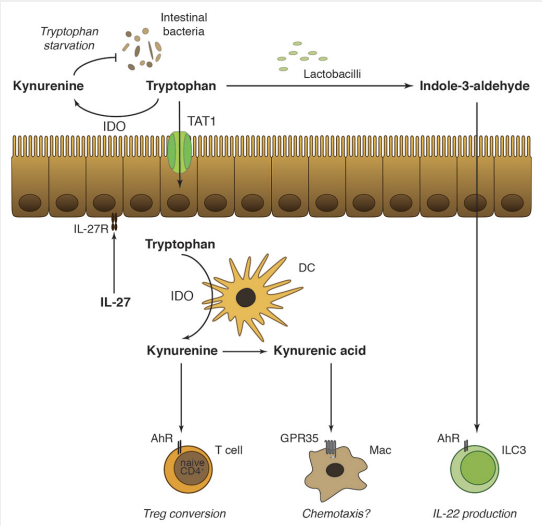


**Figure 3**

**Constituents of the gut microflora and immune system of the host metabolise the essential amino acid tryptophan.** The essential amino acid tryptophan is shuttled via the T-type amino acid transporter 1 (TAT1) across the intestinal barrier, metabolized by indoleamine-2,3-dioxygenase (IDO) expressed in dendritic cells (DC) to kynurenine and kynurenic acid. Kynurenine supports the conversion of T cells to regulatory T cells (Treg); kynurenic acid binds to the G-protein coupled receptor (GPR) 35 expressed by macrophages (Mac). Tryptophan is also metabolized by constituents of the microflora to indole-3-aldehyde, which binds to the aryl hydrocarbon receptor (AhR) facilitating IL-22 production by innate lymphoid cells (ILC)

### Sensing of amino acids by the immune system

The twenty-two amino acids taken up by our bodies from the diet are used to synthesise proteins and are also a source of energy after oxidation to urea and carbon. Nine amino acids are called essential amino acids, because our body cannot synthesise them. Amino acids metabolised by the intestinal microflora can influence the immune system [105]. The essential aromatic amino acid tryptophan is metabolised in the gut by lactobacilli to indole-3-aldehyde (as summarised in fig. 3) [106].Tryptophan is also transported across the intestinal barrier by the T-type amino acid transporter TAT1 (Slc16a10) [107]. Tryptophan is metabolised by indolamin-2,3-dioxygenase (IDO) expressed by intestinal macrophages and dendritic cells to kynurenine [108]. Kynurenine can be further metabolised by the kynurenine transferases I, II and III to kynurenic acid [105]. The tryptophan metabolites indole-3-aldehyde and kynurenine are recognised by the aryl hydrocarbon receptor (AhR) [109]. Kynurenic acid is recognised by the G-coupled receptor GPR35 [110]; the kynurenine metabolite niacin is sensed by the G-coupled receptor GPR109A [74]. Tryptophan is hence consumed from the local tissue-specific microenvironment by the IDO pathway [111], which is modulated by the cytokine IL-27 [112]. Reduced tryptophan concentrations activate cellular stress response pathways, such as the general control nonderepressible (GCN)-2-kinase and the mammalian target of rapamycin (mTOR) pathways [113]. In addition, tryptophan metabolites are recognised by specific receptors, such as the AhR receptors and the GPR35 receptor [111]. Overall, the activation of IDO-dependent pathways favours immune sup-

pression and tolerance [105, 114]. In the gut, the IDO pathway suppresses colitis [115].

There are also other amino acids involved in the regulation of immune response [76]. Besides its role in the hepatic urea cycle, arginine is also a substrate for nitric oxide and arginase, which are expressed by myeloid cells and granulocytes [116, 117]. Granulocyte-derived arginase controls chronic infections by inhibiting cytokine production by T cells and T cell proliferation [118]. Thus, amino acids, the host immune response and intestinal microorganisms have likely developed multiple pathways by which they interact with each other. We are currently just beginning to dissect the different pathways and do not well understand the importance of these interactions for the development of diseases, such as IBD.

## Macrophages express metabolite-sensing molecules

When the expression of metabolite-sensing molecules are analysed in available databases, it is striking that not only the gut epithelium but also macrophages have high expression levels of the metabolite sensing receptors GPR35 and GPR120, and the purinergic receptors P2Y and P2X. Located right beneath the intestinal epithelium with the ability to extend processes between intestinal epithelial cells into the intestinal lumen, phagocytes are optimally positioned to sense metabolites derived from the intestinal microbiota [119]. Intestinal macrophages are continuously replaced by monocyte-derived cells [17]. In adults, macrophages are low proliferating cells and produce immunosuppressive cytokines, such as IL-10, in homeostatic conditions [120]. Intestinal macrophages are highly phagocytic cells with bactericidal activity enabling them to kill bacteria and other microorganisms that have entered the host [121]. They do not release proinflammatory cytokines after engulfing a microorganism to avoid uncontrolled inflammation in the gut [122]. Recent work has highlighted the importance of GPR35, variants of which have been associated with IBD [123]. Along the intestinal tract the highest concentrations of this receptor are observed in the ileum. In the same region, intestinal macrophages have processes that enter the intestinal lumen to sense microbes and microbial-derived metabolites [124]. GPR35 is also the receptor for the chemokine CXCL17 (dendritic cell- and monocyte-attracting chemokine-like protein) [125]. Small and large intestinal tissues express CXCL17. It is likely that GPR35 may position macrophages in the gut to sample and recognise bacterial derived products and metabolites. However, formal proof of the importance of GPR35 for the migration of monocyte-derived cells has not been shown. The development of knockout cell lines and mouse strains could be potentially of great value for the dissection of the function of GPR35 in intestinal tissues.

**Table 1:** Metabolite-sensing receptors and their ligands (adapted from references [69, 141]).

| Receptor / intracellular target | Cell | Ligand | Origin | Effect |
|---|---|---|---|---|
| GPR41 / FFAR3 | Adipocytes, enteroendocrine L cells | Formate, acetate, propionate, butyrate, pentanoate | Commensal bacteria (digestion of fibres) | DC maturation, leptin production, regulation of energy balance |
| GPR43 / FFAR2 | Innate immune cells, enteroendocrine L cells, intestinal epithelial cells, white adipose tissues | Formate, acetate, propionate, butyrate, pentanoate | Commensal bacteria (digestion of fibres) | Gut homeostasis, tumour suppressor, anti-inflammatory |
| GPR109A (NIACR1, HM74) | Macrophages, intestinal epithelial cells, neutrophils, adipocytes | Butyrate and nicotinic acid (niacin) | Commensal bacteria (digestion of fibres, tryptophan metabolism) | DC migration, Gut homeostasis, tumour suppressor, anti-inflammatory |
| GPR120 (FFAR4) | Macrophages, enteroendocrine cells in the colon | Long-chain fatty acids | Diet, metabolism of commensals | Inhibition of TNF and IL-6, insulin secretion |
| GPR40 | Beta-cells (pancreatic islets), enteroendocrine K cells | Medium- to long-chain fatty acids | Diet, metabolism of commensals | Insulin secretion, anti-inflammatory |
| GPR84 | Immune cells | Medium-chain fatty acids | Diet, metabolism of commensals | Not known |
| GPR35 | Monocytes/macrophages, NKT cells, mast cells | Kynurenic acid, kysophosphatidic acid, pamoic acid, CXCL17 | Tryptophan metabolism | Associated with IBD |
| GPR91 (SUCNR1) | DCs, kidney, adipose tissues | Succinate | Succinate | Proinflammatory, haematopoiesis, activation of the renin angiotensin system |
| IDO1 | Macrophages, DCs | Tryptophan | Diet, metabolism of commensals | Promoting $T_{reg}$ differentiation |
| Purinergic (P2Y and P2X) receptors | Myeloid cells | ATP | Commensals | Th17 differentiation |
| FXR, TGR5 | Epithelial cells | Secondary bile acids | Bile acid metabolites through commensals | Promotion of $T_{reg}$ cells, inhibition of Th17 cells, anti-inflammatory properties |
| Retinoid acid receptors | DCs | Retinoic acid | Diet | Gut homing of T cells |
| FR (B9) | $T_{reg}$ cells | Vitamins B9 and B12 | Commensal microbiota | Maintenance of $T_{reg}$ cells |
| Peroxisome proliferator-activated receptors gamma and alpha | Intestinal epithelial cells | Polyunsaturated fatty acids | Commensal microbiota | Inhibition of NF-κB pathway |

ATP = adenosine triphosphate; DC = dendritic cell; FR = folate receptor; FXR = farnesoid X receptor; GPR = G protein coupled receptor; IBD = inflammatory bowel disease; IDO = indolamine 2,3-dioxygenase; IL = interleukin; NKT cell = natural killer T cell; TGR5 = transmembrane G coupled receptor 5; Th17 = IL-17 producing T helper cells; TNF = tumour necrosis factor; $T_{reg}$ = regulatory T cells

We have summarised the complex networks that regulate the recognition of microbial metabolites by the host. In the next section we discuss strategies targeting those for potential therapeutic interventions in the clinic.

## Clinical perspectives of potential interventions on interactions of microbial-derived metabolites and the host

At the moment, most treatments used for intestinal diseases, such as IBD, modulate the immune response of the host. Steroids, thiopurines, TNF antagonists and integrin blockers act in this way [126]. In future, direct interventions on host-microbe interactions may be an attractive option for the treatment of intestinal diseases. Currently, there are several ways being discussed on how host-microbe interactions can be influenced for therapeutic strategies, such as the modification of the diet, the application of antibiotics, the administration of probiotics and faecal microbiota transplantation. In general, first clinical trials are being undertaken to investigate these interventions in certain conditions, such as IBD and irritable bowel syndrome (IBS). However, information on detailed molecular mechanisms is still in its infancy. In a crossover study with IBS patients, a diet with low fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAPs) reduced gastrointestinal symptoms as compared with a regular diet [127]. FODMAPs define a group of short-chain carbohydrates, such as oligosaccharides (fructans and galactans), disaccharides (lactose), monosaccharides (fructose, glucose in excess), and polyols (sorbitol, mannitol) that are incompletely resorbed in the small intestine and then fermented in the colon [128]. A FODMAP diet is associated with reduced numbers of *Bifidobacteria* in the intestinal microbiota. The absolute numbers of bacteria in individuals on the FODMAP diet was reduced; the numbers of butyrate-producing bacteria (*Akkermansia muciniphila, Ruminococcus gnavus*) and the *Clostridium* cluster XIVa were decreased [129]. It is likely that bacterial-derived metabolites in individuals with a FODMAP diet are reduced, which affects immune responses, motility and nociception.

### Antibiotics

Antibiotics can be used for the treatment of patients with IBD [130], IBS [131], small intestinal bacterial overgrowth [132] and pouchitis [133]. In a multicentre double blinded study in patients with moderately active Crohn's disease, treatment with the nonabsorbable antibiotic rifaximin induced remission in 62% of patients [130]. In the placebo-treated group 43% of patients were in remission after the 12-week follow-up period [130]. Most patients with Crohn's disease need intestinal resection at some point during the course of their disease. Treatment of patients with metronidazole for 3 months after resection may prevent recurrence of the disease [14].

When these patients receive active care with early endoscopy after 6 months, the therapy can be adapted to the endoscopic findings with thiopurines or TNF antagonists. The active care group in this study showed a better outcome than patients not receiving early endoscopy [14]. In two double-blinded placebo-controlled trials in patients with IBS without constipation, the patients received rifaximin at a dose of 550 mg three times daily for 2 weeks and were followed up for a 10-week post-treatment period [131]. Patients treated with rifaximin showed a significant reduction of symptoms, especially bloating. This means that a short period of treatment with rifaximin leads to a sustained benefit in IBS patients without constipation. However, the use of antibiotics in patients with chronic intestinal conditions needs to be considered carefully. In particular, the occurrence of antibiotic resistance needs to be taken into account. In current clinical practice we do not test changes in the composition of the microbiota in patients treated with antibiotics. In treatment-naïve paediatric Crohn's disease patients, the analysis of rectal biopsies revealed an increased abundance of *Enterobacteriaceae, Pasteurellacaea, Veillonellaceae,* and *Fusobacteriacea*e, and decreased abundance in *Erysipelotrichales, Bacteroidales,* and *Clostridiales* [23]. The degree of dysbiosis correlated with disease activity. Earlier use of antibiotics amplified the dysbiosis associated with Crohn's activity. This means that the careful analysis of patients and defining of subgroups will be required in future to identify the individuals who benefit from treatments with antibiotics and to avoid treatments in patients who will not respond or even relapse.

### Probiotics

Preparations of live bacteria are effective for the treatment of IBD. For example, *E. coli* Nissle 1917 can be used for the induction and maintenance of remission in patients with moderate ulcerative colitis with the same efficacy as treatment with mesalazine [88]. VSL#3 probiotic mixture contains eight different *Lactobacillus* strains (*Lactobacillus acidophilus, L. bulgaricus, L casei, L. plantarum, Streptococcus thermophilus, Bifidobacterium breve, B. infantis, and B. longum*). Randomised controlled clinical trials showed that VSL#3 could prevent recurrence of pouchitis in chronic relapsing pouchitis [134]. The addition of VSL#3 to mesalazine and steroids in newly diagnosed ulcerative colitis patients may induce higher remissions rates [135]. In general, probiotics are considered as safe medications, but they also can harm. When germ-free immunocompromised animals are mono-colonised with *E. coli* Nissle, the animals die within 10 days [136]. *E. coli* Nissle is characterised by the expression of H1 flagella and type 1 fimbriae that mediate adhesion to epithelial cells. To survive in the gastrointestinal tract with its dense population of microbes, a probiotic needs to be extremely fit. Perhaps the H1 flagella and type 1 fimbriae offer *E. coli* Nissle an advantage in this particular microenvironment, but may also explain the potential pathogenicity in selected conditions [137]. Most strains do not colonise and are just expelled. This means that probiotics needs to be given repeatedly and may explain disappointing results in clinical trials.

### Faecal microbiota transplantation

Faecal microbiota transplantation (FMT) is successful for the treatment of recurrent *Clostridium difficile* infections [138]. There have been case reports that FMT is also be-

neficial for other gastrointestinal disorders associated with dysbiosis, such as IBD. Two recent randomised controlled studies have reported the efficacy of FMT for the treatment of ulcerative colitis [139, 140]. In one study, remission was observed in patients with mild to moderate active ulcerative colitis [139]. Seventy-five individuals received weekly FMT from six donors or placebo (water) via retention enema. Interim analysis showed a negative result. The additional analysis of 22 participants, who had already been enrolled into the study, after the interim analysis, gave a significant positive outcome for the endpoint remission. These 22 participants had received the FMT from one donor. This indicates that the faecal microbiota transplants vary significantly between donors and influences the results of the transplantation. In the study by Rossen et al. patients with mild to moderate active ulcerative colitis were treated with donor stool or autologous FMT (infusion of their own stool) via a nasoduodenal tube [140]. There was no difference between the two groups [140]. The different route of administration of the FMT (nasoduodenal tube vs enema) and the fact that immunosuppression was allowed may explain the different outcome of the study by Moayyedi et al., compared with the study by Rossen et al. [139, 140]. The quantity and quality of species reaching the colon may be altered after administration of FMT via naso-duodenal tube. Also, immunosuppression may facilitate the engraftment of the transplanted microbiota. We still do not have enough information on transplantation of the microbiota and how their metabolites affect the host. In complex intestinal diseases, such as IBD, the effects of FMT seem to be less beneficial than for *C. difficile* infections, because the underlying immune mechanism in the genetically predisposed host may affect the mcicrobiome. Whether the transplant perpetuates in the recipient is not known. The success of the transplantation depends on the composition of the microbiota in the donor. However, a healthy donor flora is not defined. This means that the criteria for selecting appropriate donors have not been established yet.

## Conclusions

Advances in high-throughput sequencing technologies have helped us to understand the complexity of the intestinal microbiome. We begin to appreciate that not only the microorganisms by themselves but also the metabolites of these organisms affect the functions of our own bodies. Recent research has started to elucidate the receptors by which the host recognises the metabolites produced by constituents of the microbiome. However, the acquired knowledge on host-microbiome relationships is only starting to be used in clinical practice for the treatment of intestinal diseases, such as IBD. In future, the development of novel ligands or antagonists of metabolite-recognition receptors may offer interesting approaches for the treatment of intestinal diseases.

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## Figures (large format)

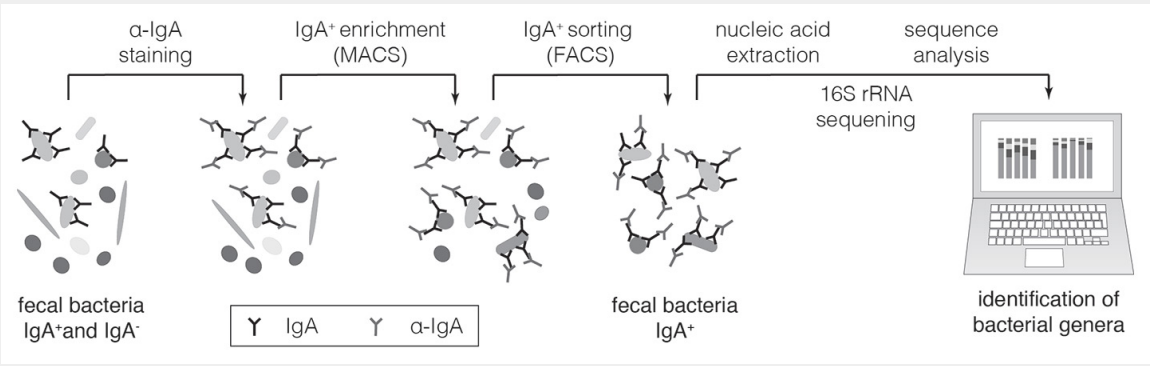


**Figure 1**

**Identification of disease-associated bacterial consortia in the gut.** The bacteria coated with IgA in faecal material can be labelled with an antibody directed against IgA. IgA-coated bacteria are then enriched by magnetic-activated cell sorting (MACS), purified by fluorescence-activated cell sorting (FACS) and sequenced by 16S ribosomal RNA sequencing to identify the IgA coated genera.

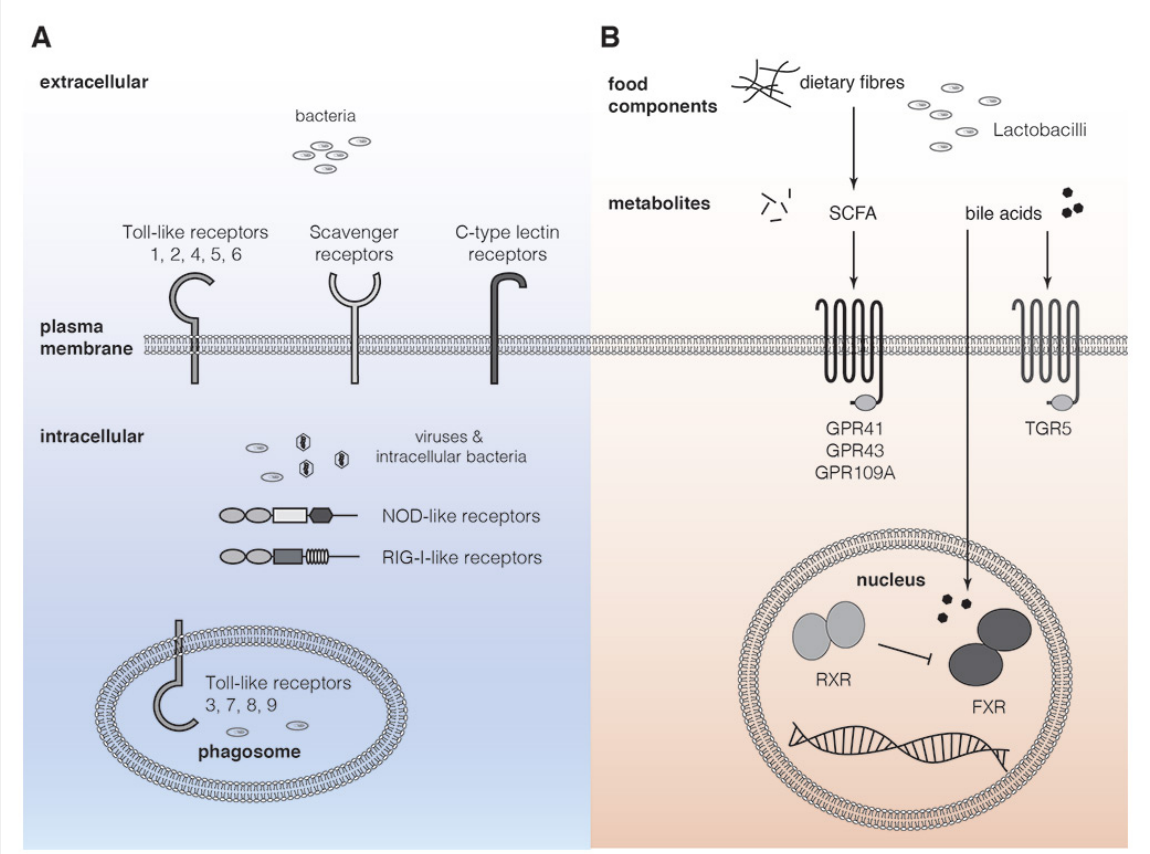


**Figure 2**

Not only conserved structural motifs of microorganisms, but also bacteria-derived metabolites are recognised by the host immune system. A. Pattern recognition receptors, such as Toll-like receptors, the scavenger receptors, the C type lectins, the RIG-I-like or the NOD-like receptors recognise pathogen-associated molecular patterns, which are highly conserved structural motifs or molecules common to microbes and viruses. B. Intestinal bacterial consortia metabolize food products and bile acids to short chain fatty acids (SCFA), secondary bile acids or vitamins. The G protein coupled receptor (GPR) recognizes SCFA, the intracellular farnesoid X receptor (FXR) and the transmembrane G coupled receptor 5 (TGR5) sense secondary bile acids and retinoic acid is recognized by the retinoid X receptor to modulate host responses.

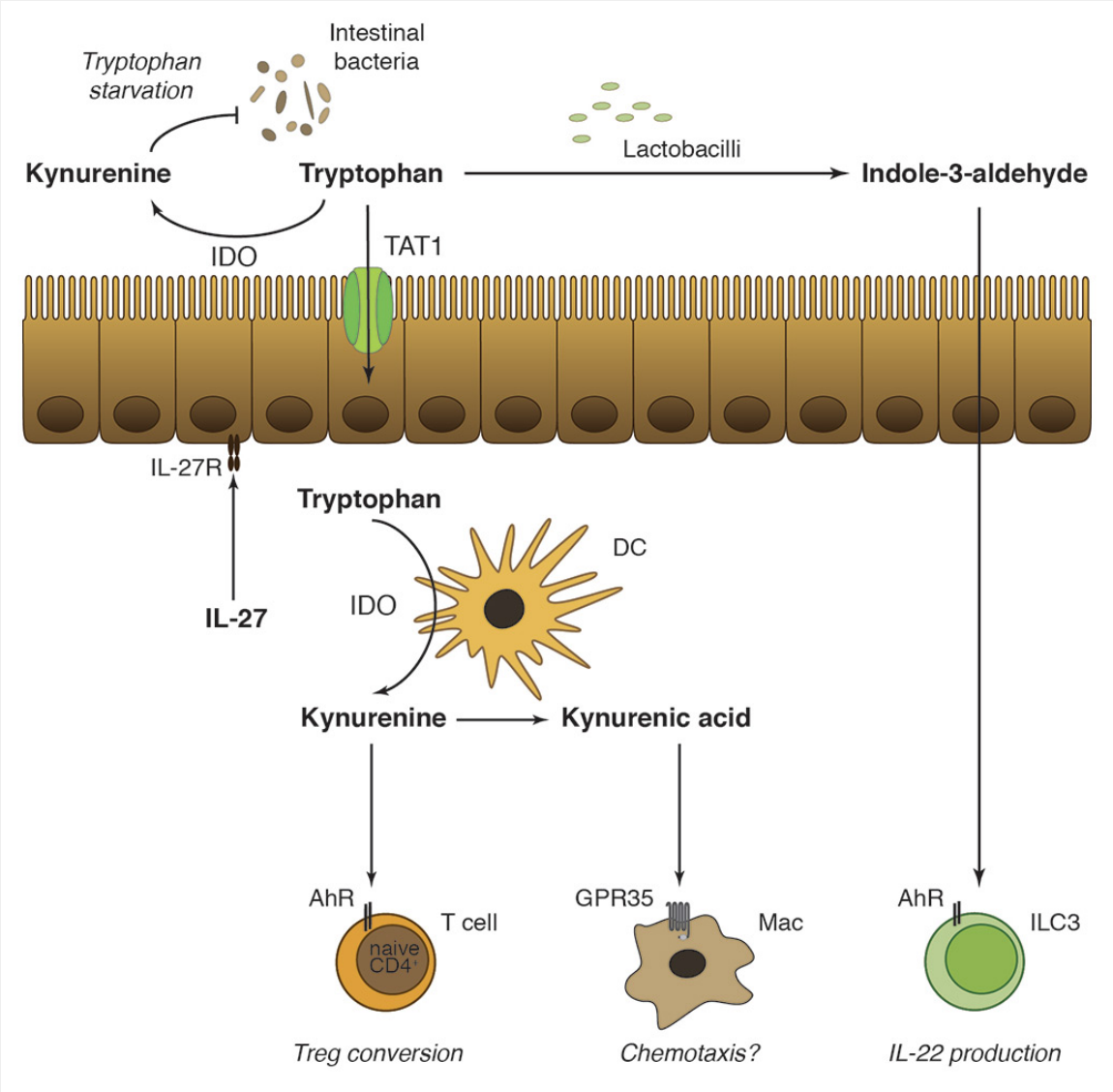


**Figure 3**

**Constituents of the gut microflora and immune system of the host metabolise the essential amino acid tryptophan.** The essential amino acid tryptophan is shuttled via the T-type amino acid transporter 1 (TAT1) across the intestinal barrier, metabolized by indoleamine-2,3-dioxygenase (IDO) expressed in dendritic cells (DC) to kynurenine and kynurenic acid. Kynurenine supports the conversion of T cells to regulatory T cells (Treg); kynurenic acid binds to the G-protein coupled receptor (GPR) 35 expressed by macrophages (Mac). Tryptophan is also metabolized by constituents of the microflora to indole-3-aldehyde, which binds to the aryl hydrocarbon receptor (AhR) facilitating IL-22 production by innate lymphoid cells (ILC)