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Host–microbe interactions: the difficult yet peaceful coexistence of the microbiota and the intestinal mucosa

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Abstract

The immune system has evolved to live in a collaborative relationship with the microbiota, while still serving its seminal function to fight off invasive pathogenic bacteria. The mechanisms that rule the interactions between the intestinal microbiota and the intestinal immune system are the focus of intense research. Here, we describe how the innate immunity is, to a great extent, in charge of the control of the microbiota in the intestine and relies on non-specific receptors called pathogen-recognition receptors. While the microbiota has a well-defined effect on the host immune homoeostasis, it has become clear that the opposite is also true, i.e., the mucosal immune system has the capacity to shape the microbial population. The mechanisms that rule the reciprocal regulation between host immunity and commensal bacteria (including specific bacteria) are currently being elucidated and will be described here. A better knowledge of how the host and bacteria interact and how the intestinal microbiota and the immune system are co-regulated will provide the basis for a better understanding of intestinal and systemic immunopathologies and for the development of new therapeutic approaches.

Key words: **Host–microbe interactions: Intestinal microbiota: Innate immunity: Pathogen-recognition receptors**

The classical immunological concept viewed micro-organisms as pathogens that cause and propagate disease. Therefore, the host immune system should recognise and eliminate them while tolerating self-molecules to preserve homoeostasis. However, many bacteria are not pathogenic and can behave as commensal (innocent bystanders), or even offer a benefit to the host. Among the body sites with significant presence of prokaryotes, the gastrointestinal tract stands out as the home of trillions of micro-organisms, which may include a combination of commensal, harmful and beneficial strains at any given time point. Despite the enormous bacterial load carried by the gastrointestinal tract and the sheer variety of species present, an exquisite balance is maintained at almost all times. The intestine is a comfortable place for bacteria, which are provided with a stable microenvironment rich in nutrients, and these in turn provide the host with essential nutrients (such as vitamin K or biotin), collaborate to aid in the digestion of food, prevent the expansion of pathogenic micro-organisms and even cooperate in intestinal development and in the modulation of the host immune responses. In consequence, the immune system has evolved to live in a collaborative relationship with the microbiota, while still serving its seminal function to fight off invasive pathogenic bacteria.

The aim of the present study is to describe the mechanisms that rule the interactions between the intestinal microbiota and the intestinal immune system, and to describe the reciprocal regulation between host immunity and commensal bacteria, which allows the host to shape intestinal microbiota and the commensal bacteria to regulate host immune homoeostasis.

Alone or in the company of others

It is well known that we men travel with a heavy luggage made up of approximately 10^{14} prokaryotic organisms, mostly bacteria but also viruses and fungi. That is, every one of us has about ten micro-organisms per each 'own' eukaryotic cell. This extra weight is not uniformly scattered in our body, but distributed in well-defined areas: the skin, the conjunctiva, the vagina, the upper respiratory tract and, especially, the

Abbreviations: DSS, dextran sodium sulphate; GF, germ-free; IEC, intestinal epithelial cells; NLR, nucleotide-binding and oligomerisation domains-like receptors; NOD, nucleotide-binding and oligomerisation domains; PPR, pathogen-recognition receptors; SFB, segmented filamentous bacteria; Th, T helper; TLR, Toll-like receptors; Treg, regulatory T cells.

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gastrointestinal tract. The latter is the home of the largest bacterial population, which is maximal in the cecum, followed by the colon and then ileum, jejunum and finally duodenum[1]. All these sites are characterised by direct or indirect contact with the outer world, which is ultimately limited, and controlled, by specialised surfaces called mucosas.

The realisation of this fact immediately prompts the question, what are these germs for? The answer to this question is not as easy as it might seem. The study of laboratory animals in germ-free (GF) conditions, available now for approximately 50 years, soon revealed that mice and rats survive perfectly well without bacteria. Reproduction and overall appearance and physiology are essentially normal. In fact, it was shown early on that GF mice survive much longer than the conventionally reared mice[2], and this seemed to be the case also for rats[3]. This effect may be dependent on age, so that absence of bacteria at an early age extends life, while it may shorten it at later stages[4].

GF conditions are also related to lower oxygen consumption and metabolic rate. In fact, body growth is affected in rats beginning at 6 months of life, when GF animals become less efficient at thriving and stabilise at 85% of the normal weight[5]. These effects resemble those of food restriction (it should be noted that GF rats spontaneously show reduced food intake), and this circumstance prompted investigators to formally compare the two conditions in a large study conducted with rats[3]. It was shown that both dietary restriction and germ exclusion prolong life, and further significant benefits could be obtained by combining the two conditions.

The influence of bodily bacteria, and specifically gut microbiota, at different levels has been the subject of increasing attention and a number of effects have been now established. Areas affected include brain development and behaviour, metabolism, obesity, food digestion and overall resistance to stress and injury[6,7]. Thus, while bacteria are not necessary for our body, they have a profound (albeit subtle) effect on us. At any rate, we live in a world with bacteria, and so the natural consequence is to be colonised by them to a certain extent. But, how do we contain bacteria in these selected sites?

The intestinal barrier – sensing bacteria by the intestinal immune system

First, and foremost, the intestinal mucosa is a physical barrier that separates the lumen, which is in contact with the outside world, from the internal medium. The core of this barrier is the intestinal epithelium, a monolayer made up of two main cell types: enterocytes and goblet cells. The former are majoritary and are specialised in transport functions, while the latter are mucus secretory cells. Intestinal epithelial cells (IEC) are sealed by tight junctions. Bacteria, viruses and fungi are efficiently restricted to the lumen by this barrier, and its efficiency is enhanced by the addition of functional and immune factors. Intestinal motility, especially in the colon, influences the luminal population mostly by maintaining an appropriate flux, i.e., by facilitating bacterial removal in faeces (bacteria constitute approximately 50% of faecal dry weight). Failure of this mechanism may lead, in certain circumstances, to toxic megacolon, a critical condition characterised by bacterial overgrowth and risk of sepsis and intestinal perforation. Another important element is the secretion of mucus by goblet cells, creating a microenvironment in close proximity to the epithelial surface that limits bacterial contact both physically and chemically, by acting as decoy ligands for bacterial receptors.

As in the rest of the body, the intestinal mucosa is provided with innate and adaptive immune responses, but they have specific characteristics. One of them is that the immune response is mediated by both cells in the intestinal epithelium and the *lamina propria,* i.e., the layer located immediately underneath the epithelium. The intestinal epithelium is composed of four different cell types of epithelial lineage, the already mentioned enterocytes and goblet cells, plus the hormone-producing enteroendocrine cells and Paneth cells. Enterocytes and Paneth cells are the main players in the immune area. Paneth cells, located at the base of the crypts, produce antimicrobial peptides, thus limiting bacterial presence at the crypt space. Enterocytes are important players but their role is far from being well defined. As explained later, they may help shape the immune response in a number of ways, and they can also react directly with bacterial products because of their privileged position. In addition, the epithelium overlaying mucosal lymphoid follicles (called Peyer's patches in the small intestine) is composed of specialised M cells, which display atrophied transport capacities and instead act as dedicated sampling instruments, passing luminal antigens into the follicle through transcytosis. Oddly enough, intestinal pathogens usually target these cells as point of entry to the mucosa, and this may be also the case for probiotic strains[8–10]. However, it is likely that minute passage of bacteria at these and other points occurs normally to facilitate some degree of host–microbiota contact[11].

Underlying the intestinal epithelium, dendritic cells and macrophages in the *lamina propria* contribute decisively to the innate immune response. Dendritic cells' cytoplasmic extensions are interdigitated among the epithelial cells in order to sample antigens and present them to T cells in the *lamina propria* and the underlying lymphoid follicles. Dendritic cells can also travel to draining lymph nodes to interact with T cells. Interspersed in the intestinal epithelium there are specific T cells (intraepithelial lymphocytes) that, together with the Peyer's patches/lymphoid follicles and *lamina propria* T cells and B cells (mainly IgA-producing B cells), form the intestinal adaptive immune system.

The intestinal mucosa maintains a state of so called 'physiological inflammation', i.e., a low level activation of immune cells with infiltration of the lamina propria but devoid of clinical symptoms. This is a direct consequence of the presence of bacteria, as it is absent in GF animals. Another key difference here is epithelial turnover, which is normally quite high (the epithelium is entirely renewed every 5–7 d) and substantially reduced in GF conditions.

Innate immunity in the intestinal mucosa

The innate immunity is, to a great extent, in charge of the control of the microbiota in the intestine. Innate immunity in the

intestine and elsewhere relies on non-specific receptors, as opposed to the specific recognition of antigens used by the adaptive arm of the immune system (Fig. 1). These receptors were initially called pathogen-recognition receptors (PPR) and bind pathogen-associated molecular patterns, i.e., not specific molecules but types of molecules whose structure differs substantially from eukaryotic ones. However, these are not associated with pathogenicity, and the denomination of microbial-associated molecular patterns was suggested instead. The picture has been complicated further by the realisation that these receptors can in fact bind internal structures, which are produced specially in the context of tissue damage and inflammation, and therefore are referred to as damage-associated molecular patterns. These terms are used exchangeably.

PPR comprise Toll-like receptors (TLR), nucleotide-binding and oligomerisation domains (NOD)-like receptors (NLR) and the helicase family (retinoic-inducible gene I and differentiation-associated gene or melanoma differentiation-associated protein 5 (MDA5)). These receptors activate signalling cascades that finely tune the production of antimicrobial products and cytokines, depending on the signals delivered by the microbiota[12]. As shown later, PPR signalling helps to regulate antigen-specific adaptive immune response. The best studied PPR are TLR and NLR.

Toll-like receptors. TLR are type I transmembrane proteins expressed by innate immune cells of the intestinal epithelium and the *lamina propria*, either at the cell surface or in endosomes. TLR consist of at least eleven members in men that recognise not only microbial components, including proteins, lipids and nucleic acids derived from bacteria, viruses and parasites, but also damaged host cell components such as nucleic acids and other 'internal' ligands. Cell and molecular localisation of TLR together with their ligands are shown in Table 1.

When examining TLR function in the intestine, one is confronted with the fact that innate immune cells, including enterocytes, express these receptors and are obviously exposed to an endless supply of ligands, yet no inflammatory response develops. Hence, TLR-mediated responses in the intestine are finely regulated. TLR are involved in intestinal homoeostasis, including the regulation of the epithelial barrier, by modulating the production of IgA, the maintenance of intestinal integrity tight junctions and the expression of antimicrobial peptides.

The fine regulation of TLR responses is exemplified by TLR9. TLR9, an intracellular protein in immune cells, is expressed on the cell surface of IEC, both on the apical and the basolateral membrane. *In vitro* studies in IEC lines have described that the basolateral stimulation of TLR9 mobilises an inflammatory cascade, while the apical stimulation induces a signal that curtail inflammatory responses to basolateral stimulation via different TLR, and therefore induces tolerance[13]. Other TLR may be restricted to the basolateral

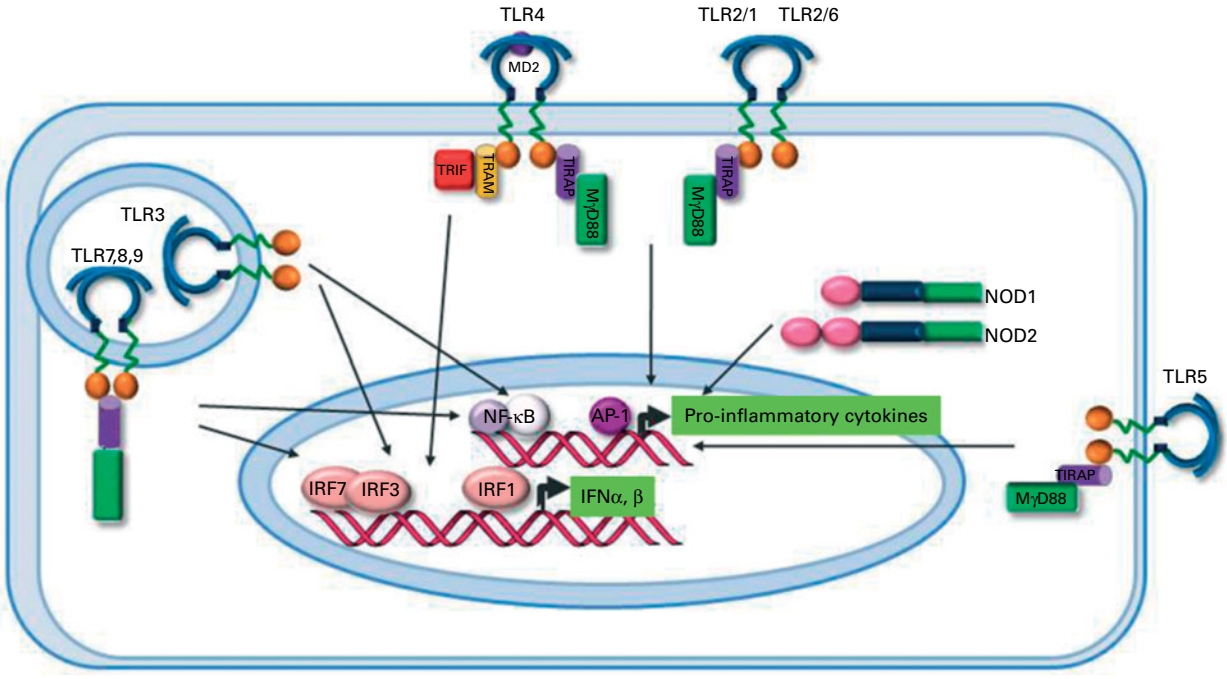

Fig. 1. Toll-like receptors (TLR) and oligomerisation domain receptors (NOD) transduction pathways (adapted from Ishii *et al.*[53]). TLR are found in the cell membrane and in endosomes. In the cell membrane of the enterocyte, TLR form homo- or heterodimers that sense microbial wall components (TLR2/6, TLR2/1 and TLR4), endosomal nucleic acids (TLR3, 7, 8 and 9) and flagellin (TLR5). TLR4 needs a co-receptor, MD2, to fully sense lipopolysaccharide and viral proteins. Four adaptor proteins are known to be involved in TLR4 signal transduction: MyD88, Toll/IL-1 receptor (TIR)-domain-containing adaptor protein (TIRAP), TIR-domain-containing adaptor protein-inducing interferon β (IFNβ) (TRIF) and TRIF-related adaptor molecule (TRAM). Activation of TLR4 can activate MyD88-dependent or -independent responses. MyD88/TIRAP-mediated responses lead to the activation of NF-κB and activator protein 1 (AP-1) and the production of pro-inflammatory cytokines. The MyD88-independent pathway is mediated by TRAM/TRIF that induce IFN regulatory factors (IRF) and the production of different IFN. Endosomal TLR induce NF-κB/AP-1 and IRF-mediated responses, while TLR2/1, TLR2/6 and TLR5 activate only NF-κB/AP-1. NOD1 and NOD2 are intracellular receptors that recognise/activates microbial wall components. Intracellular activation of NOD AP-1 and NF-κB to induce the production of pro-inflammatory cytokines.

Table 1. Expression patterns of Toll-like receptors (TLR) and their activators[53,55–57]

TLR	Ligands	Cell expression	Cellular localisation
TLR1/2	Bacterial lipopeptides Protozoan parasite proteins (T cruzi Tc52, profillin)	Most cell types including DC and IEC	Plasma membrane
TLR2	Bacterial lipoprotein/lipopeptides, peptidoglycan, lipoteichoic acid, porins, zymosan Viral structural proteins (Lipoarabinomannan) Helmint lipids Fungi cell wall components Endogenous HSP60, HSP70, HSP96, HMGB1, hyaluronic acid	IEC, Paneth cells, peripheral mononuclear leukocytes, DC, monocytes and T cells	Plasma membrane
TLR3	Viral single-stranded and double-stranded RNA, mRNA Poly(I:C), Poly(I:C_{12}U) Endogenous mRNA	IEC, DC, NK cells ant T cells	Endosomes
TLR4	Bacterial lipopolysaccharide Viral envelope proteins Protozoan parasites Glycoinositolphospholipids (*Trypanosoma cruzi*) Fungi cell wall components Endogenous HSP22, HSP60, HSP70, HSP96, HMGB1β-defensin 2, extra domain A of fibronectin, hyaluronic acid, heparan sulphate, fibrinogen surfactant protein A	IEC, Paneth cells, macrophages, DC, and T cells	Plasma membrane
TLR5	Flagellin	IEC, Paneth cells, monocytes, DC, NK cells and T cells	Plasma membrane
TLR6/TLR2	Bacterial diacyl lipopeptides, lipoteichoic acid Phenol-soluble modulin, zymosan	IEC, high expression in B cells and DC, low in monocytes and NK	Plasma membrane
TLR7	Viral single-stranded RNA Endogenous RNA	IEC, B cells, DC, monocytes and T cells	Endolysosome
TLR8	Viral single-stranded RNA Endogenous RNA	IEC, monocytes, DC, NK cells and T cells	Endolysosome
TLR9	Baterial, viruses and protozoan parasites unmethylated CpG motifs Protozoan parasite hemozoin (*Plasmodium*) Endogenous CpG DNA oligodeoxynucleotides	IEC, Paneth cells, DC, B cells, peripheral mononuclear leukocytes, macrophages, NK and microglial cells	Endolysosomes and plasma membrane
TLR10	Unknown, may interact with TLR2 and TLR1	B cells, DC, monocytes and T cells	Intracellular
TLR11	Cell surface uropathogenic bacteria, profillin-like molecule CpG DNA from *Toxoplasma gondii*		Plasma membrane

CpG, cytosine-guanine containing single stranded oligodeoxynucleotides; DC, dendritic cells; HMGB1β, high mobility group box 1 beta; HSP, heat shock protein; IEC, intestinal epithelial cells; NK, natural killer.

membrane or to intracellular locations, thus limiting responses to invading bacteria. However, it is not entirely clear how TLR responses are regulated in basal conditions.

The model of colitis induced by the administration of dextran sodium sulphate (DSS) has been shown to be very useful in studying host–microbial interactions. Although the pathogenesis of DSS colitis is not completely understood, it is widely accepted that the administration of DSS disrupts the intestinal barrier, possibly via interaction with surface lipids, and alters intestinal permeability, allowing intestinal microbiota to gain access to the intestinal mucosa. It has been shown that the administration of ligands for TLR5, 2, 3 and 9 protects against DSS-induced colitis, while knock-out mice for TLR2, 4 and MyD88 are more susceptible to DSS colitis[14]. Furthermore, TLR5 knock-out mice develop colitis spontaneously. These results are the opposite of what would be expected based on the direct effects of TLR activation, and therefore suggest that TLR limit inflammation indirectly. On the other hand, studies that show that monoclonal antibody blockade of TLR4 suppresses DSS colitis[15] and that constitutive activation of TLR4 in IEC in transgenic mice augments DSS-induced colitis[14] indicate the need to limit TLR responses in order to avoid excessive inflammatory responses. However, interpretation of this evidence is complicated by the fact that DSS also stimulates monocytes.

Nucleotide-binding and oligomerisation domains-like receptors. NLR are a large family of cytoplasmic proteins comprising over twenty members. Among the NLR family members, NOD1 and NOD2 were the first identified and are sensors of bacterial components involved in the modulation of the intestinal inflammatory and apoptotic response[16]. NOD1 is expressed in IEC and recognises Gram-negative peptidoglycan[17]. An elegant study showed that signalling through NOD1 constitutes the major pathway to activate and up-regulate NF-κB and *NF-κB* genes in cells infected with intracellular bacterial pathogens that do not activate TLR. This way, NOD1 in IEC provides the intestine with a backup mechanism to fight intracellular invasive Gram-negative enteric bacteria that can bypass TLR activation[18].

NOD2 is expressed in IEC, monocytes and Paneth cells and recognises muramyl dipeptide, derived from peptidoglycan, common to both Gram-positive and Gram-negative bacteria[19]. NOD2 is required for the secretion of antimicrobial peptides by Paneth cells. Paneth cells express a wide array of antimicrobial peptides, including α-defensins, lysozyme, phospholipase A_2 (which has antimicrobial properties independent of its catalytic activity) and lectin $RegIII_\gamma$, that constitute an autonomous defence mechanism against harmful bacteria. $RegIII_\gamma$ is produced also by intraepithelial lymphocytes and has been proposed to be essential for preventing bacterial contact with the epithelium[20,21]. *NOD2* gene mutations are among the strongest genetic factors associated to Crohn's disease. These patients show an imbalance in intestinal microflora and an inability to clear intestinal pathogens. Interestingly, NOD2-deficient mice are unable to efficiently kill bacteria, and show an altered intestinal microbiota and an increase in the faecal content of bacteria[22]. It is therefore likely that NOD2 mutations in Crohn's disease may increase disease susceptibility by altering interactions between ileal microbiota and mucosal immunity[23].

NLR can assemble, in response to several stimuli, to form large multimolecular complexes that control the activation of the proteolytic enzyme caspase 1. Caspase 1 in turn cleaves the cytokine precursors pro-IL-1β and pro-IL-18, this is critical for the release of the biologically bioactive forms (IL-1β and IL-18), and triggers pro-inflammatory antimicrobial responses. These complexes are called inflammasomes. In general, NLR inflammasomes contain the common adaptor apoptosis-associated speck-like protein containing a CARD (ASC). So far, four inflammasomes have been characterised in mouse models, named after the PPR regulating its activity: NLR family, pyrin domain containing 1 (NLRP1), NLR family, pyrin domain containing 3 (NLRP3), NLR family, CARD domain containing 4 (NLRC4) and absent in melanoma 2 (AIM2) (a non-NLR-containing inflammasome)[24]. NLRP3 is by far the best studied inflammasome and is activated by a wide range of pathogen-associated molecular patterns including muramyl dipeptide, bacterial RNA, the double-stranded RNA analog poly(I:C) or lipopolysaccharide[24,25]. Several signs of metabolic stress (monosodium urate crystals, extracellular glucose), environmental pollutants (silica, asbestos), UV radiation and skin irritants also activate NLRP3[24,25]. How these very different stimuli activate NLRP3 is still not clear, but it has been hypothesised that they do not bind directly to the inflammasome. A proposed mechanism of action in macrophages implies two signals[24] (Fig. 2); the first signal would be provided by microbial molecules or endogenous cytokines (TNF or IL-1β) via receptor ligation (TLR, TNF receptor, NOD1 or NOD2) and subsequent NF-κB activation and induction of NLRP3 expression, a prerequisite for inflammasome activation, and a second signal provided by certain bacterial toxins and particulate matters, which would directly activate NLRP3. Interestingly, mice lacking NLRP3 inflammasome components (namely ASC, caspase-1 and NLRP3) are more susceptible to DSS colitis, and missense mutations in NLRP3 have been reported in Crohn's disease[26–28].

How host-microbiota homoeostasis is maintained

Men naturally acquire an intestinal microbiota after birth and the bacteria are kept at bay by the barrier function of the mucosa. Microbial attacks and probably minor breaches in the mucosa are handled by the innate and adaptive immune systems, which fight invading micro-organisms, usually with no or minor clinical symptoms. The epithelial layer has a remarkable capacity to reseal newly made lesions by a combination of enhanced proliferation and a mechanism called restitution, so that neighbouring cells literally stretch out to reach cells in the other side of the gap. However, it is unclear how frequent minor episodes are, and it is possible that low numbers of bacteria trespass the barrier and reach the mesenteric lymph nodes or further (a process called translocation). A number of different studies have found microbiota-derived micro-organisms in the spleen or liver of normal animals, suggesting that this is indeed the case[11,29]. Translocation

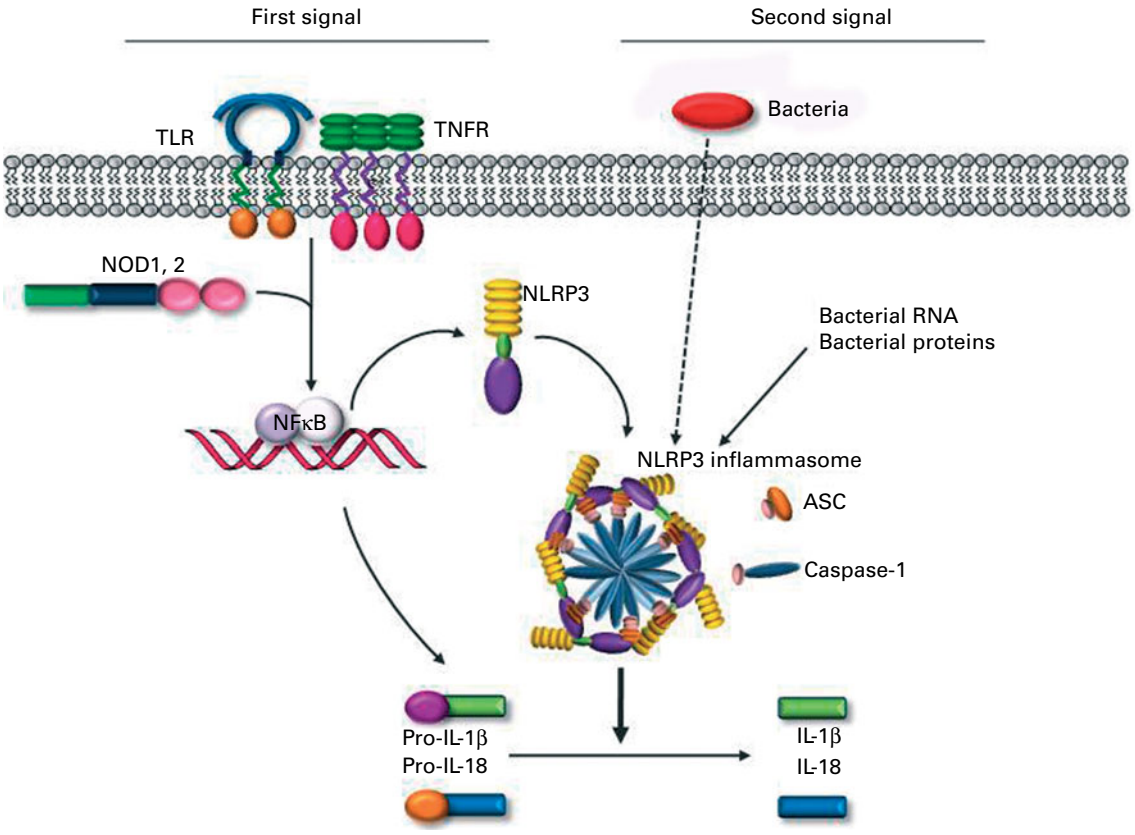

Fig. 2. Inflammasome activation in macrophages (adapted from Franchi *et al.*[24] and Davis & Ting[54]). Two signals have been proposed to activate the inflammasome in macrophages. First, signal is to be provided by microbial molecules or endogenous cytokines (TNF or IL-1β) via receptor ligation (Toll-like receptors (TLR), TNF receptor (TNFR), oligomerisation domain receptors 1 or 2 (NOD1 or NOD2)). This activates NF-κB and subsequently induces NOD-like receptor family, pyrin domain containing 3 (NLRP3) expression. A second signal provided by certain bacterial toxins and particulate matters directly activates NLRP3 inflammasome, and caspase-1 which is activated to cleave Pro-IL-1β and Pro-IL-18.

has been demonstrated also in an *in vitro* model of M cells, and data indicate that these cells can respond differently to commensal and harmful bacteria[30]. This phenomenon is incompletely understood.

At any rate, point blank attacks on the mucosa are accounted for by a relatively small group of bacteria and other micro-organisms (enteropathogens), while the vast majority of the microbiota behave as either neutral (commensals) or even beneficial to the host. It is becoming clear that the very composition of the intestinal microbiota has a determinant role in homoeostasis. GF and gnotobiotic animals are animals in which no bacteria or only certain known strains of bacteria or micro-organisms are present, respectively (by definition GF animals are gnotobiotic, but the term is used preferentially for animals with a restricted, well-defined bacterial population). Studies with these types of animals have shown a broad influence of the microbiota in the development and modulation of the gut immune function. Intestinal colonisation as such modulates innate and adaptive immune functions, stimulating the production of microbicidal peptides[31], secretory IgA, the differentiation of naive T-helper (Th) cells into effector Th1, Th2 and Th17 cells and the development of regulatory T cells (Treg)[32]. But, individual members of the intestinal microbiota may also exert distinct effects at this level, and this hypothesis has become a major focus of interest[33]. For instance, segmented filamentous bacteria (SFB) are a group of uncultivable bacteria that colonise the intestinal epithelium of weaning rodents and stimulate the development of the immune response. Mice that are colonised with SBF-deficient microbiota have weaker IgA antibody and T-cell responses, lack Th17 cells and, more importantly, cannot control colonisation by *Citrobacter rodentium*, an enteroinvasive pathogen[32,34,35].

In addition, beneficial bacteria may act by growth competition and stabilisation of the microbiota. There may be other mechanisms operating as well, such as the release of bacteriocins[36] or the expression of polysaccharide A[37]. These properties form the basis of the use of some bacterial strains as probiotics.

Chemical and immune barriers shape the microbial population

While the microbiota has a well-defined effect on the mucosal immune system, it has become clear that the opposite is also true, i.e., the mucosal immune system has the capacity to shape the microbial population. As indicated earlier, Paneth cells located at the base of the crypts are the major producers of microbicidal peptides in the small intestine and cecum[38] that are responsible for controlling intestinal barrier

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penetration by both commensal and pathogenic bacteria[31]. Regulation of microbicidal peptide production by Paneth cells is dependent on the intestinal flora and is regulated by PPR. Thus, oral administration of TLR ligands for TLR9, 3, 4 and 5 induces the degranulation of Paneth cells[38]. Furthermore, it has been shown that Paneth cells have an autonomous mechanism to detect potentially invasive bacteria that involves cell-intrinsic activation of MyD88 and the induction of antimicrobial peptide production[31]. This mechanism is important in limiting bacterial translocation and dissemination of microbes across the mucosal barrier. The dependence of Paneth cells on bacterial stimuli to produce antimicrobial peptides and modulate the microbiota is further shown in an experiment in which mice were treated with vancomycin. The antibiotic down-regulated the production of the lectin $RegIII_{\gamma}$, a microbicidal peptide, allowing the growth of antibiotic-resistant bacteria, as the ability of these mice to kill antibiotic-resistant bacteria was reduced. Treatment of these animals with the TLR4 ligand lipopolysaccharide restored the production of $RegIII_{\gamma}$[39]. Similarly, mice with disrupted IL-17c signalling show lower expression of genes encoding antibacterial molecules by epithelial cells, increased microbiota and higher mortality secondary to infection[40].

The production of IgA is clearly dependent on the microbiota and changes in its composition alter the IgA pattern[41]. In line with the observations mentioned earlier, GF mice display reduced faecal IgA and lower numbers of IgA-positive cells in the *lamina propria;* specific pathogen-free animals treated with antibiotics show a comparable reduction of the levels of IgA to those of GF mice[42]. Also, bacterial strains might not be equivalent as IgA inducers. Thus, in a gnotobiotic mice model infected only with *Bacteroides thetaiotaomicron,* a commensal bacteria, a sharp increase of IgA_2 (>75-fold) was observed after infection[43].

Secretory IgA, produced by B cells, is a specialised antibody type which is released into the intestinal lumen, where it has a prolonged lifespan compared to regular antibodies and forms an immunological barrier against luminal micro-organisms. The intestine is the highest producer of antibodies in the body. IgA-deficient mice develop compensatory mechanisms but still are less resistant to a variety of infections, highlighting the importance of this element. Dimeric IgA is transcytosed to the intestinal lumen by the polymeric Ig receptor, whose expression is stimulated by the microbiota. The extracellular part of this receptor remains associated with IgA after secretion and forms secretory IgA, which is produced in enormous quantities (3–5 g/d)[44]. These secretory molecules form immune complexes with bacteria that are retained in the mucus, thus being protected from host-derived inflammatory mediators. Two types of IgA are produced in the lamina propria in men, IgA_1 and IgA_2. The production of IgA_1 is T-cell dependent and antigen specific, while IgA_2 has a more limited repertoire (lipopolysaccharide and polysaccharides *v.* proteins) and appears to be highly effective, displaying a higher resistance to hydrolysis and being therefore specially suited to the intestinal environment[12,38,45,46]. However, IgA_2 responses tend to be less sustained. IgA_2 class switching has been associated with TLR activation in human IEC via expression of B-cell-activating factor and a proliferation-inducing ligand[12,38]. Other cell types may also be involved, including dendritic cells, monocytes/macrophages and granulocytes. In mice, similar mechanisms probably work by an increase of B-cell recruitment to the *lamina propria*, class switching and higher secretion of IgA in the small intestine. In both human subjects (IgA_2) and mice, a so called 'natural IgA' is produced, i.e., a non-specific secretory IgA (meaning without any known specificity) produced in the absence of antigenic stimulation in normal conditions.

Secreted IgA contributes to protection of the host from systemic translocation of bacteria or bacterial products, and it may help control commensal bacteria in order to maintain the intestinal homoeostasis. In addition, IgA regulates the balance of commensal bacteria and consequently the composition of the intestinal microflora[42]. Activation-induced cytidine deaminase knock-out mice lack IgA-producing plasma cells. In these animals, the lack of IgA in the intestine induces the expansion of aerobic bacteria, particularly SFB (an example of 'commensal status' depending on the experimental conditions), and the administration of IgA reverts these effects[33]. IgA can also regulate bacterial gene expression. Thus, Rag −/− mice, that lack T and B cells, and therefore IgA, were used to study the effect of IgA on *B. thetaiotaomicron* growth rate and gene expression in gnotobiotic conditions[43]. In these conditions, a strong immune response was induced by *B. thetaiotaomicron,* which is otherwise commensal to the host. This response was ablated in the presence of IgA, even though it did not affect the growth rate of *B. thetaiotaomicron*. In turn, IgA modulated bacterial gene expression, inhibiting the immune response. Thus, IgA may be required for keeping commensal bacteria as actual 'commensal' to the host[42]. However, it should be noted that recombination activating gene (Rag) and Severe Combined ImmunoDeficient (SCID) mice, both lacking T and B cells, do not develop intestinal inflammation in standard conditions.

T cells have also been implicated in microbiota modulation[33]. Intestinal bacteria are necessary for the induction of IL-10, a protective anti-inflammatory cytokine that is mainly produced by Treg[47,48]. Treg can be generated both in the thymus and in the periphery (induced Treg). The importance of induced Treg is exemplified in mice lacking these cells, which suffer a Th2-dependent inflammation at the intestinal mucosa and alteration of the commensal microbiota[49]. Some specific commensal microbiota-derived factors promote induced Treg cell functions. Tolerogenic *lamina propria* $CD103^+$ dendritic cells promote the induction of IL 10-secreting Treg. To acquire a tolerogenic phenotype, CD103 + dendritic cells are stimulated by several factors expressed by IEC, such as TGF-β, thymic stromal lymphopoietin or retinoic acid, depending, in part, on bacterial stimulation[50]. Capsular polysaccharide A from *Bacteroides fragilis,* a commensal bacterium, can further promote the induction of induced Treg by tolerogenic dendritic cells by a mechanism that involves TLR2 activation[33,37,51].

GF mice exhibit poor Th17 differentiation. This process requires the presence of specific commensal bacteria, such

as cytophaga–flavobacter–bacteroidetes or SFB, and is inhibited when mice are treated with antibiotics and in mice that are colonised by an SFB-deficient microbiota[32,35,52]. The induction of Th17 by SFB has been shown to be mediated by the production of bacterial ATP, which activates a subset of dendritic cells that produce IL-1β, IL-16 and IL-23[33], or by serum amyloid protein, which is produced in response to SFB. Interestingly, it is possible to induce Th17 in mice that show spontaneous low levels of this cell type simply by giving them bacteria from mice with high Th17 levels. Absence of Th17-cell-inducing bacteria is accompanied by an increase in Foxp3 + Treg in the lamina propria (LP), suggesting that the microbiota is a major regulator of the Th17:Treg balance in the mucosa[52]. Th17 (and Th1) cells play an important role in intestinal barrier function, producing cytokines that recruit and activate macrophages and neutrophils, which in turn eliminate penetrating bacteria. In addition, Th17 cells have been shown to induce the production of bacterial defensins.

Conclusions

The gut maintains a complex relationship with the intestinal microbiota, probably more obliged than strictly symbiotic. At any rate, the host and the luminal micro-organisms live together as reasonably good neighbours, with occasional rough encounters that are usually of no consequence. The combination of an efficient, self-repairing barrier, abundant mucus secretion, continuous luminal flow of contents and a vigorous, yet finely regulated, immune system is capable of keeping a massive foreign population contained within the limits of the mucosa. The innate immune response plays a prominent role in appropriate barrier function. This delicate equilibrium represents a well-balanced opposition of considerable forces. However, this equilibrium can be altered substantially, resulting typically in inflammatory responses, as in inflammatory bowel disease. Thus, intestinal inflammation may be the consequence of both an enhanced immune response and a defect in barrier function.

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