Review

Innate Immunity

16(6) (2010) 391–404

ISSN 1753-4259 (print)
10.1177/1753425909357577

Telling apart friend from foe: discriminating between commensals and pathogens at mucosal sites

Naren Srinivasan

Sir William Dunn School of Pathology, University of Oxford, Oxford, UK

From the moment we are born, we are exposed to a vast variety of microbes. The intestine in particular is perhaps inhabited by the largest number of microbes, consisting of both established commensals as well as sporadic pathogens. Mucosal surfaces form an important barrier against microbial invasion. Together with the physical barrier that they provide, mucosal surfaces also rely on innate immune functions to sense luminal microbes and signal accordingly to generate protective immune responses. However, since innate immune recognition is microbial specific and antigen-independent, the contact with both beneficial commensals and harmful pathogens creates the need for discrimination between the two. The mechanisms governing the ability of the mucosal immune system to discriminate between commensals and pathogens have long been unclear; however, recent discoveries have shed some light on this distinction. This review will summarize the current theories put forth to explain how the mucosal immune system maintains tolerance towards commensals while retaining the ability to mount inflammatory responses against pathogens.

Keywords: discrimination, innate-immunity, intestinal homeostasis, mucosal surfaces, tolerance

INTRODUCTION

Inflammation – a consequence of microbial recognition

The innate immune system is the first line of defence against pathogens. Innate immune responses depend on germ-line encoded receptors known as pattern recognition receptors (PRRs) borne on leukocytes.[1] Pattern recognition receptors mediate the recognition of microbes by binding to highly conserved, invariant motifs known as pathogen associated molecular patterns (PAMPs) such as peptidoglycan, lipopolysaccharide (LPS) and β-glucans.[1] Toll-like receptors (TLRs) are the primary PRRs involved in bacterial recognition in the mucosal epithelium, they were also the first family of PRRs to be described. Toll-like receptors are type 1 integral glycoproteins divided into two domains – an extracellular domain of numerous leucine-rich repeats (LRRs) and an intracellular TIR (Toll/IL (interleukin)-1 receptor] domain.[2] A consequence of microbial recognition through some classes of PRRs (*e.g*. TLRs) is the establishment of acute inflammation. This is mediated through the production of pro-inflammatory cytokines, chemokines, and interferons, including IL-1, IL-6, IL-12, IL-18 and tumour necrosis factor-α (TNF-α), which are required for microbial killing and clearance.[3] Some of these cytokines, including IL-1 and IL-6, are responsible for mediating the early induced response, characterized by the production of acute phase proteins (*e.g*. C-reactive protein by the liver) that selectively bind and rupture microbial surfaces. Second, IL-1 and IL-6 are potent pyrogens that reduce bacterial growth and stimulate host defence mechanisms. Finally, mediators such as IL-8 and TNF-α are involved in selectively increasing the permeability of blood vessels in order to aid the recruitment of leukocytes (mainly neutrophils) to the site of infection.

An important hallmark of innate immune recognition concerns the ligand specificity of PRRs; PAMPs form a type of molecular signature which is conserved across

Received 6 June 2009; Revised 12 October 2009, 18 November 2009; Accepted 19 November 2009

Correspondence to: Naren Srinivasan, Brasenose College, Radcliffe Square, Oxford OX1 4AJ, UK. E-mail: naren.srinivasan@path.ox.ac.uk

microbial species. Thus, each PRR can bind to a variety of micro-organisms possessing a common invariant PAMP.[4] However this 'advantage' poses a puzzling paradox; if all microbes possessing a particular PAMP are treated as intruders towards which antimicrobial responses are mounted, then how do we co-exist with commensal bacteria? Commensals play important roles in host metabolism, pathogen antagonism and in development of the host immune system.[5–7] In return, hosts provide commensals with nutrient-rich micro-environments where they can thrive. This symbiotic relationship is most manifest in the human gut, wherein approximately 1000 species reside at extremely high densities.[8]

Fortunately, in spite of the lack of any apparent PRR-mediated discrimination, we are still able to tolerate commensals while mounting the necessary protective inflammatory responses against pathogens. We can thus infer that certain mechanisms must exist to distinguish between commensals and pathogens. Evidence for such mechanisms is found in pathologies that occur as a consequence of the dysregulation of this discrimination. Nowhere is this better highlighted than in the inflammatory bowel diseases (IBD), Crohn's disease and ulcerative colitis, in which normal flora of the intestinal tract are thought to drive inflammation.[9]

Intestinal epithelial cells (IECs), which form the interface between the flora in the gut lumen and the host connective tissue, possess a wide range of PRRs.[10,11] The gut lumen to which the PRRs are in contact is heavily colonized by PAMP-rich, non-pathogenic, commensal organisms.[12] So how then is immune hyporesponsiveness maintained despite the apparent PAMP–PRR interactions? Inappropriate immune responses, in general, are avoided by what is known as tolerance. Tolerance is typically functional against allergens and self-antigens, the failure of which results in allergic reactions and autoimmune disorders. Similarly, commensal gut bacteria are tolerated by the host, the breakdown of which results in IBD. In the classical sense, tolerance is mediated by peripherally induced and thymus-derived regulatory T-cells (T-regs) that protect against excessive inflammation ensuing from translocating microbes. Tolerance may also encompass the mechanisms by which the host reduces interactions with its normal flora and commensals actively dampen innate pro-inflammatory signalling. In this review, I use the term 'tolerance' in both senses.

The basis of discrimination

The basis of discrimination can be divided into three broad categories: (i) the nature of the microbe; (ii) the nature of the intestinal epithelial cells; and (iii) the immune cells inhabiting the gut sub-epithelium. As the roles of dendritic cells (DCs) and regulatory T-cells in regulating intestinal homeostasis have been reviewed elsewhere,[13,14] I discuss here the first two categories of discrimination.

THE NATURE OF THE MICROBE

Assessibility to immune cells and the role of virulence factors

The simplest method of discrimination relies on differences in invasiveness between commensals and pathogens which result in the former's physical exclusion from the sub-epithelium, ultimately translating into the absence of chronic inflammation.[15] Every environment requires a unique set of adaptations from its inhabitants to maintain successful colonization. The intestinal tract is no different and has developed numerous attributes to subvert microbial invasion (Fig. 1). It is lined by a monolayer of columnar epithelial cells, known as intestinal epithelial cells (IECs), which are attached to each other via tight junctions that serve to limit diffusion via intercellular gaps. The apical surfaces of IECs are lined by microvilli creating a characteristic brush border, lately shown to contribute to mucosal tolerance by detoxifying LPS through the expression of intestinal alkaline phosphatases.[16] A glycocalyx on the apical surface of IECs and a 150-μm thick mucous layer of pore-forming mucins provide additional physical barriers that reduce the motility of microbes.[15,17] The mucous layer is composed of two sublayers, an inner and outer layer composed principally of the glycoprotein mucin-2 (Muc2).[17] In attest to its role in bacterial exclusion, it was found that the inner colonic mucous layer in wild-type mice is devoid of bacteria while Muc2-deficient mice display deep bacterial penetration down to sterile crypts and consequently develop colitis by 7 weeks of age.[17] Furthermore, the production of antimicrobial peptides such as defensins plays a role in the prevention of colonization.[18] The layer below the IECs houses the gut-associated lymphoid tissue (GALT), which is divided into inductive and effector regions.[19] The inductor region is comprised of Peyer's patches, mesenteric lymph nodes and isolated lymphoid follicles, all replete with T-cells.[6] The area above the Peyer's patch is known as the sub-epithelial dome (SED) and contains professional antigen-presenting cells (APCs) such as dendritic cells (DCs), responsible for bridging the gap between innate and adaptive immune responses.[20]

As mentioned, the triggering of inflammation requires engagement of a PAMP to its cognate PRR. This further necessitates that the PAMP on the microbe be delivered

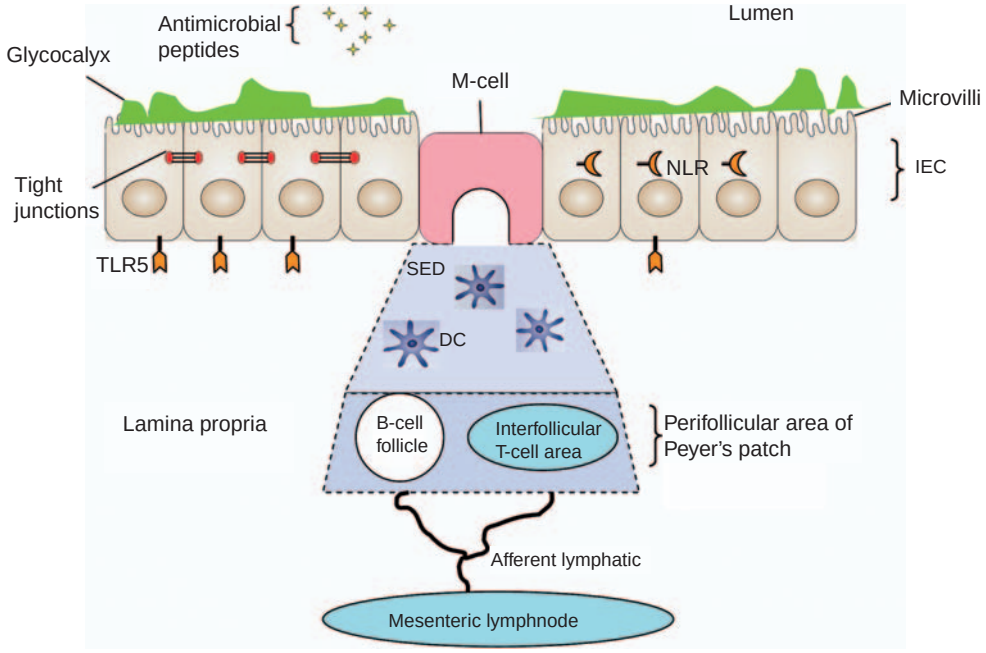

Fig. 1. The intestinal epithelial-cell (IEC) barrier has evolved numerous adaptations to subvert microbial invasion: a brush border of microvilli to hinder microbial attachment, intercellular tight junctions that hold adjacent plasma membranes tightly together, a mucin-rich glycocalyx which provides a physical barrier to penetration, and the secretion of antimicrobial peptides such as defensins. The IEC barrier is interrupted periodically along its length by inductive sites known as M-cells which lack a glycocalyx and brush border to allow easy sampling of microbes. Immediately below M-cells lies the sub-epithelial domain (SED) which houses dendritic cells (DCs) and other antigen-presenting cells (APCs) which process the delivered antigen. These APCs may then present antigen to T-cells and B-cells housed either within the perifollicular area or within the mesenteric lymph nodes (MLNs).

in close apposition to the PRR, a process dependent on the efficient colonization of the epithelium. Successful pathogens possess virulence factors that facilitate the adherence to (*e.g.* adherins), and breaching of, IECs eventually leading to their dissemination within the underlying Peyer's patches where they are able to elicit systemic inflammatory responses. For example, the parasite *Entamoeba histolytica* secretes a cysteine protease enzyme that cleaves Muc2 so allowing its penetration past the mucous layer.[20,21] The lack of such virulence factors in commensals results in their exclusion from Peyer's patches and thus is thought to account partly for the absence of commensal-induced chronic inflammation.[15,19] For example, pathogenic strains such as *Shigella flexneri*, normally able to cross the epithelial barrier, lose their ability to do so when the genes coding for virulence factors (*e.g.* invasins) are inactivated.[22] Such a method of achieving tolerance is often described as tolerance by exclusion, since non-pathogenic microbes are excluded from the subepithelium. This mechanism seems to be an evolutionarily efficient means of ensuring discrimination since the lack of virulence factors is a defining characteristic of commensals inimitable by pathogens. The non-detection of avirulent microbes is a theme also reflected in the distribution of PRRs in IECs described later.

Role of γδ T-cells in maintaining gut homeostasis

The mucus layer and the integrity of the gut epithelium are crucial in preventing the translocation of luminal antigens, flora and the ensuing inflammation. Indeed, many IBD models, such as the dextran sodium sulphate (DSS) model, induce inflammation by damaging the epithelium. However, the gut epithelium is a monolayer likely to be frequently sloughed off by mechanical abrasion or pathogens as earlier mentioned, leaving the underlying immune cells exposed to a vast number of commensals, begging the question of how chronic inflammation is avoided? Recent studies implicate γδ intra-epithelial T-cells as maintaining homeostasis following mucosal injury.[23] γδ T-cells are found in relatively high abundance in between IECs, basolateral to tight junctions. Soon after mucosal disruption, γδ T-cells secrete cryoprotective factors (*e.g.* keratinocyte growth factor involved in epithelial wound healing), bactericidal proteins (*e.g.* Reg IIIγ to kill invading bacteria), and chemokines (to recruit leukocytes to assist in this).[23] Importantly, secretion of many of these factors is dependent on the detection of commensals or commensal-mediated tissue damage,[23] by innate immune pathways such as the Myd88 pathway. Embedded in this observation is the fact that when

means of differentiation on the basis of virulence are removed, commensals and pathogens are homogeneously perceived by the innate immune system. However, the finding that $\gamma\delta$ T-cells are not activated upon pathogen challenge,[24] questions this notion and suggests the involvement of additional factors yet to be clarified.

Compartmentalization of PRRs

Intestinal epithelial cells form a monolayer of columnar epithelial cells whose apical surface is in constant contact with the gut lumen and its inhabitants. Both TLRs and NLRs are expressed by IECs; yet, despite their constant exposure to PAMPs such as LPS, chronic inflammation is avoided.[10,11] An explanation for this apparent paradox lies in the expression pattern of PRRs in IECs. Lipopolysaccharide is well established as the ligand and trigger for TLR4-induced pro-inflammatory signaling.[25] Toll-like receptor 4 requires two additional proteins for LPS-dependent signalling, MD-2 and CD14.[26] Mucosal IECs have been reported to express no MD-2 and low levels of TLR4 protein which could account for the observed LPS-specific hyporesponsiveness.[26] In contrast, other research has shown that TLR4–MD-2 complexes are expressed; however, they remain restricted to crypt epithelial cells and mononuclear cells of the lamina propria.[27] Intestinal crypts maintain a sterile environment to protect resident stem cells from infection by maintaining a high concentration of antimicrobial mediators such as defensins, secreted by specialized cells known as paneth cells.[15,28] Incidentally, apart from the microbicidal functions of defensins, they have also been implicated in neutralizing LPS and so blocking TLR4-mediated pro-inflammatory signaling.[29] This tactical compartmentalization of TLR4 may serve as an intricate mechanism of circumventing commensally-induced immune activation, since only micro-organisms capable of attachment to deeper layers of the mucosal epithelium and disruption of the crypt barrier would elicit a pro-inflammatory response.[28] Such an invasion would, in turn, demand that the micro-organism possess virulence factors thereby ensuring that only pathogen-specific immune responses are elicited.

Additionally, sub-cellular compartmentalization of NOD1, a member of the NOD (nucleotide binding and oligomerization domain)-like receptor (NLR) family, and TLR4 on IECs, two chief PRRs involved in pathogen recognition, has been observed.[10,30,31] Thus, only internalized bacteria, capable of survival within the cell, would trigger PRR recognition. While this accounts for the discrimination between intracellular pathogens and commensals, it still leaves unanswered the question as to how extracellular pathogens and commensals are distinguished from each other. Research into the mode of NOD-1 activation by certain *Helicobacter pylori* strains has shed some light on this subject. NOD-1 has been established as the receptor for a peptidoglycan-derived motif known as GM tri-DAP.[10,30] The receptor NOD-1 is localized in the cytoplasm of IECs (Fig. 1) and thus requires the internalization of PAMP.[32] While this is facilitated by facultative intracellular pathogens, how extracellular bacteria activated NOD-1 was not known until recently. Viala *et al*.[33] have shown that the *H. pylori* (an extracellular bacterium) activation of NOD-1 is dependent on a type-IV secretory system, which functions as a molecular syringe that delivers both virulence factors and PAMPs into the cytoplasm of the host cell. Secretory systems form one of many ways of introducing bacterial-derived products into a host, the two most important of which are type-III and type-IV systems, which possess homologies to flagella and conjugation systems, respectively.[32,34] The introduction of bacterial protein into eukaryotic cells is a hallmark of pathogenicity and, accordingly, many methods exist to facilitate it. Since non-pathogenic bacteria generally lack these mechanisms, they may not be able to facilitate the introduction of PAMPs into intracellular compartments, thereby preventing any PAMP–PRR engagement and the ensuing pro-inflammatory signalling cascade.

Furthermore, certain PRRs, such as TLR5, are compartmentalized on the basolateral surface of epithelial cells (Fig. 1).[12] Such a mechanism achieves the same result as the above strategies, that is, it provides a detection system that is set off only in the event of a breach of the epithelial barrier. Consequently, this limits potentially destructive pro-inflammatory responses to incidents where it is appropriate.

In summary, the strategic compartmentalization of PRRs presents another form of tolerance by exclusion. It can be argued, that the architecture of the intestinal immune system has evolved in a way that creates a sampling bias.[35] That is, by restricting PRRs to areas that are inaccessible to non-pathogens, the host biases the detection of PAMPs originating from invasive pathogens.[35] As a result, the host avoids chronic inflammation against beneficial commensals while still able to mount inflammatory responses against invasive pathogenic bacteria. However, on a cautionary note, the discoveries that commensals are indeed detected by TLRs,[36] and sampled by intestinal DCs,[37] suggests that tolerance by exclusion only offers a partial reason for the hyporesponsiveness against commensals.

Sensing virulence factors

Complementary to the exclusion of avirulent microbes, the innate immune system is capable of detecting the

activities of virulence factors and, hence, limiting inflammatory responses to microbes that bear them (pathogens). This stems from Matzinger's Danger hypothesis, which states that cells of the innate immune system are primarily activated by (endogenous) 'danger' signals, originating from injured or stressed cells.[38] Thus the innate immune system is not only involved in providing protection against pathogens, but also in the maintenance of homeostasis through the recognition of stressed or injured cells. Indeed, most PRRs are well equipped to detect danger signals, possessing affinities for both exogenous and endogenous ligands, *e.g*. TLR4 for LPS and heat-shock protein 70 (Hsp70). The Danger hypothesis is attractive from our perspective because it provides an ostensibly simple solution to our paradox; cells undergoing stress display danger signals which PRRs, in turn, recognize and respond to accordingly. And, since virulent pathogens, but not quiescent commensals, cause cell stress, immune responses are restricted to them.

A number of danger signals, their PRRs, and modes of action have since been revealed, the best characterized of which is a member of the NLR family known as the NALP3 (Nacht domain, leucine-rich repeat, and pyrin domain-containing protein 3) inflammasome.[39] When NALP3 is activated, it assembles a multiprotein complex collectively known as the inflammasome, which mediates the activation of a protease, caspase-1. In turn, caspase-1 mediates the processing and release of IL-1β, IL-18, and cellular death. The activation of the NALP3 inflammasome has been shown to be caused by a multitude of factors, but is thought to operate via a two-signal mechanism.[39] Signal 1 can be activated by TLR agonists (PAMPs), and results in the NF-κB-dependent synthesis of pro-IL-1β.[39] Numerous *in vitro* studies have found that Signal 2 constitutes a danger signal which may be triggered by different stimuli, including ATP, various bacterial toxins (*e.g*. listeriolysin O, nigericin, aerolysin, urate crystals) and particulate matter (*e.g*. silica).[39] While the mechanisms of action for these stimuli have not been deduced in their entirety, some general principles have been uncovered. Adenosine-5'-triphosphate (ATP) has been shown to act by activating the purinergic receptor P2X7 which ultimately results in a K^+ efflux that is critical to the activation of the inflammasome.[40] Accordingly, the bacterial pore-forming toxins (maitotoxin and nigericin) are K^+ ionophores which are sufficient to activate the inflammasome.[41] In contrast, particulate matter such as silica and asbestos are thought to activate the inflammasome by reactive oxygen species (ROS) generated in response to a rupturing of the phagosome in what is known as 'frustrated' phagocytosis.[42,43]

Precisely how a change in ionic gradient or damage to the lysosome can lead to the activation of the inflammasome has been widely speculated,[44] but largely remains a mystery. Nevertheless, we can draw some important conclusions from these findings that are germane to our argument. While Signal 1 may be derived from both commensal and pathogenic microbes, it would appear that Signal 2 can only be delivered by a virulent, pathogenic microbe. Indeed pathogens such as *Aeromonas hydrophila* secrete toxins such as aerolysin which permeabilize the cell membrane causing a decrease in intracellular K^+ and, thereby, activating the NALP3 inflammasome.[45] Additionally, the intracellular pathogen *Listeria monocytogenes* secretes listeriolysin O which disrupts the lysosomal membrane (not unlike asbestos and silica) enabling its escape into the cytosol.[41] Man-made facial recognition systems analyze a number of distinctive features of a face (*e.g*. the width of a nose, distance between eyes, length of a jaw line, *etc*.), before accurately deducing the identity of the person. Similarly, this two-signal requirement may serve to avoid a false-positive error, that is, inflammatory responses towards avirulent microbes, by including more characteristics into the recognition algorithm.

Commensal-derived metabolites

Gastric digestion is an inefficient process, unable to metabolize a large proportion of complex polysaccharides that are commonly consumed, such as starches and oligosaccharides.[6] We depend upon commensal bacteria to make these carbon sources accessible. Besides the important contribution to mammalian metabolism, commensal-derived metabolites are thought to have an immune-modulatory role.[20] For example, butyrate, a by-product of starch digestion, has been shown to inhibit strongly the expression of pro-inflammatory cytokines such as IL-12 and TNF-α, while inducing the release of the regulatory cytokine IL-10.[6] Recently, a study conducted by Kumar *et al*.[46] has provided a possible mechanism through which butyrate and other short-chain fatty acids may act. The study showed that butyrate, acting via an unknown receptor, promoted the loss of neddylated $SCF^{\beta TrCP}$ which consequently attenuated the NF-κB pathway (see below).[46]

Modification of PAMPs

It is ironic that commensals characterized by their salutary effects on host health should share adaptations with notoriously deadly pathogens for avoiding immune detection (summarized in Table 1). For example, the lipid A component of LPS from Gram-negative bacteria is known to be recognized by the TLR4–MD2 complex.[47] This complex is specific for hexa-acylated lipid A molecules with fatty acid side chains that are 12–16

Table 1. Bacterial strategies of dampening inflammatory responses

Mechanism of action	Bacterial species	References
Blocking IκB poly-ubiquitination	salmonellae, *Lactobacillus casei*	57, 60
PPAR-γ mediated anti-inflammatory signalling	*Bacteroides thetaiotamicron*	58
Preventing IκB phosphorylation	*Yersinia enterocolitica*	62
Promoting T-regulatory responses	*Bacteroides fragilis*	67, 68
Modification of PAMPs : LPS flagellin	*Rhodobacter sphaeroides*, *Wolinella succinogenes*, *Helicobacter pylori*	55
Secretion of metabolites, *e.g.* butyrate	*Faecalibacterium prausnitzii*	89

carbons in length.[48] Certain bacterial strains, for example *Rhodobacter sphaeroides*, contain penta-acylated lipid A that have species-specific antagonistic effects on LPS from *Escherichia coli*.[49] Pathogenic strains including species of *Legionella* also exploit this specificity of TLR4 by altering their lipid moieties.[3] Other species of *Bacteroides*, a common gut commensal, have structurally distinct LPS domains with penta-acylated lipid A,[50] relatively longer fatty acid side chains (15–17 carbons) and a monophosphorylated disaccharide backbone as opposed to a biphosphorylated backbone in enterobacterial species.[51] Presumably due to lower affinity for TLR4, the LPS derived from *Bacteroides* is 100–1000-fold less endotoxic than enterobacterial LPS.[52,53]

This mechanism of evading PRR detection through the alteration of PAMPs has also been described in other TLR-subfamilies; TLR5 which has be shown to have ligand specificity for bacterial flagellin,[54] provides one such example. The flagellated members of the ε clade of proteobacteria have been shown to evade TLR5 recognition by mutating TLR5 recognition sites on flagellin, while employing compensatory mutations to retain functionality of the flagellum.[55] Interestingly, *Wolinella succinogenes* and *H. pylori*, a commensal and a pathogen respectively, both employ this tactic to avoid detection by TLR5.[55]

Anti-inflammatory signalling

Innate immunity works on the principle of 'sensing and signalling'; the detection of bacterial PAMPs initiates various signalling pathways ultimately concluding in protective responses such as inflammation that resolve an infection. A key signalling co-ordinator of inflammatory and immune responses is nuclear factor kappa B (NF-κB) which is the master transcription factor responsible for the expression of pro-inflammatory mediators during the start of inflammation, and anti-inflammatory mediators during its resolution.[56] In the absence of an activation signal, NF-κB remains localized in the cytoplasm bound in a complex with its inhibitor, IκB.[56] In the case of TLRs, the binding of a PAMP to the LRR domains triggers an intracellular signalling cascade that results in the phosphorylation and subsequent ubiquitination of IκB which ultimately leads to its proteasomal degradation (Fig. 2).[57] The degradation of IκB exposes the nuclear localization signal on NF-κB thereby transporting NF-κB to the nucleus where it mediates activation of pro-inflammatory gene expression.[57] Another family of PRRs capable of stimulating this cascade is the intracellular NOD family which includes NOD1 and NOD2 receptors.[57] Pathogens have evolved numerous mechanisms of evading pro-inflammatory responses; thus, it is not surprising to find commensals with homologous, although distinct, immune-modulatory functions.[58] Tolerance realized in this manner is known as tolerance by constraint.

Bacteroides thetaiotaomicron is a common, commensal, anaerobic, gut bacterium which possesses just such a method of regulating epithelial pro-inflammatory signalling; it promotes the nuclear export of the peroxisome-proliferation-activated-receptor-γ (PPARγ) which is a nuclear receptor known to associate with the transcriptionally active RelA/p65 subunit of NF-κB (Fig. 2).[58] The PPARγ-dependent nuclear export of RelA reduces the availability of NF-κB, ultimately resulting in decreased transcription of NF-κB-dependent genes.[8,58] However, precisely how this bacterium drives PPARγ export remains to be shown. In accordance with this finding, it has been shown that, in the colonic epithelia of ulcerative colitis patients, the expression of PPARγ can be reduced by up to 60%.[59]

Another way in which commensals may regulate epithelial responses is through the inhibition of the post-translational modifications of IκB needed for NF-κB-mediated gene expression.[60] For instance, commensal salmonellae and *Lactobacillus casei* have been shown to prevent IκB poly-ubiquitination, while the pathogenic *Yersinia* spp. inhibit NF-κB nuclear localization by blocking IκB phosphorylation (Fig. 2).[57,60–62]

Recently, the mechanism behind this commensal-mediated regulation has been elucidated. It is known that the poly-ubiquitination of IκB (the NF-κB inhibitor) is

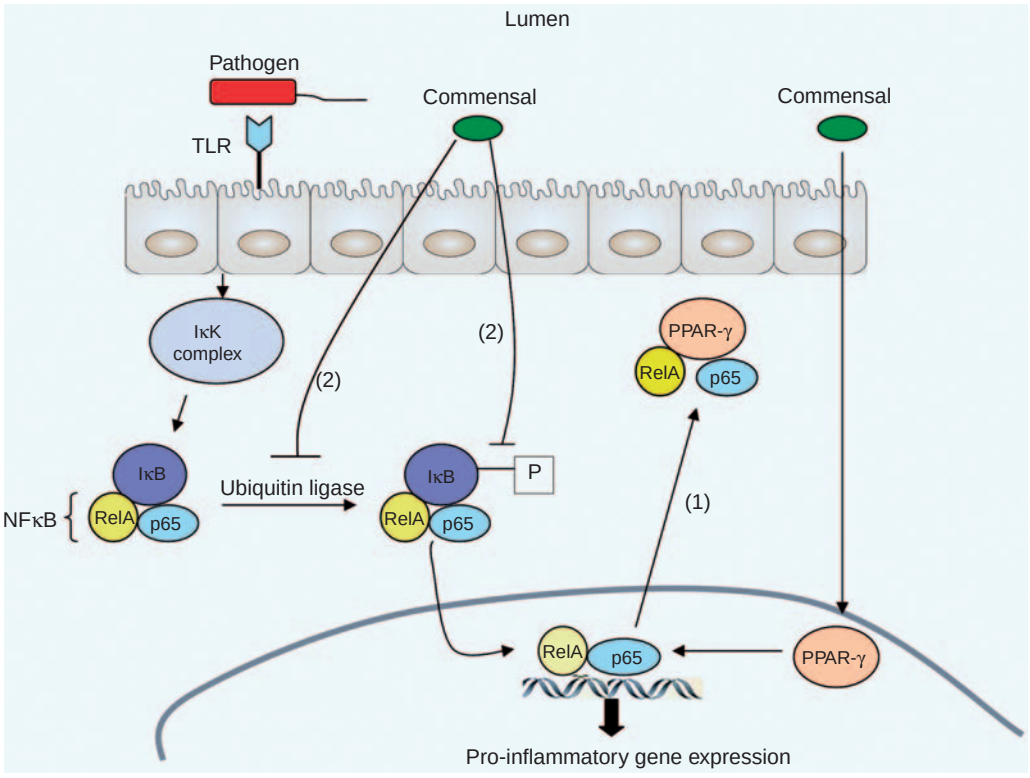

Fig. 2. Mechanisms of anti-pro-inflammatory signalling. Toll-like receptor (TLR) mediated recognition of pathogenic bacteria induces pro-inflammatory signalling via the canonical pathway; first, TLR–PAMP engagement activates the IκB–kinase complex (IκK), this in turn mediates the phosphorylation of IκB (the inhibitor of transcription factor NF-κB). IκB is then polyubiquitinated and subsequently degraded by a proteasome, releasing the transcription factor NF-κB for nuclear localization where it mediates pro-inflammatory gene expression. Certain commensals can block this pathway via two main mechanisms: promoting the nuclear export of NF-κB through peroxisome-proliferation-activated-receptor-γ (PPAR-γ) signalling (1), inhibiting the polyubiquitination of IκB, or preventing IκB phosphorylation (2).

catalyzed by an ubiquitin ligase known as E3 ubiquitin ligase–β-transducin-repeat-containing protein ($SCF^{\beta TrCP}$).[63] This enzyme is itself regulated by the ubiquitin homolog, NEDD8, which mediates a covalent modification known as neddylation that has been shown to be necessary for $SCF^{\beta TrCP}$ activity.[63] Indeed, one way in which commensals block NF-κB signalling is through reducing the neddylation of $SCF^{\beta TrCP}$.[64] The driver behind reduced neddylation has been revealed to be bacterial-induced ROS.[64] Puzzlingly, ROS are also defined as activators for the NALP3 inflammasome and associated with Crohn's disease.[65] The gastrointestinal tract is densely populated by commensals, and pathogens make up a tiny transitory fraction of this population. Thus, the mucosal immune system is constantly receiving (low levels of) signalling from commensals (*e.g.* LPS), whilst pathogens would ostensibly deliver more concentrated (due to their proximity), infrequent signals. What emerges from these observations is that, at steady-state, a physiological set point level of ROS may be maintained by commensal-derived PRR signalling, whilst upon infection the balance slants towards inflammation. This further implies that PRR signalling outputs may be scalable and supports the idea that inflammation in general has a physiological role in maintaining homeostasis (reviewed by Medzhitov[66]).

However, precisely how a commensal's ability to stimulate ROS in intestinal epithelia differs from that of a pathogen remains to be answered. The possibility that hitherto undiscovered PAMPs that are unique to commensals may offer an explanation for the anti-inflammatory signaling.[8] Consistent with this hypothesis is the recent discovery that polysaccharide A (PSA) functions as a microbial symbiosis factor in certain *Bacteroides* strains, by mediating the suppression of pro-inflammatory IL-17 whilst also inducing the production of the anti-inflammatory, IL-10.[67] Given that PSA is a complex sugar, it is reasonable to suggest that it is recognized by PRR(s) much like β-glucans are detected by Dectin-1. Thus far, characterization of PSA has focused on its MHC-II dependent presentation to $CD4^+$ T-cells, which are induced to produce IL-10.[68] Signalling through PRRs can induce DC maturation and the production of cytokines and, by doing so, couple innate and adaptive immunity. Therefore, identifying the PRR(s) responsible for PSA recognition may further our understanding of how the unique anti-inflammatory profile is evoked.

It is important to realize that the existence of the tolerance by constraint mechanisms described above imply that commensals are in fact recognized by the host, in contrast to the tolerance by exclusion hypothesis. Since, if they were not, then there would no need to evolve mechanisms of dampening host pro-inflammatory responses against commensals in the first place. How to reconcile these two seemingly contradictory hypotheses will become apparent below.

The human intestine harbours a multitude of microbes involved in complex relationships with the host which range from mutualistic (benefiting both parties) to parasitic (benefiting a single party at the expense of another).[69] It is widely argued that the emergent properties of a microbial gut community *i.e*. the sum of their individual activities, which favour host fitness, are selected for.[70] Mutualists, which by definition favour host fitness (as well as their own), are thus selected over parasites. The immune system can mediate this selection for mutualists as demonstrated by its ability to respond to microbe-derived metabolites (see above), and the immune system's dependency on certain microbes for normal development.[7] The studies described here highlight the various mechanisms through which chronic inflammation is avoided in the gut, and are the result of millions of years of co-evolution between hosts and commensals. Finally, the finding that enteropathogenic microbes and resident gut commensal flora share some of these mechanisms may reflect the parallel evolution between commensals and pathogens in response to similar immune selection pressures (Table 1). Ultimately, however, most enteropathogenic infections are zoonotic (acquired from another primary host) and transient in contrast with the life-long colonization of mutualists; as such, these pathogens possess certain emblematic traits enabling their discrimination from commensals.

The Nature of Intestinal Epithelial Cells

IEC regulation of adaptive immune responses

Thus far, two general mechanisms of tolerance have been described, namely, tolerance by exclusion and tolerance by constraint. Tolerance by exclusion maintains that commensally-derived PAMPs do not engage with host PRRs; in contrast, tolerance by constraint proposes that commensals are recognized by PRRs but they have evolved mechanisms of down-regulating the resultant pro-inflammatory responses. The reality may involve the collusion of both mechanisms.

Recently, two seminal studies have shown that commensals are detected at steady-state in the gut by TLRs[36] and NOD-1.[7] Furthermore, the studies showed that detection was not simply an unfortunate corollary of the nature of innate immune detection, but in fact necessary for the maintenance of intestinal homeostasis as well as the development of the gut-associated lymphoid tissue (GALT), respectively. This confirms that commensal recognition does occur and raises yet another enigmatic paradox.[5,36] If indeed commensal organisms are sampled, then how is the subsequent induction of an adaptive immune response avoided? Although tolerance by constraint provides a partial solution, not all commensals may have evolved mechanisms of attenuating pro-inflammatory signalling. It is the general opinion of most that the nature of the pathogen determines the type of immune response evoked, *e.g*. a Th2 response to a parasite. However, it has recently been proposed that the tissue in which the response occurs plays a previously unappreciated role controlling effector class.[71] In line with this, an explanation to hyporesponsiveness to commensals may lie in the anti-inflammatory nature of the gut mucosa.

First, it is useful to consider how antigens enter the GALT. Three main methods of are possible – via IECs, via dendritic cells (DCs) and through M cells.[20] Phagocytosis by IECs is unlikely to be a major means of entry, at least for commensals, due to the reasons already discussed, *i.e*. a lack of virulence factors. However, the latter two present possible modes of entry for commensals. M cells are specialized cells that cover the Peyer's patches and are responsible for delivering antigens via transcytosis to the underlying subepithelial dome (SED), where they are engulfed by macrophages or DCs.[20] Dendritic cells are also capable of directly sampling luminal contents through the extension of dendrites in between IEC tight junctions.[72] Here, I shall briefly discuss the unique phenotypes of intestinal macrophages and DCs, and how they are imprinted in the gut.

Intestinal macrophages

Macrophages and efficient phagocytes found in great numbers particularly at entrances from the external environment, *e.g*. alveolar macrophages in the lungs. Typically, macrophages express a variety of PRRs which enable the engulfment and subsequent killing of foreign microbes through numerous bactericidal mechanisms, *e.g*. respiratory burst. Additionally, macrophages, in their capacity as antigen-presenting cells (APCs), are responsible for eliciting adaptive immune responses. The assortment of cytokines accompanying antigen presentation plays an instructive role in determining the type of adaptive immune response that is evoked, *i.e*. Th1, Th2, Th17, *etc*.

While resident macrophage phenotype is likely to vary according to anatomical location, intestinal macrophages, found in the lamina propria (LP) have a particularly distinct phenotype. Their characterization in humans has revealed that these cells lack expression of a number of PRRs including CD14, scavenger receptor B, complement (C3) receptor (CR3), CR4 as well as receptors for IgG, IgA, integrin or the growth factor cytokines IL-2 and IL-3.[73] Intriguingly, the macrophages did not secrete cytokines IL-1, IL-6, IL-10, IL-12, TNF-α or TGF-β, when stimulated with an array of inflammatory stimuli but retained avid phagocytic and bacteriocidal activity.[73] This distinctive phenotype of intestinal macrophages was found to be imprinted via stromal-cell derived factors, in particular TGF-β (see below).[73]

Importantly, the 'inflammatory anergic' phenotype of intestinal macrophages is another possible mechanism by which mucosal inflammation is reduced in the intestinal mucosa. The 'acid test' for this theory is that the breakdown of the proposed mechanism should result in IBD. Indeed, this prediction was found to hold true in a new finding that implicates a unique $CD14^+$ macrophage subset from the LP of Crohn's disease patients playing a causative role in mediating inflammation.[74] Fascinatingly, relative to normal control patients, $CD14^+$ macrophages were found in greater numbers in the intestines of ulcerative colitis patients and even more so in Crohn's disease patients.[74] This subset of macrophages expressed numerous PRRs shown be absent on $CD14^-$ macrophages. Furthermore, in contrast to $CD14^-$ macrophages, $CD14^+$ macrophages produced large quantities of pro-inflammatory cytokines IL-23, TNF-α, IL-6 in response to stimulation with commensal bacteria.[74] Finally, $CD14^+$ macrophages were shown to stimulate, via a TNF-α and IL-23 synergy, IFN-γ secretion by T-cells.[74] This observation further supports the mechanism of TGF-β-mediated anergy, since IFN-γ is involved in the suppression of TGF-β signalling.

Dendritic cells

Dendritic cells are professional APCs which can activate lymphocytes either in the GALT or at mesenteric lymph nodes (MLNs). They are responsible for either delivering tolerogenic (signals promoting a non-inflammatory adaptive immune response, *e.g.* regulatory T-cell responses) or immunogenic signals (signals that skew the adaptive immune response towards an inflammatory phenotype, *e.g.* Th17 responses) to lymphocytes and, therefore, are at the interface between adaptive and innate immunity. Gut mucosal DCs fall into numerous subpopulations but are thought to have two general phenotypes – tolerogenic, non-inflammatory (resident) DCs and immunogenic DCs which are recruited upon pathogen invasion.

Non-inflammatory gut mucosal DCs contribute to tolerance by two principal means – promoting the differentiation of forkhead box P3 ($FoxP3^+$) T-regs able to suppress commensal-specific adaptive immune responses,[75] and inducing B-cells to secrete immunoglobulin A (IgA) which prevents commensals from breaching the gut mucosal barrier, thereby averting the induction of a systemic immune response.[37] Secretory IgA has also been implicated in the neutralization of LPS, which reduces signalling via TLR4.[76] The non-inflammatory phenotype of DCs is not an inherent property of all myeloid DCs, rather DCs are seen as being 'educated' by IECs.[77] Intestinal epithelial cells control the DC phenotype through the secretion of various mediators, the foremost of which are discussed briefly below.

Thymic stromal lymphoprotein

Thymic stromal lymphoprotein (TSLP) is a cytokine which promotes the polarization of naïve T-cells into Th2 cells by preventing the production of IL-12 (a Th1 skewing cytokine) by DCs.[78] It was shown that IEC-conditioned DCs were unable to drive Th1 responses and also acquired the ability to secrete IL-6, the cytokine responsible for driving the development of IgA secreting plasma cells.[78] Consistent with the proposed anti-inflammatory role of TSLP is the observation that nearly 70% of Crohn's disease patients have undetectable levels of TLSP.[78] Finally, other studies have shown that IEC-derived TLSP has the ability to drive the development of regulatory T-regs from naïve-cells in the gut.[79]

Transforming growth factor-β

Transforming growth factor-β and IL-10 are other anti-inflammatory cytokines that are known to be constitutively produced by IECs.[80,81] It has been shown that TGF-β can inhibit NF-κB-mediated gene expression in both intestinal macrophages[73] and DCs.[82] High concentrations of TGF-β have been recently shown to promote the DC-mediated differentiation of naïve $CD4^+$ T-cells into T-regs,[75,83] while also dampening the expression of IL-23 receptor,[83] thereby reducing the expansion of Th17 cells known to mediate inflammation in IBD. Maturation into T-regs was also dependent on retinoic acid (RA),[75] and since RA synthesizing enzymes are expressed by IECs,[84,85] this may be yet another level through which inflammation is kept in check. Together with its indirect role in establishing the anti-inflammatory environment of the GALT, IECs have lately been shown to induce T-reg expansion directly through MHC II-dependent antigen presentation.[85]

Interestingly, this expansion was independent of TGF-β and RA suggesting a distinct mechanism from that employed by DCs.

Interleukin-10

Interleukin-10 is a well-known anti-inflammatory cytokine and the IL-10 deficient mice commonly used as models of intestinal inflammation confirm its role in the gut. Apart from the typical, known producers of IL-10 such as T-regs, DCs, macrophages and B-cells, it was recently found that IL-10 is constitutively synthesized and secreted by the intestinal epithelial lining.[81] Interleukin-10 dampens inflammation primarily through its up-regulation of BCL3, which is an inhibitor of NF-κB.[81] Indeed, when IL-10 was depleted from human mucosal *ex vivo* explants, a down-regulation of BCL3 was noted along with a mutual increase in IFN-γ, TNF-α and IL-17.[81] Interestingly, the trigger for IFN-γ production was found to be LPS, suggesting that IL-10 prevents the injurious consequences of LPS and perhaps translocation of other PAMPs *in vivo*.[81] This final observation supports the finding that IL-$10^{-/-}$ mice fail to develop colitis when reared in germ-free conditions.[86]

Prostaglandin E_2

Lastly, the secretion of prostaglandin E_2 (PGE_2) and other metabolites by IECs has been implicated in the inhibition of IFN-α and IL-12 production.[87] Once again, the signalling pathway through which PGE_2 mediates its function is unclear. Together, these findings show that the tolerogenic nature of certain subclasses of DCs and the anergic phenotype of macrophages occupying the mucosa is dependent on a dialogue with IECs that is mediated via various immune-modulatory molecules. In summary, at steady state (in the absence of an infection), resting APCs promote T-reg differentiation and an overall anti-inflammatory micro-environment (Fig. 3). This is achieved through the secretion of a variety of

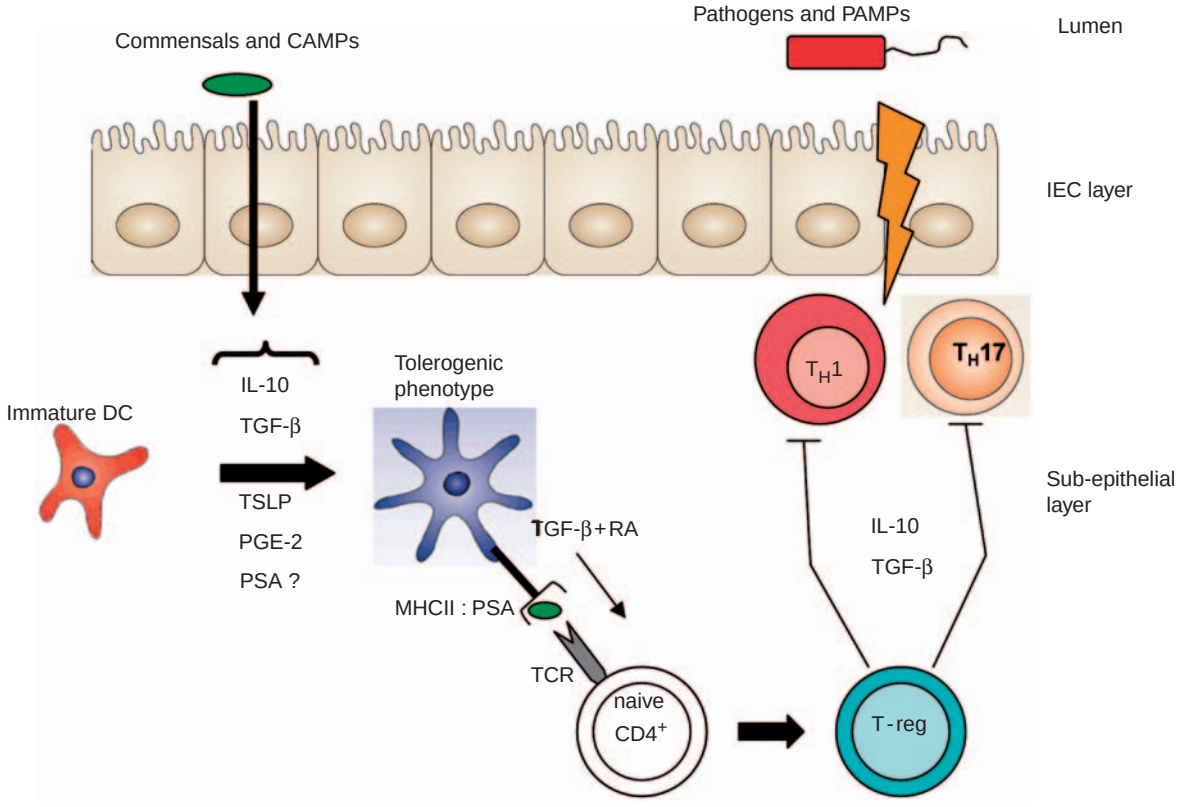

Fig. 3. Interactions between cells in the mucosal immune system that enable the maintenance of homeostasis. Dendritic cells (DCs) in the intestinal tract are shaped by numerous factors including IL-10, TGF-β, thymic stromal lymphoprotein (TSLP) and of prostaglandin (PGE-2). Some of these factors are constitutively secreted by IECs while others may be triggered or enhanced by commensal-derived signalling or commensal-associated molecular patterns (CAMPs). DCs acquire a unique phenotype that promotes polarization of naïve T-cells towards a regulatory T-cell phenotype (T-reg), a process dependent on TGF-β and retinoic acid (RA). Another means by which commensals may favour the skewing towards T-regs is through DC presentation of commensal-specific antigens such as polysaccharide A (PSA) which have unique unexplored properties. T-regs balance the inflammation (bolt) mediated via Th1 and Th17 cells induced by pathogen infection and pathogen-associated molecular patterns (PAMPs), *e.g.* LPS derived from commensals and pathogens.

soluble factors discussed here. Upon invasion, *i.e*. a breaching of the IEC barrier, pathogens signalling through innate immune receptors, activate APCs which, in turn, promote the development of effector T-cells and also the over-riding of the suppressive T-reg response.[88]

Inflammatory Bowel Disease: a Breakdown of Tolerance?

Inflammatory bowel disease is thought to be the consequence of a hyperactive response towards commensals, shown by the quelling of inflammation in most IBD models when mice are reared in germ-free conditions; however, exactly how commensals deliver the pathological signals in IBD is unknown. At least three possibilities exist: (i) dysbiosis (a breakdown in symbiosis); (ii) a weakened mucosal epithelium; and (iii) defective innate immune signalling. Evidence in support of each of these reasons exists.

Dysbiosis

If the balance between anti-inflammatory mutualists and other non-mutualists is skewed away from the former, inflammation may ensue. To this end, it has been shown that the abundance of *Faecalibacterium prausnitzii*, an anti-inflammatory commensal, is decreased in Crohn's disease patients.[89] Furthermore, the lack of early exposure to microbes may result in the underdevelopment of the intestinal immune system and/or a deficiency in the T-reg repertoire, both of which might be contributing factors in IBD.[7]

Epithelial damage

A loss-of-function mutation in NOD2, a cytosolic PRR, renders paneth cells unable to secrete α-defensins, which allows unfettered microbial growth in the gut and subsequent penetration of the intestinal epithelium wherein excessive immune activation can take place.[90,91]

Defective innate immune signalling

Genome-wide association studies have identified polymorphisms in NOD2 associated with Crohn's disease.[92,93] The ligand for NOD2 is muramyl dipeptide (MDP) found in peptidoglycan. Signalling through NOD2 activates the NF-κB pathway, and curiously also inhibits signalling through various TLRs.[94] A gain-of-function mutation in NOD2 may initiate inflammation by enhancing the NF-κB–dependent responses towards commensal-derived MDP. Moreover, a loss-of-function mutation may contribute to inflammation through the loss of TLR inhibition.[13] Defects in autophagy have also been found to be associated with Crohn's disease.[95]

Finally, the above-mentioned factors are not mutually exclusive; that is, dysbiosis and epithelial damage may themselves be consequences of chronic inflammation resulting from defective innate immune signalling. So it remains to be deduced which is a causative factor and which a secondary effect (Box 1).

Conclusions

What emerges from this discussion is that intestinal immune homeostasis is the result of a delicate balance between tolerance towards commensals and the induction of protective inflammatory responses against invasive pathogens. When this balance is skewed, it results in IBD pathologies such as Crohn's disease and ulcerative colitis. The maintenance of this balance is achieved through two general means – tolerance by exclusion and tolerance by constraint (summarized in Box 2). These mechanisms achieve a basal level of

Box 1. Future questions

Future studies will examine how IECs are differentially activated by commensals and pathogens, and elucidate how this differential activation translates into tolerogenic conditioning of mucosal DCs. In this regard, the recent identification of commensal-associated molecular patterns (CAMPs) such as PSA may hold the answer.[67] An additional area that has been largely overlooked in this field concerns gut immunity towards fungi. Most studies performed thus far have focused on TLR and NOD signalling in response to bacterial commensals. However, up to 2% of the microbes inhabiting the gut are fungi and representatives of all four major phyla have been found.[96] This raises questions into the consequence of IEC expression of C-type lectin receptors (CLRs) such as Dectin-1, which have a well established role in fungal detection,[97] and the role of Spleen tyrosine kinase (Syk)-dependent signalling (an adaptor associated with Dectin-1) in the maintenance of intestinal homeostasis. Fungi are notorious for their pathogenic relationships with humans and their beneficial contributions are vague. Nevertheless, many fungal species are mutualists in non-human hosts, *e.g*. the abundant anaerobic fungi involved in cellulose degradation in cows,[98] suggesting a possible analogous beneficial role in human hosts. Lastly, it remains to be studied if protozoans have any effects on intestinal homeostasis. To this end, helminth worms are recognized inducers of Th2 responses,[99] which may counteract the Th1 profiles characteristic of IBD.

Box 2. Summary of mechanisms used in discrimination

The host immune system and the normal flora that inhabit the gut have coevolved mechanisms that foster a peaceful co-existence. These are summarized below:

The nature of the microbe

Virulence factors – the prerequisite of virulence factors to stimulate the innate immune system means that only pathogenic microbes can induce inflammation

Modification of PAMPs – commensals, under selection pressure from the immune system, mutate their PAMPs to evade detection by PRRs

Anti-inflammatory signalling – commensals are able to attenuate the NF-κB pathway using various strategies

The architecture and nature of the GALT

Reduced or basolateral expression of PRRs on intestinal epithelial cells

Inflammatory 'anergic' phenotype of intestinal macrophages

Non-inflammatory intestinal DCs that promote regulatory T-cell responses

commensal-induced tonic signalling needed for maintaining intestinal homeostasis, while at the same time preventing the onset of classical overt inflammation. This ultimately allows the discrimination between commensals and pathogens.

Acknowledgements

The author thanks Professors Gordon Brown, Muazzam Jacobs, Siamon Gordon and Laura Roden for their advice and contributions to this manuscript. Funding was provided by the Mellon Mays Undergraduate Fellowship (MMUF) and Professor Gordon Brown.

References

1. Janeway Jr CA, Medzhitov R. Innate immune recognition. *Annu Rev Immunol* 2002; **20**: 197–216.
2. Palsson-McDermott EM, O'Neill LA. Building an immune system from nine domains. *Biochem Soc Trans* 2007; **35**: 1437–1444.
3. Roy CR, Mocarski ES. Pathogen subversion of cell-intrinsic innate immunity. *Nat Immunol* 2007; **8**: 1179–1187.
4. Medzhitov R. Recognition of microorganisms and activation of the immune response. *Nature* 2007; **449**: 819–826.
5. Macpherson AJ, Harris NL. Interactions between commensal intestinal bacteria and the immune system. *Nat Rev Immunol* 2004; **4**: 478–485.
6. Magalhaes JG, Tattoli I, Girardin SE. The intestinal epithelial barrier: how to distinguish between the microbial flora and pathogens. *Semin Immunol* 2007; **19**: 106–115.
7. Bouskra D, Brezillon C, Berard M *et al*. Lymphoid tissue genesis induced by commensals through NOD1 regulates intestinal homeostasis. *Nature* 2008; **456**: 507–510.
8. Pedron T, Sansonetti P. Commensals, bacterial pathogens and intestinal inflammation: an intriguing *menage a trois*. *Cell Host Microbe* 2008; **3**: 344–347.
9. Sartor RB. The influence of normal microbial flora on the development of chronic mucosal inflammation. *Res Immunol* 1997; **148**: 567–576.
10. Girardin SE, Boneca IG, Carneiro LA *et al*. Nod1 detects a unique muropeptide from Gram-negative bacterial peptidoglycan. *Science* 2003; **300**: 1584–1587.
11. Philpott DJ, Girardin SE. The role of Toll-like receptors and Nod proteins in bacterial infection. *Mol Immunol* 2004; **41**: 1099–1108.
12. Gewirtz AT, Simon Jr PO, Schmitt CK *et al*. *Salmonella typhimurium* translocates flagellin across intestinal epithelia, inducing a proinflammatory response. *J Clin Invest* 2001; **107**: 99–109.
13. Coombes JL, Powrie F. Dendritic cells in intestinal immune regulation. *Nat Rev Immunol* 2008; **8**: 435–446.
14. Coombes JL, Robinson NJ, Maloy KJ, Uhlig HH, Powrie F. Regulatory T cells and intestinal homeostasis. *Immunol Rev* 2005; **204**: 184–194.
15. Sansonetti PJ. War and peace at mucosal surfaces. *Nat Rev Immunol* 2004; **4**: 953–964.
16. Bates JM, Akerlund J, Mittge E, Guillemin K. Intestinal alkaline phosphatase detoxifies lipopolysaccharide and prevents inflammation in zebrafish in response to the gut microbiota. *Cell Host Microbe* 2007; **2**: 371–382.
17. Johansson ME, Phillipson M, Petersson J, Velcich A, Holm L, Hansson GC. The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. *Proc Natl Acad Sci USA* 2008; **105**: 15064–15069.
18. Vaishnava S, Behrendt CL, Ismail AS, Eckmann L, Hooper LV. Paneth cells directly sense gut commensals and maintain homeostasis at the intestinal host-microbial interface. *Proc Natl Acad Sci USA* 2008; **105**: 20858–20863.
19. Nagler-Anderson C. Man the barrier! Strategic defences in the intestinal mucosa. *Nat Rev Immunol* 2001; **1**: 59–67.
20. Artis D. Epithelial-cell recognition of commensal bacteria and maintenance of immune homeostasis in the gut. *Nat Rev Immunol* 2008; **8**: 411–420.
21. Lidell ME, Moncada DM, Chadee K, Hansson GC. *Entamoeba histolytica* cysteine proteases cleave the MUC2 mucin in its C-terminal domain and dissolve the protective colonic mucus gel. *Proc Natl Acad Sci USA* 2006; **103**: 9298–9303.
22. Sansonetti PJ, Tran Van Nhieu G, Egile C. Rupture of the intestinal epithelial barrier and mucosal invasion by *Shigella flexneri*. *Clin Infect Dis* 1999; **28**: 466–475.
23. Ismail AS, Behrendt CL, Hooper LV. Reciprocal interactions between commensal bacteria and gamma delta intraepithelial lymphocytes during mucosal injury. *J Immunol* 2009; **182**: 3047–3054.
24. Fahrer AM, Konigshofer Y, Kerr EM *et al*. Attributes of gammadelta intraepithelial lymphocytes as suggested by their transcriptional profile. *Proc Natl Acad Sci USA* 2001; **98**: 10261–10266.

25. Beutler B. Tlr4: central component of the sole mammalian LPS sensor. *Curr Opin Immunol* 2000; **12**: 20–26.
26. Abreu MT, Vora P, Faure E, Thomas LS, Arnold ET, Arditi M. Decreased expression of Toll-like receptor-4 and MD-2 correlates with intestinal epithelial cell protection against dysregulated proinflammatory gene expression in response to bacterial lipopolysaccharide. *J Immunol* 2001; **167**: 1609–1616.
27. Ortega-Cava CF, Ishihara S, Rumi MA *et al*. Strategic compartmentalization of Toll-like receptor 4 in the mouse gut. *J Immunol* 2003; **170**: 3977–3985.
28. Lotz M, Menard S, Hornef M. Innate immune recognition on the intestinal mucosa. *Int J Med Microbiol* 2007; **297**: 379–392.
29. Motzkus D, Schulz-Maronde S, Heitland A *et al*. The novel beta-defensin DEFB123 prevents lipopolysaccharide-mediated effects *in vitro* and *in vivo*. *FASEB J* 2006; **20**: 1701–1702.
30. Chamaillard M, Girardin SE, Viala J, Philpott DJ. Nods, Nalps and Naip: intracellular regulators of bacterial-induced inflammation. *Cell Microbiol* 2003; **5**: 581–592.
31. Hornef MW, Normark BH, Vandewalle A, Normark S. Intracellular recognition of lipopolysaccharide by Toll-like receptor 4 in intestinal epithelial cells. *J Exp Med* 2003; **198**: 1225–1235.
32. Gregorio E, Rappuoli R. Inside sensors detecting outside pathogens. *Nat Immunol* 2004; **5**: 1099–1100.
33. Viala J, Chaput C, Boneca IG *et al*. Nod1 responds to peptidoglycan delivered by the *Helicobacter pylori* cag pathogenicity island. *Nat Immunol* 2004; **5**: 1166–1174.
34. Hueck CJ. Type III protein secretion systems in bacterial pathogens of animals and plants. *Microbiol Mol Biol Rev* 1998; **62**: 379–433.
35. Skoyles J. How gut sampling and microbial invasiveness/noninvasiveness provides mucosal immunity with a nonmolecular pattern means to distinguish commensals from pathogens: a review. 2008; available from Nature Precedings <http://hdl.handle.net/10101/npre.2008.1895.1>
36. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R. Recognition of commensal microflora by Toll-like receptors is required for intestinal homeostasis. *Cell* 2004; **118**: 229–241.
37. Macpherson AJ, Uhr T. Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria. *Science* 2004; **303**: 1662–1665.
38. Matzinger P. The danger model: a renewed sense of self. *Science* 2002; **296**: 301–305.
39. Mariathasan S, Monack DM. Inflammasome adaptors and sensors: intracellular regulators of infection and inflammation. *Nat Rev Immunol* 2007; **7**: 31–40.
40. Hogquist KA, Nett MA, Unanue ER, Chaplin DD. Interleukin 1 is processed and released during apoptosis. *Proc Natl Acad Sci USA* 1991; **88**: 8485–8489.
41. Mariathasan S, Weiss DS, Newton K *et al*. Cryopyrin activates the inflammasome in response to toxins and ATP. *Nature* 2006; **440**: 228–232.
42. Dostert C, Petrilli V, Van Bruggen R, Steele C, Mossman BT, Tschopp J. Innate immune activation through Nalp3 inflammasome sensing of asbestos and silica. *Science* 2008; **320**: 674–677.
43. Hornung V, Bauernfeind F, Halle A *et al*. Silica crystals and aluminum salts activate the NALP3 inflammasome through phagosomal destabilization. *Nat Immunol* 2008; **9**: 847–856.
44. Franchi L, Eigenbrod T, Munoz-Planillo R, Nunez G. The inflammasome: a caspase-1-activation platform that regulates immune responses and disease pathogenesis. *Nat Immunol* 2009; **10**: 241–247.
45. Gurcel L, Abrami L, Girardin S, Tschopp J, van der Goot FG. Caspase-1 activation of lipid metabolic pathways in response to bacterial pore-forming toxins promotes cell survival. *Cell* 2006; **126**: 1135–1145.
46. Kumar A, Wu H, Collier-Hyams LS, Kwon YM, Hanson JM, Neish AS. The bacterial fermentation product butyrate influences epithelial signaling via reactive oxygen species-mediated changes in cullin-1 neddylation. *J Immunol* 2009; **182**: 538–546.
47. Shimazu R, Akashi S, Ogata H *et al*. MD-2, a molecule that confers lipopolysaccharide responsiveness on Toll-like receptor 4. *J Exp Med* 1999; **189**: 1777–1782.
48. Miller SI, Ernst RK, Bader MW. LPS, TLR4 and infectious disease diversity. *Nat Rev Microbiol* 2005; **3**: 36–46.
49. Golenbock DT, Hampton RY, Qureshi N, Takayama K, Raetz CR. Lipid A-like molecules that antagonize the effects of endotoxins on human monocytes. *J Biol Chem* 1991; **266**: 19490–19498.
50. Berezow AB, Ernst RK, Coats SR, Braham PH, Karimi-Naser LM, Darveau RP. The structurally similar, penta-acylated lipopolysaccharides of *Porphyromonas gingivalis* and *Bacteroides* elicit strikingly different innate immune responses. *Microb Pathog* 2009; **47**: 68–77.
51. Weintraub A, Zahringer U, Wollenweber HW, Seydel U, Rietschel ET. Structural characterization of the lipid A component of *Bacteroides fragilis* strain NCTC 9343 lipopolysaccharide. *Eur J Biochem* 1989; **183**: 425–431.
52. Weintraub A, Larsson BE, Lindberg AA. Chemical and immunochemical analyses of *Bacteroides fragilis* lipopolysaccharides. *Infect Immun* 1985; **49**: 197–201.
53. Mancuso G, Midiri A, Biondo C *et al*. *Bacteroides fragilis*-derived lipopolysaccharide produces cell activation and lethal toxicity via Toll-like receptor 4. *Infect Immun* 2005; **73**: 5620–5627.
54. Hayashi F, Smith KD, Ozinsky A *et al*. The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. *Nature* 2001; **410**: 1099–1103.
55. Andersen-Nissen E, Smith KD, Strobe KL *et al*. Evasion of Toll-like receptor 5 by flagellated bacteria. *Proc Natl Acad Sci USA* 2005; **102**: 9247–9252.
56. Lee WJ. Bacterial-modulated signaling pathways in gut homeostasis. *Sci Signal* 2008; **1**: pe24.
57. Neish AS. Molecular aspects of intestinal epithelial cell-bacterial interactions that determine the development of intestinal inflammation. *Inflamm Bowel Dis* 2004; **10**: 159–168.
58. Kelly D, Campbell JI, King TP *et al*. Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR-gamma and RelA. *Nat Immunol* 2004; **5**: 104–112.
59. Dubuquoy L, Jansson EA, Deeb S *et al*. Impaired expression of peroxisome proliferator-activated receptor gamma in ulcerative colitis. *Gastroenterology* 2003; **124**: 1265–1276.
60. Neish AS, Gewirtz AT, Zeng H *et al*. Prokaryotic regulation of epithelial responses by inhibition of IkappaB-alpha ubiquitination. *Science* 2000; **289**: 1560–1563.
61. Tien MT, Girardin SE, Regnault B *et al*. Anti-inflammatory effect of *Lactobacillus casei* on *Shigella*-infected human intestinal epithelial cells. *J Immunol* 2006; **176**: 1228–1237.
62. Ruckdeschel K, Harb S, Roggenkamp A *et al*. *Yersinia enterocolitica* impairs activation of transcription factor NF-kappaB: involvement in the induction of programmed cell death and in the suppression of the macrophage tumor necrosis factor alpha production. *J Exp Med* 1998; **187**: 1069–1079.
63. Collier-Hyams LS, Sloane V, Batten BC, Neish AS. Bacterial modulation of epithelial signaling via changes in neddylation of cullin-1. *J Immunol* 2005; **175**: 4194–4198.
64. Kumar A, Wu H, Collier-Hyams LS *et al*. Commensal bacteria modulate cullin-dependent signaling via generation of reactive oxygen species. *EMBO J* 2007; **26**: 4457–4466.

65. Petrilli V, Dostert C, Muruve DA, Tschopp J. The inflammasome: a danger sensing complex triggering innate immunity. *Curr Opin Immunol* 2007; **19**: 615–622.
66. Medzhitov R. Origin and physiological roles of inflammation. *Nature* 2008; **454**: 428–435.
67. Mazmanian SK, Round JL, Kasper DL. A microbial symbiosis factor prevents intestinal inflammatory disease. *Nature* 2008; **453**: 620–625.
68. Mazmanian SK, Kasper DL. The love–hate relationship between bacterial polysaccharides and the host immune system. *Nat Rev Immunol* 2006; **6**: 849–858.
69. Sachs JL, Simms EL. Pathways to mutualism breakdown. *Trends Ecol Evol* 2006; **21**: 585–592.
70. Ley RE, Peterson DA, Gordon JI. Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell* 2006; **124**: 837–848.
71. Matzinger P. Friendly and dangerous signals: is the tissue in control? *Nat Immunol* 2007; **8**: 11–13.
72. Rescigno M, Urbano M, Valzasina B *et al*. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nat Immunol* 2001; **2**: 361–367.
73. Smythies LE, Sellers M, Clements RH *et al*. Human intestinal macrophages display profound inflammatory anergy despite avid phagocytic and bacteriocidal activity. *J Clin Invest* 2005; **115**: 66–75.
74. Kamada N, Hisamatsu T, Okamoto S *et al*. Unique CD14 intestinal macrophages contribute to the pathogenesis of Crohn disease via IL-23/IFN-gamma axis. *J Clin Invest* 2008; **118**: 2269–2280.
75. Coombes JL, Siddiqui KR, Arancibia-Carcamo C *et al*. A functionally specialized population of mucosal $CD103^+$ DCs induces $Foxp3^+$ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. *J Exp Med* 2007; **204**: 1757–1764.
76. Coombes JL, Maloy KJ. Control of intestinal homeostasis by regulatory T cells and dendritic cells. *Semin Immunol* 2007; **19**: 116–126.
77. Iliev ID, Matteoli G, Rescigno M. The yin and yang of intestinal epithelial cells in controlling dendritic cell function. *J Exp Med* 2007; **204**: 2253–2257.
78. Rimoldi M, Chieppa M, Salucci V *et al*. Intestinal immune homeostasis is regulated by the crosstalk between epithelial cells and dendritic cells. *Nat Immunol* 2005; **6**: 507–514.
79. Watanabe N, Wang YH, Lee HK *et al*. Hassall's corpuscles instruct dendritic cells to induce $CD4^+CD25^+$ regulatory T cells in human thymus. *Nature* 2005; **436**: 1181–1185.
80. Oshima S, Nakamura T, Namiki S *et al*. Interferon regulatory factor 1 (IRF-1) and IRF-2 distinctively up-regulate gene expression and production of interleukin-7 in human intestinal epithelial cells. *Mol Cell Biol* 2004; **24**: 6298–6310.
81. Jarry A, Bossard C, Bou-Hanna C *et al*. Mucosal IL-10 and TGF-beta play crucial roles in preventing LPS-driven, IFN-gamma-mediated epithelial damage in human colon explants. *J Clin Invest* 2008; **118**: 1132–1142.
82. Fujita S, Seino K, Sato K *et al*. Regulatory dendritic cells act as regulators of acute lethal systemic inflammatory response. *Blood* 2006; **107**: 3656–3664.
83. Zhou L, Lopes JE, Chong MM *et al*. TGF-beta-induced Foxp3 inhibits T(H)17 cell differentiation by antagonizing RORgammat function. *Nature* 2008; **453**: 236–240.
84. Saurer L, McCullough KC, Summerfield A. *In vitro* induction of mucosa-type dendritic cells by all-trans retinoic acid. *J Immunol* 2007; **179**: 3504–3514.
85. Westendorf AM, Fleissner D, Groebe L *et al*. $CD4^+Foxp3^+$ regulatory T cell expansion induced by antigen-driven interaction with intestinal epithelial cells independent of local dendritic cells. *Gut* 2009; **58**: 211–219.
86. Xavier R, Podolsky DK. Commensal flora: wolf in sheep's clothing. *Gastroenterology* 2005; **128**: 1122–1126.
87. Contractor N, Louten J, Kim L, Biron CA, Kelsall BL. Peyer's patch plasmacytoid dendritic cells (pDCs) produce low levels of type I interferons: possible role for IL-10, TGFbeta, and prostaglandin E_2 in conditioning a unique mucosal pDC phenotype. *J Immunol* 2007; **179**: 2690–2694.
88. Powrie F, Maloy KJ. Immunology. Regulating the regulators. *Science* 2003; **299**: 1030–1031.
89. Sokol H, Pigneur B, Watterlot L *et al*. *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *Proc Natl Acad Sci USA* 2008; **105**: 16731–16736.
90. Kobayashi KS, Chamaillard M, Ogura Y *et al*. Nod2-dependent regulation of innate and adaptive immunity in the intestinal tract. *Science* 2005; **307**: 731–734.
91. Wehkamp J, Harder J, Weichenthal M *et al*. NOD2 (CARD15) mutations in Crohn's disease are associated with diminished mucosal alpha-defensin expression. *Gut* 2004; **53**: 1658–1664.
92. Hugot JP, Chamaillard M, Zouali H *et al*. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* 2001; **411**: 599–603.
93. Ogura Y, Bonen DK, Inohara N *et al*. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* 2001; **411**: 603–606.
94. Watanabe T, Asano N, Murray PJ *et al*. Muramyl dipeptide activation of nucleotide-binding oligomerization domain 2 protects mice from experimental colitis. *J Clin Invest* 2008; **118**: 545–559.
95. Cho JH. The genetics and immunopathogenesis of inflammatory bowel disease. *Nat Rev Immunol* 2008; **8**: 458–466.
96. Scupham AJ, Presley LL, Wei B *et al*. Abundant and diverse fungal microbiota in the murine intestine. *Appl Environ Microbiol* 2006; **72**: 793–801.
97. Taylor PR, Tsoni SV, Willment JA *et al*. Dectin-1 is required for beta-glucan recognition and control of fungal infection. *Nat Immunol* 2007; **8**: 31–38.
98. Gordon GL, Phillips MW. The role of anaerobic gut fungi in ruminants. *Nutr Res Rev* 1998; **11**: 133–168.
99. Finkelman FD, Shea-Donohue T, Goldhill J *et al*. Cytokine regulation of host defense against parasitic gastrointestinal nematodes: lessons from studies with rodent models. *Annu Rev Immunol* 1997; **15**: 505–533.