WAR AND PEACE AT MUCOSAL SURFACES

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Abstract | That we live with numerous bacteria in our gut without any adverse effects is a remarkable feat by the body's immune system, particularly considering the wealth of sensing and effector systems that are available to trigger inflammatory or innate immune responses to microbial intrusion. So, a fine line seems to exist between the homeostatic balance maintained in the presence of commensal gut flora and the necessarily destructive response to bacterial pathogens that invade the gut mucosa. This review discusses the mechanisms for establishing and controlling the 'dialogue' between unresponsiveness and initiation of active immune defences in the gut. *Si vis pacem, para bellum*. (If you wish for peace, prepare for war.)

In addition to its functions in digestion, nutrient transport, water and electrolyte exchange, and endocrine and paracrine hormone production, the intestinal epithelium has a role in defining the barrier between the host and the external environment. This barrier is essentially devoted to permanently protecting the body against invasion and systemic dissemination of both pathogenic and commensal microorganisms. The colon is permanently exposed to a high load of commensal bacteria and, together with dendritic cells (DCs)[1], intestinal epithelial cells (IECs) are the main cells to interact with luminal bacteria[2]. The intestinal epithelium is composed of three barriers in one[3]: a physical barrier, an innate immune barrier and an adaptive immune barrier. However, this barrier is not absolute and crosstalk occurs between the microorganisms and the mucosal tissues, with IECs mediating this dialogue. Microorganisms are constantly sampled from the intestinal lumen and taken into the inductive sites (that is, the Peyer's patches and lymphoid follicles) of the intestinal immune system, and the commensal microorganisms have an important role in the maturation of the gut and its immune system. Primates have evolved together with their intestinal microflora, resulting in a situation of mutualism or 'microbial tolerance', the exact mechanisms of which have only recently begun to be unravelled. The term microbial tolerance is not used here in the classical sense of immunological tolerance to antigens but in the sense of a lack of responsiveness to a bacterial population. Central to this tolerance is the concept of innate immunity: the intestinal barrier provides a combination of sensing and defence mechanisms that achieve permanent protection against intrusion by commensal microorganisms and therefore generate a state of 'physiological inflammation'. Entero-invasive bacterial pathogens excessively stimulate these innate mechanisms of mucosal protection, thereby causing rupture and inflammatory destruction of the epithelium. Identification of the receptors that these pathogens interact with and the signalling pathways that they 'interfere' with should allow identification of molecules that are important for the regulation of intestinal inflammation. This review compares the state of 'armed peace' that prevails in the interaction between the intestinal epithelium and its commensal bacteria, and the state of 'open war' that breaks out when a bacterial pathogen trespasses the intestinal borders.

The intestinal epithelium: an interactive barrier

Lesions of the intestinal epithelium must be quickly repaired, otherwise they allow penetration of commensal or pathogenic bacteria. Protection is maintained by a highly dynamic barrier at the epithelial lining, in which tightly bound IECs are renewed by accelerated division of crypt cells that migrate upwards from the bottom of the intestinal crypts. In addition to forming a physical barrier, the epithelial lining also forms a functionally

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interactive barrier that is crucial for the development of the innate immune response: it contains commensal microorganisms and can mount fast responses to pathogens through the rapid mobilization of humoral and cellular components. It is also central to the induction and expression of the mucosal adaptive immune response.

Physical barrier. The specialized architecture of the intestinal epithelium forms a tight barrier against the penetration of microorganisms. Two main structural components are essential[4]: the MICROVILLI of the BRUSH BORDER and the tight junctions[5]. Microvilli are associated with a dense meshwork of actin filaments that is linked to the adherens and tight junctions between the IECs and thereby regulate the permeability of the tight junctions and the barrier function of the epithelium[6]. Tight junctions are characterized by the presence of transmembrane proteins, such as isoforms of claudin[7] and occludin[8]. So, bacterial components that interfere with the normal function of regulatory proteins of the microvilli or tight junctions are expected to weaken the barrier and increase its permeability.

The physical barrier that is maintained by the epithelial lining is reinforced by the presence of a layer of glycocalyx, which is formed from mucins that bind the apical membrane of IECs. A thick layer of mucus, comprising diverse mucins, forms an additional system of protection. Goblet cells, the mucus-producing cells, are present both in the crypt and villus epithelium throughout the small intestine, colon and rectum. Massive release of mucin granules is triggered by the presence of physical, chemical or infectious insults[9]. Important functions of the mucus layer are to form a semipermeable protective barrier and to help accelerate the repair of intestinal damage, particularly through intestinal trefoil factor (CD73)[10]. Mucins also interact with bacterial cell-surface polysaccharides and protein appendages (such as flagella), thereby trapping bacteria in the mucus flow so that they are washed away by intestinal PERISTALSIS[11]. In general, bacteria cannot subvert these mechanisms unless they express mucinases[12] and adherence, colonization and invasion factors[13,14]. Accessibility to the apical surface of the intestinal epithelium and rupture of the barrier are indeed the main properties that distinguish pathogenic microorganisms from commensal microorganisms.

Innate immunity. Homeostasis of the epithelium requires the maintenance of a finely tuned balance between response and tolerance to the luminal microorganisms. Disruption of this balance leads to intestinal inflammation. In this crosstalk between the bacterial microorganisms and the epithelium, two main effectors regulate the apical density of microorganisms: antimicrobial peptides and neutrophils. Antimicrobial peptides have broad activities *in vitro* against Gram-positive and Gram-negative bacteria. Their amphipathic properties allow them to interact with and lyse bacterial membranes[15]. One of the main classes, the defensins[16], can be divided into two families: α-defensins and β-defensins. The α-defensins are produced by specialized cells at the bottom of the intestinal crypts; these are known as Paneth cells and are located in the small intestine. In humans, there are two main α-defensins: human defensin 5 (HD5; also known as DEFA5) and HD6 (REF. 17). The β-defensins are ubiquitously expressed throughout the gastrointestinal tract, including the colon. They are likely to have an important regulatory role in controlling the large bacterial population that is characteristic of the colonic commensal microflora. Among the β-defensins, human β-defensin 1 (BD1; also known as DEFB1) is constitutively expressed, whereas human BD2 is expressed at low levels and upregulated in response to infection or pro-inflammatory signals[18]. The cathelicidins are another class of antimicrobial peptide, in which a cathelin domain is linked to a peptide with antimicrobial activity[19]. In humans, the only identified cathelicidin, LL37 (also known as CAMP), is constitutively expressed by the intestinal epithelium.

In the presence of microbial colonization or invasion of the epithelium, neutrophils are recruited to the basal side of IECs, thereby contributing to the barrier. Recruitment to the site of infection occurs mainly in response to a gradient of CXC-chemokine ligand 8 (CXCL8; also known as interleukin-8, IL-8), which is mainly produced by epithelial cells. Neutrophils then translocate across the epithelial lining along a gradient of a different chemoattractant, which has been identified to be pathogen-elicited epithelial chemoattractant (PEEC) using *Salmonella typhimurium*-infected IECs[20]. After they have entered the gut lumen, neutrophils can exert their antibacterial function. Increased expression of antibacterial proteins, as well as recruitment of inflammatory cells (particularly neutrophils), forms part of the sentinel role of IECs, and after infection, IECs undergo activation of pro-inflammatory pathways, particularly the nuclear factor-κB (NF-κB)- and activator protein 1 (AP1)-signalling pathways[21]. This leads to the expression of several pro-inflammatory cytokines and chemokines that coordinate the innate immune response.

Adaptive immunity. In addition to maintaining efficient physical and innate immune barrier functions, the intestinal epithelium must also take an active part in the induction of adaptive immune surveillance at the mucosal surface. This function involves the collaboration of IECs with antigen-presenting cells (APCs) and lymphoid cells. This collaboration occurs mainly in the follicle-associated epithelium (FAE), which is characterized by the presence of M (microfold) cells. These epithelial cells are dedicated to the translocation of antigens and microorganisms from the intestinal lumen to the basolateral side of the epithelium, where they are in direct contact with mucosal lymphoid tissues that can initiate immune responses[22]. Mucosal lymphoid follicles and their associated FAE are distributed throughout the digestive tract, either as visible aggregates (particularly in the distal portion of the ileum in humans, where they are known as Peyer's patches[23]) or as single lymphoid follicles (in the colon and rectum)[24]. Owing to their lack

MICROVILLI
Multiple extensions of the apical pole of intestinal epithelial cells that are caused by membranous evaginations around actin bundles. Together, these extensions form the brush border, which physically protects the apical pole, particularly against pathogens.

BRUSH BORDER
Formed by the microvilli. It provides a large increase of the apical surface, thereby increasing the capacity of the epithelial surface for absorbing nutrients and exchanging water and electrolytes.

PERISTALSIS
An activity of the intestinal muscular layer that leads to unilateral movement, promoting the movement of the intestinal content from proximal to distal zones.

of surface glycocalyx and their high endocytic activity, M cells sample commensal bacteria and transfer them to DCs, in which a small number of bacteria can survive and induce T-cell-independent IgA responses, which are thought to account for the regulation of endogenous bacteria[25–27]. Recent evidence indicates that, in addition to these specialized inductive sites for mucosal immunity, lumenal microorganisms can also be captured by DCs that extend pseudopods across the IECs of the epithelial lining and retract these processes before trafficking to immunocompetent sites with their bacterial cargo[28]. Mucosal effector mechanisms encompass a complex array of both humoral factors, particularly secretory IgA, and cellular factors[29], including B cells, T cells and lymphocytes unique to the gut-associated lymphoid tissue (GALT), such as intra-epithelial lymphocytes and lamina-propria lymphocytes.

Crosstalk between commensals and mucosae

The relationship between the intestinal epithelium and its commensal microflora is a functional paradox. To avoid inflammation, it is crucial that most of the intestinal surface must be protected against close contact with bacteria and their products, and against an excessive response if it occurs. By contrast, specialized sites sample bacteria to promote an adaptive immune response and thereby increase protection[30]. This is the function of the FAE: translocated commensal bacteria are immediately destroyed by the resident phagocytic cells, and the resulting antigens are then presented to lymphocytes to generate a specific immune response[31]. The lack of intestinal inflammation that occurs despite the dense bacterial microflora populating the gut (particularly the colon) does not reflect mutual ignorance but, instead, reflects a combination of finely tuned, apparently antagonistic processes: permanent surveillance for trespassing microorganisms, which leads to minimum responses that eradicate these invaders (with IECs having a crucial role as sentinels); and tolerance, or at least lack of reactivity. This tolerance is largely an active process. The intestinal ecosystem has therefore evolved under the 'schizophrenic' constraint of downregulating the innate inflammatory response, while ensuring a proper adaptive immune response to any accidentally intruding commensal bacteria.

Physiological inflammation. It would be incorrect to describe the intestine as being in a constant state of low level inflammation. Even the colonic mucosa, which encounters the most 'pressure' from commensal microorganisms, shows only a marked mononuclear infiltrate but no neutrophils — the latter being considered the signature of intestinal inflammation[32]. It is more appropriate to consider that, under normal conditions, the pressure of the intestinal microflora is translated (particularly by IECs) into tolerogenic signals, which are sent to immunocompetent cells, while any 'slip-up' in this process leads to an immediate, finely controlled and localized inflammatory response that goes clinically unnoticed.

The signalling loop that mediates the epithelial response to microorganisms is based on sensing of structural motifs (known as PATHOGEN-ASSOCIATED MOLECULAR PATTERNS[33], PAMPs) that are specific for prokaryotic components, such as lipopolysaccharide (LPS), lipoprotein, peptidoglycan (PGN), lipoteichoic acid, flagellin and CpG-containing (unmethylated) DNA. It should be noted that these molecular motifs are expressed by both commensal and pathogenic microorganisms. The motifs are recognized by PATTERN-RECOGNITION RECEPTORS (PRRs). The best-known PRRs are the Toll-like receptors (TLRs), which are expressed by cells of the myeloid and lymphoid lineages, as well as by epithelial and endothelial cells[34]. TLRs have recently been shown to be involved in the recognition of commensal bacteria, thereby participating in intestinal homeostasis[35]. TLRs sense extracellular PAMPs and trigger 'outside in' signalling that leads to the activation of pro-inflammatory pathways[36–39]. More recently, another family of PRRs has been recognized: the nucleotide-binding oligomerization domain (NOD) family of proteins, which are expressed intracellularly and recognize PGNs. NOD1 recognizes muramyl tripeptides from Gram-negative bacteria, which are characterized by the presence of *meso*-diaminopimelic acid. By contrast, NOD2 recognizes a minimal motif, muramyl dipeptide, that is common to the PGNs of all bacterial species, regardless of their Gram-staining characteristics[40–42]. Although there are differences in the signalling pathways that are triggered by the ligation of various PRRs, ligation of each type of PRR induces the expression of pro-inflammatory genes driven by both the transcription factor NF-κB and the associated pro-inflammatory cascades, such as the mitogen-activated protein kinase (MAPK) pathways involving p38 and JNK (JUN amino-terminal kinase)[43,44]. This leads epithelial cells to produce an array of pro-inflammatory cytokines and chemokines, among which CXCL8 is most abundant.

Responsiveness in the crypts. It is probable that the intestinal crypts — which maintain a sterile luminal content to enable permanent regeneration of the epithelium by resident stem cells — are responsive to the presence of overgrowing bacteria through sensing of released PAMPs. For example, at least in the small intestine of mice, the expression of TLR4 (REF. 45), and possibly the NOD proteins, occurs specifically in the epithelium at the bottom of the crypts[46]. Following activation of the pro-inflammatory pathways, an array of defence mechanisms account for 'cleaning up' the lumen of the crypts, including expression and release of antibacterial defensins and attraction of neutrophils (FIG. 1). This has raised an interesting paradigm that could account, in a certain category of patients, for the initial inflammatory process that is observed in Crohn's disease. It has recently been shown that ~30% of familial cases of Crohn's disease involve a loss-of-function mutation in *NOD2* (REFS 47,48), thereby rendering cells expressing the mutated NOD2 unable to respond to its agonist, muramyl dipeptide. Immunostaining of intestinal tissues has shown that NOD2 is highly expressed and is mainly localized in the Paneth cells[49], which produce α-defensins. It is possible

PATHOGEN-ASSOCIATED MOLECULAR PATTERNS (PAMPs). Molecular motifs that are characteristic of prokaryotes and are thereby recognized by the mammalian innate immune system.

PATTERN-RECOGNITION RECEPTORS (PRRs). Host receptors (such as Toll-like receptors) that can sense pathogen-associated molecular patterns and initiate signalling cascades (involving the activation of nuclear factor-κB) that lead to an innate immune response.

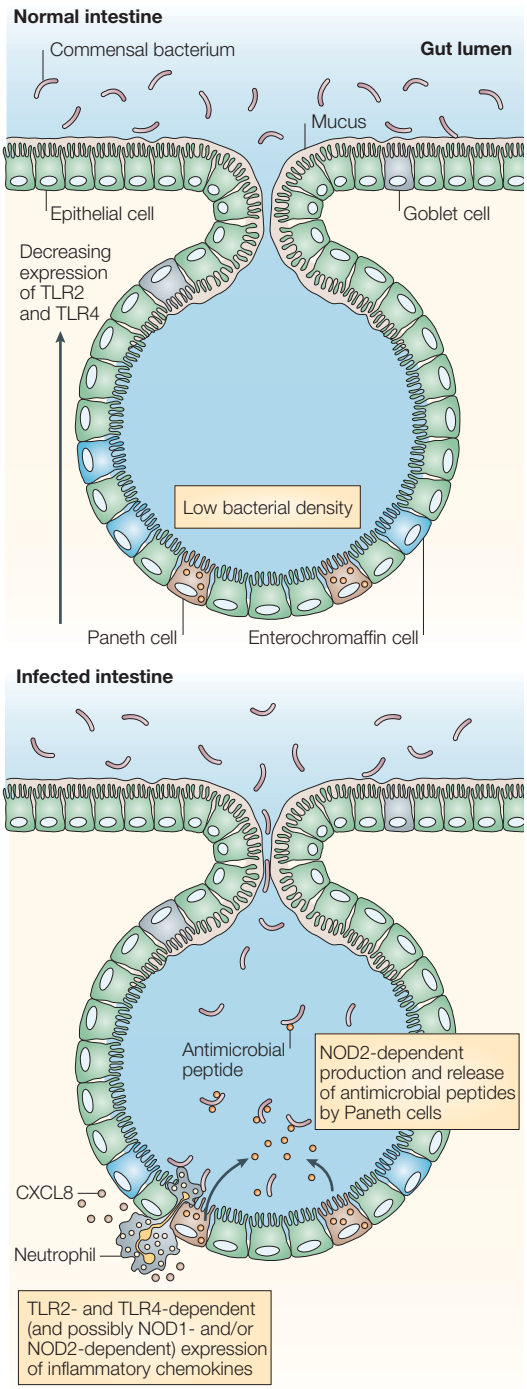

Figure 1 | **Expression of TLRs and NOD2 by luminal surface versus crypt epithelial cells in the small intestine.** This scheme shows the probable differences between the epithelial cells at the luminal surface and those in the crypts of the gut in terms of their expression of pattern-recognition receptors — such as Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain (NOD) proteins — for sensing the presence of microorganisms through their pathogen-associated molecular patterns. To protect stem cells and their environment, crypts are organized as integrated units of bacterial sensing and destruction, in which Paneth cells (through their production of defensins) have an important role. A similar pattern is likely to occur in the colon, in which Paneth cells are absent, but β-defensins are produced by epithelial cells. CXCL8, CXC-chemokine ligand 8.

that lack of expression and release of α-defensins in response to the abnormal presence of bacteria in the sterile crypts leads to uncontrolled bacterial growth, which eventually triggers extensive local inflammation, granuloma formation and transmural abscesses. This would be a typical example of the effect of a 'break-down' in the finely tuned network of physiological inflammation in the intestinal tissue.

Unresponsiveness at epithelial surfaces. Considering that the molecular motifs that activate both the TLRs and the NOD proteins are present at the surface of both commensal and pathogenic microorganisms, it is important to understand why commensal microorganisms do not generate permanent intestinal inflammation. Three principal factors are expected to account for tolerance to commensal microorganisms: properties of the bacteria themselves, characteristics of the epithelial surface and properties of the immune cells that are present in the lamina propria (BOX 1).

Although our knowledge of the ~500 bacterial species that constitute the intestinal microflora remains limited[50] (particularly because most of these species are extremely oxygen-sensitive obligate anaerobes and are therefore difficult to culture), these microorganisms all have some general characteristics — such as their lack of pathogenicity factors (for example, adhesins and invasins) — that impair their ability to colonize the epithelium. Therefore, as previously discussed, these bacteria and their released PAMPs are constantly trapped in secreted mucus and washed away by peristaltic movements. Also, many of the dominant species belong to the Gram-negative genus *Bacteroides*[51]; the lipid A (the endotoxic portion of LPS that is anchored in the outer membrane) of these bacteria is pentacylated and therefore has a low endotoxicity[52] or can even have an antagonistic action towards potently endotoxic hexacylated lipid A[53]. This is known as tolerance by ignorance or blindness, a characteristic that is also a property of the mononuclear cells that underlie the lamina propria, particularly the resident macrophages that do not express CD14 (REF. 54).

The surface of the epithelium also participates in tolerance by ignorance or blindness through several strategies. First, the TLR machinery of epithelial cells might be defective at the level of the epithelial surface, most of which is exposed to the bacterial microflora. For example, it has been shown that, although some IEC lines express detectable amounts of mRNA encoding TLR4, production of TLR4 protein is negligible[55,56], and these cells lack the cofactors that are required for LPS recognition, such as CD14 and MD2 (REF. 55). Second, the TLRs might be 'hidden', as is the case for TLR5. TLR5 recognizes the monomeric subunits of bacterial flagellin that are secreted by flagellated bacteria[57,58], and its expression is thought to be polarized to the basolateral side of IECs[38], although this is under debate[59]. So, recognition of the flagellin subunits might require delivery to the basal side of the epithelium, which could be achieved either by subversion of the barrier function of tight junctions or by transepithelial translocation of flagellin

Box 1 | **Mechanisms for intestinal tolerance to commensal bacteria**

Commensal bacteria
- Impaired ability to escape trapping in mucus
- Impaired ability to adhere and invade the epithelial barrier
- Low endotoxicity, as a result of having pentacylated lipid A (Gram-negative bacteria)

Mucosal epithelium
- Defective sensing of molecular pathogen-associated molecular patterns (except for crypt cells), as a result of reduced expression of sensing molecules (for example, Toll-like receptor 2 (TLR2), TLR4, MD2 and CD14) or sequestration of sensing molecules (for example, basolateral expression of TLR5)
- Early warning systems (for example, nucleotide-binding oligomerization domain proteins) for the detection of invading pathogens
- Permanent induction of active anti-inflammatory systems under pressure of the gut microflora. (For example, activation of the nuclear receptor PPAR-γ (peroxisome-proliferator-activated receptor-γ) results in inhibition of the nuclear factor-κB pathway)

Lamina propria
- Contains tolerogenic dendritic cells, macrophages and regulatory T cells that produce anti-inflammatory cytokines (such as interleukin-10 and transforming growth factor-β) in response to commensal bacteria

— two properties that could distinguish commensal bacteria from pathogenic bacteria. Owing to structural variations, some flagellin molecules might also have a low intrinsic ability to stimulate TLR5 present at the cell-surface of gastric cells[60]. Third, converging evidence indicates that recognition of PAMPs by the intestinal epithelium occurs intracellularly. This seems to be the case for TLR4, which has been shown to be present in the Golgi apparatus, where it co-localizes with internalized LPS. This is in contrast to TLR4 expressed by mononuclear cells, which is present at the cell surface[45]. In polarized intestinal cells, LPS has also been shown to concentrate in the apical recycling endosome, in which it can be intercepted by transcytosing LPS-specific secretory IgA — an interaction that leads to neutralization of the pro-inflammatory response to LPS[61]. NOD1-dependent recognition of muramyl tripeptides also occurs intracellularly[40]. So, another characteristic of pathogenic bacteria, in contrast to commensal bacteria, might be their capacity to carry out or facilitate introduction of PAMPs into epithelial cells; further investigation is required to confirm this possibility. An exciting example of this process is provided by *Helicobacter pylori*, which can programme gastric epithelial cells to transcribe pro-inflammatory genes, particularly those encoding chemokines (such as CXCL8) that attract neutrophils. This property is largely linked to the presence of the Cag pathogenicity island (PAI) in the *H. pylori* genome; the Cag PAI encodes a TYPE IV SECRETORY SYSTEM[62] that can translocate the Cag effector proteins to the cell cytoplasm. However, none of the Cag proteins can induce inflammation, and subsequent experiments have shown that PGN fragments introduced into gastric cells through the type IV secretory system of *H. pylori* induce NOD1-dependent activation of NF-κB and thereby transcription of the inflammatory programme. Interestingly, NOD1-deficient mice cannot regulate the density of *H. pylori* growing at their gastric epithelial surface, indicating that, after recognition of its cognate PAMP, NOD1 activates the recruitment of inflammatory cells — particularly neutrophils, which cross the epithelial barrier to eradicate the bacteria — thereby inducing chronic gastritis (J. Viala, unpublished observations) (FIG. 2).

Adding to the subtlety of the epithelial mechanisms that control intestinal inflammation, the commensal flora might participate in bacterial tolerance through an active crosstalk with the epithelium, thereby achieving tolerance by constraint. It seems that there are counter-regulatory mechanisms that might have an important role in the induction of tolerance to PAMPs. Expression and activation of molecules such as a truncated version of the TLR adaptor MyD88 (myeloid differentiation primary-response protein 88), which has a dominant-negative role in NF-κB activation[63], and IRAK-M (IL-1-receptor-associated kinase M), a negative regulator of TLR signalling[64], might have a role; however, this needs to be confirmed *in vivo*.

One target for the active induction of epithelial tolerance is the peroxisome-proliferator-activated receptor-γ (PPAR-γ), which has an anti-inflammatory function[65]. For example, non-pathogenic Gram-negative bacteria can induce the expression and activation of PPAR-γ, which then downregulates the inflammatory response. Engagement of TLR4 by the LPS of commensal Enterobacteriaceae might account for this process[66]. More recently, a study of the interactions of the probiotic *Bacteroides thetaiotaomicron* with IECs showed a novel function of PPAR-γ in its role as a negative regulator of NF-κB activation and therefore in its anti-inflammatory role. Instead of interfering with NF-κB activation in the cytoplasm, *B. thetaiotaomicron* triggers the association of PPAR-γ with the REL-A subunit of the NF-κB transcriptional complex, leading to the formation of a complex that is retro-transported from the nucleus to the cytoplasm, thereby blocking transcription of NF-κB-activated pro-inflammatory genes[67].

An alternative target for maintaining epithelial tolerance is the ubiquitylation pathway, which leads to protein degradation by the proteasome. Exposure of IECs to non-virulent *Salmonella* spp. prevents ubiquitylation of the α-subunit of inhibitor of NF-κB (IκBα) — which is normally induced by virulent *Salmonella* spp. or tumour-necrosis factor (TNF) — probably by blocking the function of the ubiquitylation complex E3 ubiquitin ligase–β-transducin-repeat-containing protein, which mediates ligation of ubiquitin molecules to the protein targeted for degradation[68]. As a consequence, IκBα is not degraded, and NF-κB does not translocate to the nucleus and thereby does not mediate the transcription of target genes. It could be argued that non-virulent *Salmonella* spp. cannot be considered as typical commensal microorganisms; nevertheless, it is possible that the ubiquitylation and proteasomal degradation of proteins that are important for regulating inflammatory cascades are probable targets for the induction of tolerance by constraint.

TYPE IV SECRETORY SYSTEM
A type of molecular syringe that Gram-negative bacteria have. It enables these bacteria to deliver DNA (by species such as *Agrobacterium*) and protein effectors (by species such as *Helicobacter pylori*) into eukaryotic cells.

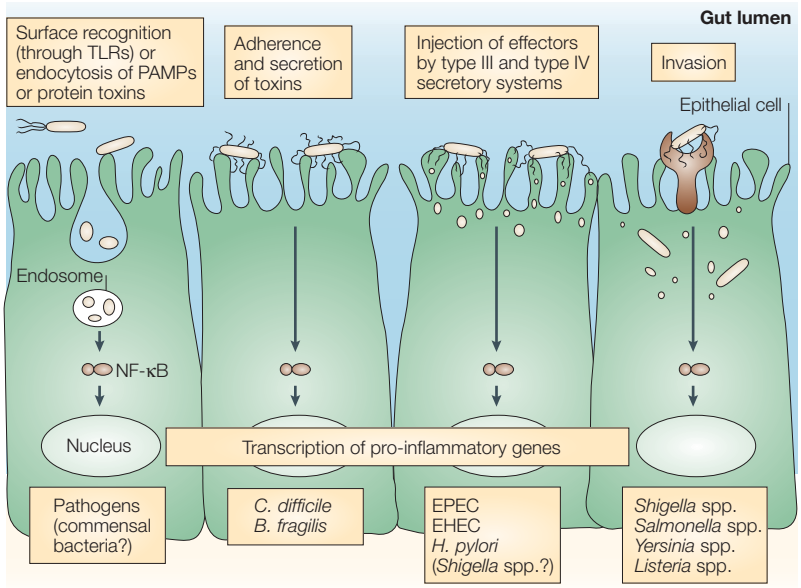

Figure 2 | **Bacteria trigger a pro-inflammatory programme in intestinal epithelial cells, using various strategies.** Pathogenic bacteria and possibly commensal bacteria can be detected by epithelial cells through cell-surface receptors (such as Toll-like receptors, TLRs) or by endocytosis of microbial products. Detection by TLRs or other intracellular pattern-recognition receptors triggers a signalling cascade that results in the activation of nuclear factor-κB (NF-κB), which translocates to the nucleus, where it promotes the transcription of pro-inflammatory genes. Some pathogenic bacteria (such as *Clostridium difficile* and *Bacteroides fragilis*) adhere to epithelial cells and secrete toxins, which induce NF-κB activation. By contrast, enteropathogenic *Escherichia coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC) and *Helicobacter pylori* inject effector molecules into the cell through type III or type IV secretory systems. A different mechanism is also used by *Shigella* spp. and *Salmonella* spp., which directly invade the cell, resulting in NF-κB activation (which is thought to be largely nucleotide-binding oligomerization domain (NOD)-protein dependent) and stimulation of an inflammatory response. PAMP, pathogen-associated molecular pattern.

Finally, recent work indicates that DNA from probiotic bacteria, such as *Bacteroides vulgatus*, limits IEC-mediated pro-inflammatory responses *in vitro* and *in vivo*[69]. This indicates that the model of TLR9 recognizing unmethylated (prokaryotic) CpG-containing DNA and not methylated eukaryotic DNA requires further refinement.

Adaptive immune tolerance. It should be stressed that, in the lamina propria of the intestine, the adaptive immune response is also oriented towards tolerance, and most microbial and food antigens do not elicit an adaptive immune response. Sampling of commensal microorganisms is achieved both by IECs of the FAE and by DCs that capture microorganisms and their antigens and carry them to immunocompetent organs. DCs can even extend dendrites through epithelial tight junctions to sample luminal antigens directly[28]. It is probable that PRRs provide an array of crucial signals that modulate the differentiation of APCs (particularly DCs), thereby strongly influencing the nature of the immune response and, in particular, its orientation towards tolerance. Control of the expression of IL-12 — which is produced by APCs in response to pathogenic microorganisms, but not commensal microorganisms — is considered an essential element of tolerogenicity[70]. In addition, T cells of thymic origin that circulate in the lamina propria and are specific for commensal antigens need to be actively suppressed to avoid uncontrolled inflammation following the translocation of these commensal antigens. The presence of regulatory T cells is a characteristic of the lamina propria. These cells, which are known as T helper 3 cells or regulatory T cells, inhibit the activation, differentiation and proliferation of other T cells. They produce IL-10 and/or transforming growth factor-β, and the neutralization of either of these two cytokines abolishes the suppressive functions of these cells[71]. The observation that IL-10-deficient mice develop enterocolitis in the presence of an intestinal flora supports the above scheme.

In summary, the lack of an inflammatory response to commensal bacteria reflects dual tolerance mechanisms — one by ignorance and one by constraint — in which a certain level of crosstalk between bacteria, the epithelium and lamina-propria cells leads to a global, although fragile, balance that actively regulates intestinal inflammation in a negative manner. It is probable that 'breaks' in these subtle and intricate mechanisms of tolerance to the commensal microflora, possibly occurring in genetically predisposed individuals, account to a large extent for inflammatory bowel diseases (IBDs)[72], such as Crohn's disease and ulcerative colitis.

Crosstalk between pathogens and mucosae

In contrast to commensal microorganisms, enteric bacterial pathogens subvert, sometimes invade and often cause inflammatory destruction of the intestinal epithelium. Pathogens need to gain access to the epithelial surface to colonize it and (for some) to then disrupt this barrier and invade the mucosa, as well as to overcome host-defence mechanisms that are triggered by their aggressive behaviour. Although some pathogenic microorganisms, such as *Vibrio cholerae*, colonize the epithelial surface[73], the ultimate pathogenic activity is mucosal invasion, and this is the focus of this review. Despite this, pathogenic bacteria that colonize the epithelial surface[73] can cause inflammation through the production of cytotoxins, such as those secreted by *Clostridium difficile* and *Bacteroides fragilis*. Alternatively, colonization occurs through close adherence to, and effacement of, the brush border, followed by injection of effectors through a TYPE III SECRETORY SYSTEM (TTSS), such as that used by enteropathogenic *Escherichia coli* (EPEC) and enterohaemorrhagic *E. coli* (EHEC). The latter also produce cytotoxins known as Shiga-like toxins. Rupture of the intestinal barrier by Gram-negative enteroinvasive pathogens can lead to three main outcomes, depending on the intrinsic virulent properties of the pathogen[74]: 'local' mucosal invasion, as exemplified by *Shigella* spp.[75]; 'loco-regional' infection, as carried out by *Yersinia* spp.[76]; or a complex infectious pattern combining both 'local' mucosal invasion and systemic dissemination, as exemplified by *Salmonella* spp.[77]. There are two main routes for bacteria to cross the intestinal epithelium. The first, 'assisted crossing', involves M cells of the FAE, whereas the second could be described as 'head-on crossing', in which bacteria have developed the ability to attack the epithelium. The latter strategy encompasses several tactics that require crosstalk

TYPE III SECRETORY SYSTEM (TTSS). A molecular syringe that is prevalent in pathogenic and symbiotic Gram-negative bacteria. TTSSs can deliver effector molecules into eukaryotic target cells.

between invasive microorganisms and the epithelium. This dialogue that is established between Gram-negative entero-invasive pathogens and the intestinal epithelium is mainly mediated by effectors secreted into cells by a TTSS[78].

Use of M cells by entero-invasive pathogens. A large range of bacterial pathogens exploit M-cell transport to invade the intestinal mucosa[23,30]. Most of the molecular interactions that mediate translocation through M cells are not yet clear. As well as the generic, non-specific mechanisms that facilitate the capture and transport of any luminal particle, specific mechanisms of adherence and internalization are likely to exist but are probably redundant. For example, the product of the *lpf* fimbrial operon of *S. typhimurium*[79] and the Inv adhesin of *Yersinia pseudotuberculosis* bind with high affinity to the β_1-integrins expressed apically by M cells[80]. For *Shigella* spp., the TTSS allows extensive translocation of bacteria through the FAE compared with the limited background translocation of a non-invasive mutant[81]. Similarly, *S. typhimurium spi1* (*Salmonella* PAI 1) encodes a TTSS that injects effectors, which reorganize the cytoskeleton of the cell, thereby mediating the translocation of bacteria through M cells[82].

Although translocation through M cells seems to be an easy process for these microorganisms, bacterial survival in the Peyer's-patch or the lymphoid-nodule environment is a challenge because incoming bacteria (particularly in the dome area of the lymphoid follicles) face a dense group of phagocytic cells, both resident cells and those that are quickly recruited[83]. This highlights that invasive microorganisms not only need to cross the epithelial lining but also need to resist innate immune defences to survive phagocytosis and killing and to establish infection. Various strategies to subvert host immune defences can be described using the examples of *Yersinia*, *Shigella* and *Salmonella* spp.

Yersinia spp. have a dual strategy (FIG. 3) that is both antiphagocytic, owing to intracellular injection of YopE, YopH and YopT, which inactivate the actin cytoskeleton, and anti-inflammatory, owing to the block in TNF production that is mediated by YopP, which then inhibits further recruitment of the pro-inflammatory infiltrate, particularly monocytes and neutrophils[84]. Alternatively, phagocytosed *Yersinia* spp. can cause YopP-dependent apoptosis of the host cell. These Yop proteins encoded by the virulence plasmid of *Yersinia* spp. and injected through the TTSS therefore function as effector proteins that 'anaesthetize' the innate immune response, thereby achieving immediate bacterial survival. This strategy is reflected in the clinical symptoms of infection with *Yersinia* spp., which are dominated by the formation of ileal and mesenteric abscesses in which bacteria are mainly extracellular[85].

Following translocation through M cells, the behaviour of *Shigella* spp. differs markedly from the behaviour of *Yersinia* spp.[86] (FIG. 4). After translocation across the FAE, *Shigella* spp. ensure their immediate survival by causing apoptosis of macrophages and monocytes[87,88] through IpaB-mediated activation of caspase-1 (REFS 89,90). However, this mechanism also triggers early mucosal inflammation through the release of mature IL-1β and IL-18, as a result of caspase-1-mediated cleavage of their precursors. This inflammatory process is a 'double-edged sword' with regard to successful *Shigella* infection. On the one hand, it disrupts the impermeable epithelial barrier and facilitates bacterial invasion in the area surrounding the initial point of epithelial translocation[91,92]. The role of IL-1β in this process of disruption has been clearly established[93,94]. On the other hand, IL-18 is required for the control of infection with *Shigella* spp.[94], probably through stimulating the production of interferon-γ[95]. In addition, neutrophils produce elastase, which actively degrades virulence factors such as Ipa proteins and IcsA. These events disarm the microorganisms[96] and eventually cause their death, in particular by trapping the bacteria extracellularly in intertwined filamentous structures known as neutrophil extracellular traps (NETs), which are composed of DNA and antimicrobial molecules (including histones and proteases)[97]. In response to these defence mechanisms, *Shigella* spp. need mechanisms to resist being killed. One of these is their ability to invade epithelial cells and then spread from cell to cell[98]. This mechanism of 'underground' colonization offers good protection against phagocytes and accounts for the clinical pattern of shigellosis, which is characterized by extensive epithelial

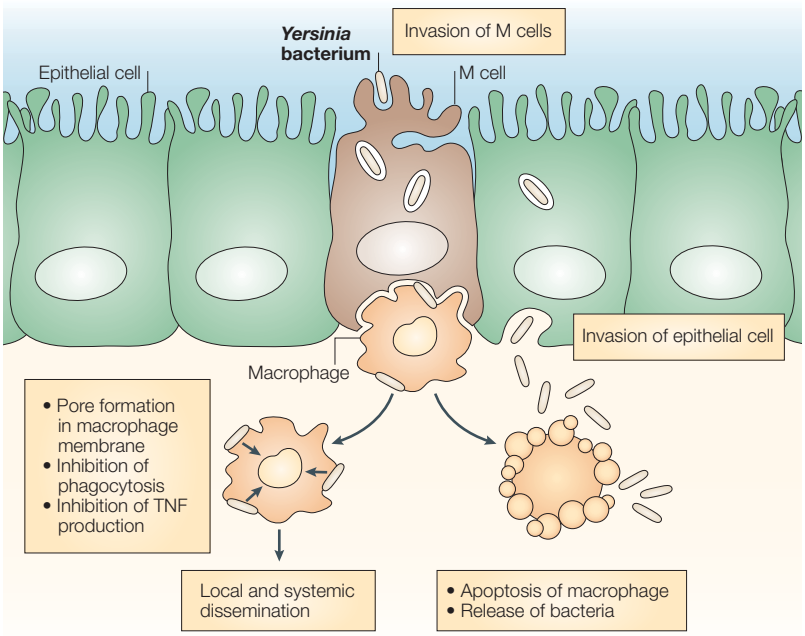

Figure 3 | **Steps of *Yersinia* spp. translocation of the intestinal epithelium and development of the infectious process leading to mesenteric lymphadenitis.** *Yersinia pseudotuberculosis* and *Yersinia enterocolitica* cross the epithelial barrier mainly through the M (microfold) cells of the follicle-associated epithelium of Peyer's patches in the ileal portion of the small intestine. To survive, these bacteria resist phagocytosis by macrophages through the injection of Yop effectors into these cells using a type III secretory system, leading to paralysis of the actin cytoskeleton. *Yersinia* spp. also downregulate inflammation, thereby avoiding humoral and cellular effectors of the innate immune response. *Yersinia* spp. might also cause apoptosis of macrophages. As a result, bacteria resist host immune defences in subepithelial tissues, allowing them to invade the epithelium and infect distant mesenteric lymph nodes. TNF, tumour-necrosis factor.

destruction. Recent evidence indicates that *Shigella* spp. can also manipulate the inflammatory response. Mutation in the chromosomal gene *shiA* results in increased inflammatory destruction compared with that caused by the homologous wild-type strain, indicating an as-yet-unknown mechanism of downregulating inflammation in response to mucosal invasion[99]. Recently, my research group has shown that OspG, one of the plasmid-encoded *Shigella* proteins that are injected into cells through the TTSS, interferes with proteasomal degradation of IκB, thereby downregulating the severity of inflammation. This was confirmed by the observation that an *ospG* mutant causes much less inflammation than the wild-type strain (D. W. Kim, unpublished observations). Downregulation of the expression of bactericidal peptides also reflects the capacity of *Shigella* spp. to manipulate the innate immune response to manage survival. Early in the course of shigellosis, it was shown that expression of the antibacterial peptides LL37 and BD1 was reduced or abrogated in biopsies from patients with bacillary dysentery[100].

Interestingly, *Salmonella* spp. establish another pattern of interaction that involves a combination of extra- and intracellular strategies that also rely on expression of a TTSS (FIG. 5). The initial step is similar to that of *Shigella* spp.; the SipB effector encoded by *spi1* (similar to the IpaB protein of *Shigella* spp.) causes macrophage apoptosis through activation of caspase-1 (REF. 101). However, the subsequent steps are considerably different.

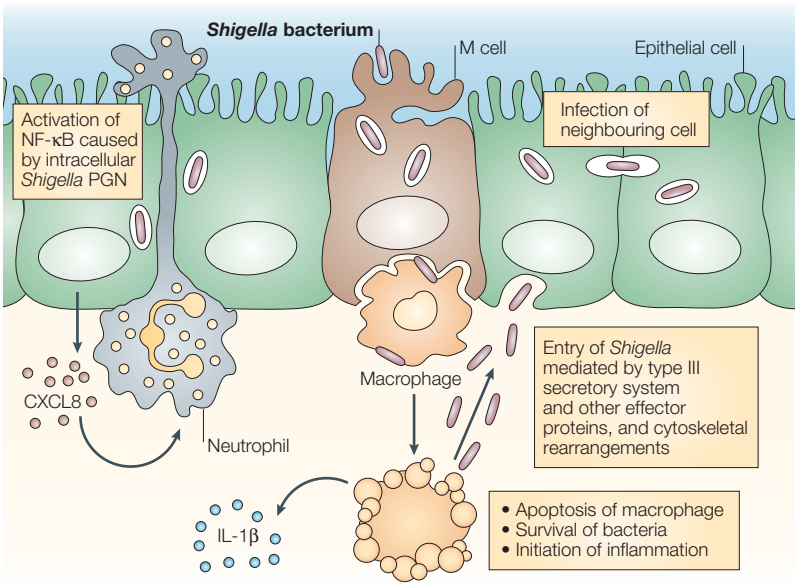

Figure 4 | **Steps of *Shigella* spp. translocation of the intestinal epithelium and development of the infectious process leading to bacillary dysentery.** *Shigella* spp. cross M (microfold) cells of the follicle-associated epithelium that covers the lymphoid nodules associated with the colonic mucosal tissues. In this subepithelial location, *Shigella* spp. cause extensive apoptosis of macrophages. This process allows escape of bacteria into the tissues and efficient basolateral entry to epithelial cells, followed by cell-to-cell spreading, which generates efficient intracellular colonization. Caspase-1-mediated apoptosis can also initiate inflammation through the release of mature interleukin-1β (IL-1β). The inflammatory mechanism is considerably amplified by the presence of intracellular bacteria that activate the NOD1 (nucleotide-binding oligomerization domain protein 1) pathway through the release of peptidoglycan (PGN). CXCL8, CXC-chemokine ligand 8; NF-κB, nuclear factor-κB.

They are characterized by a switch in strategy, in which the bacteria eventually 'accept' phagocytosis by resident macrophages of the dome and the surrounding area; however, through expression of Spi2 and its dedicated effectors, *Salmonella* spp. remodel their phagosome in such a way that they reside in a self-made and distinct intracellular compartment, known as the SCV (*Salmonella*-containing vacuole)[102–105]. The *Salmonella*-encoded protein SifA has an important role in mediating the recruitment of vesicles to increase the amount of SCV membrane, thereby avoiding rupture of the vacuole and transition to a lysosome[106]. This strategy is important for the capacity of *Salmonella* spp. to further disseminate in their host and cause septicaemia — a strategy reinforced in *Salmonella typhi* by expression of a capsule, the Vi antigen.

Direct invasion of the epithelial lining. As well as crossing the FAE, entero-invasive bacteria can also cross the villous epithelium of the small intestine or the superficial epithelium of the colon by direct attack of the epithelial lining, such as is carried out by *Listeria monocytogenes*. Several surface proteins contribute to entry of *L. monocytogenes* to epithelial cells *in vitro*. However, internalin A (InlA) is the main mediator of a 'zipper' type of internalization[107]. InlA allows direct crossing of the villous epithelium in the small intestine, because unlike *Yersinia*, *Shigella* and *Salmonella* spp., *L. monocytogenes* shows no tropism for the FAE[108]. Entry to cells involves interaction between InlA and human epithelial (E)-cadherin, a transmembrane glycoprotein that is normally involved in homophilic E-cadherin–E-cadherin interactions at the adherens junctions of polarized epithelial cells. The crucial role of InlA–E-cadherin interactions *in vivo* has been confirmed using a transgenic mouse model that expresses human E-cadherin[109]. After cellular invasion, *L. monocytogenes* escapes from the vacuole following membrane rupture mediated by listeriolysin O (LLO); it then moves through the cytoplasm, and then from cell to cell, in an actin-dependent process. In addition to its role in membrane lysis, LLO is also a signalling molecule that can activate pro-inflammatory pathways[110]. Despite this, the level of intestinal inflammation that is observed during the intestinal phase of listeriosis does not match the degree of inflammation observed in the course of shigellosis or salmonellosis. So, it is possible that either the pro-inflammatory effectors of *L. monocytogenes* are far less potent than those of its Gram-negative counterparts or *L. monocytogenes* has a strong compensating anti-inflammatory function.

Infection with *Salmonella* spp. can be considered as a model of crosstalk between apically established bacteria and IECs, leading not only to transepithelial translocation following Spi1-mediated entry to the cell but also to reprogramming of IECs, which induces them to express important mediators of the innate immune response, particularly chemokines that attract neutrophils. As discussed earlier, the sensing of flagellin by TLR5 is likely to be the main effector mechanism, leading not only to NF-κB-dependent activation of the expression of CXCL8 and other chemokines (which

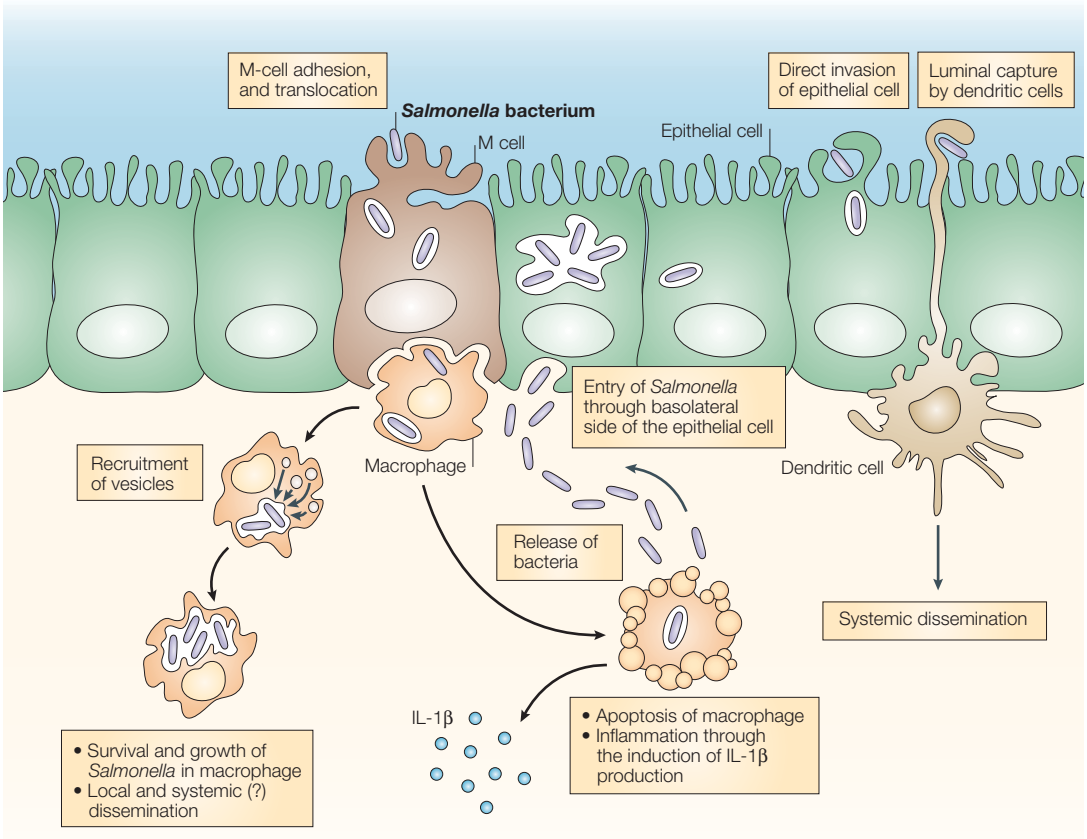

Figure 5 | **Strategies that allow *Salmonella* spp. to cross the intestinal barrier, survive in intestinal tissues and spread systemically.** *Salmonella* spp. cross M (microfold) cells of the follicle-associated epithelium mainly in the Peyer's patches of the ileal portion of the small intestine but possibly also in the colon. In this subepithelial location, *Salmonella* spp. might cause macrophage apoptosis through effectors injected using a type III secretory system that is encoded by Spi1 (*Salmonella* pathogenicity island 1), thereby also triggering inflammation. *Salmonella* spp. also switch to expression of Spi2, which encodes a type III secretory system that allows injection of effector proteins from the endocytic vacuole into the cell cytoplasm, thereby enabling bacteria to modify the vacuole to a *Salmonella*-containing vacuole, which supports bacterial survival and multiplication. This provides bacteria with the capacity to both invade epithelial cells basolaterally, owing to expression of Spi1 effectors, and to disseminate systemically. Alternatively, *Salmonella* spp. can also directly enter intestinal cells by the apical pole of the cell or be captured by dendritic cells that emit pseudopods between epithelial cells. The latter process promotes systemic dissemination of *Salmonella* spp. IL-1β, interleukin-1β.

recruit neutrophils and mononuclear cells to subepithelial tissues[37,57]) but also to a high level of expression of CC-chemokine ligand 20 (which is required for the recruitment of immature DCs), thereby establishing an immediate link between innate and adaptive immunity[59,111]. Furthermore, these events might contribute to the process of DC-mediated capture of luminal bacteria. It is also probable that, after *Salmonella* spp. have undergone cellular invasion, they activate a NOD-dependent inflammatory cascade — although this has not yet been convincingly shown, possibly because intracellular bacteria remain trapped in a closed vacuole from which PGN cannot easily diffuse into the cytosol. Recent evidence indicates that pathogens such as *Salmonella* that remain trapped in a vacuole are sensed and activate pro-inflammatory and apoptotic pathways[112].

Clearly, a gradient of CXCL8 formed by basolateral secretion is required for recruitment of neutrophils to the epithelium. However, this gradient cannot easily account for the transepithelial migration of neutrophils[113]. The interaction of *Salmonella* with the apical surface of IECs induces the production of PEEC, which has recently been shown to be identical to hepoxilin A3 (REF. 114) — an eicosanoid of which the synthesis might be under the control of an Arf6-dependent pathway induced by the *Salmonella* invasin SipA[115]. The dogma that *Shigella* spp. exclusively translocate the epithelial lining through the FAE might not be so clear cut. *In vivo* Shigellae might also invade the mucosa in areas devoid of FAE structures. This strategy could involve injection through their activated TTSS (from their extracellular position) of effector proteins that disorganize the cohesion of the epithelial layer or even induce cytotoxicity and cell killing, thereby opening avenues for translocation to subepithelial tissues. In addition, a few bacteria might eventually be able to enter epithelial cells apically, as observed in the small intestine of guinea pigs[116] and, more recently, in the colon of mice (M. L. Bernardini, unpublished observations) and following intrarectal

inoculation[117]. However, it is probable that mucosal inflammation triggered by bacteria colonizing the epithelial surface leads to a disruption of the epithelial lining in which neutralization of neutrophil infiltration[92], IL-1β[93] or CXCL8 (REF. 118) not only abrogates mucosal inflammation and epithelial destruction but also blocks bacterial invasion of the epithelium. Indeed, the question is how do *Shigella* spp. recruit an inflammatory infiltrate through crosstalk with the apex of IECs. Unlike *Salmonella* spp., *Shigella* spp. do not have flagella, so they cannot activate the TLR5-dependent pathway that seems to be important for clearance of *Salmonell*a spp. It is possible that mutations in the operons that are required for flagellar biosynthesis preserve *Shigella* spp. against surface eradication by an excessive innate immune response. As previously suggested, similar to *H. pylori*, *Shigella* spp., through their TTSS, might introduce PGN into the IEC cytoplasm and initiate a NOD1-dependent response, inducing expression of CXCL8 and other pro-inflammatory cytokines and chemokines. Other options for inducing an inflammatory response are possible, including expression of various Osp and IpaH proteins, some of which, after injection into cells, might have a pro-inflammatory property. At present, the evidence indicates that Osp proteins have an anti-inflammatory effect. Again, this might be part of a process in which the bacteria need to balance excessive inflammation, which could kill the host and therefore the bacteria, and insufficient inflammation, which does not allow 'unlocking' of the epithelial barrier for efficient invasion.

Assistance of bacterial invasion by DCs. *Salmonella* spp. have been shown to follow two main pathways of translocating the mouse intestinal epithelium. The first is the 'classical' pathway, involving translocating the FAE in Peyer's patches, whereas the second, used by some bacteria, involves translocating the regular villous epithelium using a mechanism that is CD18 dependent[119]. This latter pathway indicates that bacteria are transported by phagocytic cells, and it leads to systemic infection. The cells that mediate translocation are probably DCs that internalize bacteria from their location across the intestinal epithelium in the lamina propria[28]. Using this mechanism, DCs can open the tight junctions between epithelial cells and extend dendrites that sample luminal bacteria. As this process develops, the integrity of the epithelial barrier is preserved, owing to the expression of junctional proteins (such as claudins, occludin and zona occludens 1) by the DCs, and these proteins establish transient tight junctions with the epithelial cells. The degree to which this DC-assisted process takes part in the global process of *Salmonella* translocation and whether it also participates in the translocation of other invasive pathogens, such as *Shigella* spp. and *L. monocytogenes,* remains to be shown. Apart from these mechanistic aspects of bacterial invasion, DCs provide a crucial link between the innate and the adaptive immune response[120].

Conclusion

This review attempts to make sense of the crosstalk that is established between the bacterial populations of the gut and the intestinal mucosa of the host. IECs emerge as sentinel cells that are not only able to discriminate between 'friends' (the commensal microorganisms) and 'foes' (the pathogens) but are also able to translate this recognition process into signals to the mucosal innate immune system that tip the balance towards tolerance in the presence of commensal microorganisms and inflammation aimed at microbial destruction in the presence of pathogens. Despite the fact that both commensal microorganisms and pathogens express common motifs that are recognized by dedicated receptors, I have emphasized several factors that are important in the modulation of intestinal homeostasis. PAMPs are not equal in their signalling capacity, and their diversity needs to be further explored in the context of their potential to trigger innate immune responses. In addition, areas of the gut are not equal in their capacity to respond to microorganisms. For example, the intestinal crypt must remain relatively germ-free, because it is a 'sanctuary' where stem cells provide constant renewal of the epithelium. So, the crypts are more reactive to the presence of microorganisms and, accordingly, express PRRs at higher levels and in more strategic positions, such as at the apex of IECs. Therefore, it is not surprising that intestinal inflammation is often initiated in the crypts, as indicated by cryptitis being the principal initial sign of the development of IBDs. Finally, bacterial pathogens are different from commensal bacteria, as has now been confirmed by genomic analysis. They express virulence factors — such as adherence molecules, invasion systems, enzymes and toxins — that provide them with the capacity not only to establish close contact with the epithelial surface but also to colonize and /or invade and cause destructive damage to the host mucosa. It is now clear that dysfunction in this delicate network of extracellular and intracellular sensors, which can discriminate between commensal and pathogenic bacteria, leads to pathological consequences, such as IBDs. The association of mutations in the *NOD2* gene with Crohn's disease provides a good demonstration of this emerging concept[121].

The complexity and intricate nature of the signalling networks involved in the homeostatic and pathogenic mechanisms need to be modelled and analysed *in vitro*. Issues such as the sampling of PAMPs by epithelial cells and the parallel mechanisms of sensing and signalling can only be addressed by *in vitro* approaches. However, only *in vivo* approaches allow us to study the complexity of these processes. One big handicap is the lack of relevant mouse models that accurately reproduce the diseases caused by human-specific pathogens. Therefore, two main priorities emerge: the requirement for humanized mice that develop human-like diseases, and the sustained development of molecular, imaging and sampling tools for global analysis of *in vivo* processes.

1. Rescigno, M., Rotta, G., Valzasina, B. & Ricciardi-Castagnoli, P. Dendritic cells shuttle microbes across gut epithelial monolayers. *Immunobiology* **204**, 572–581 (2001).
2. Kagnoff, M. F. & Eckmann, L. Epithelial cells as sensors for microbial infection. *J. Clin. Invest.* **100**, 6–10 (1997).
3. Madara, J. L., Nash, S., Moore, R. & Atisook, K. Structure and function of the intestinal epithelial barrier in health and disease. *Monogr. Pathol.* **31**, 306–324 (1990).
4. Staehelin, L. A., Mukherjee, T. M. & Williams, A. W. Freeze-etch appearance of the tight junctions in the epithelium of large and small intestine of mice. *Protoplasma* **67**, 165–184 (1969).
5. Turner, J. R. in *Microbial Pathogenesis of the Intestinal Epithelial Cell* (ed. Hecht, G. A.) 1–22 (American Society for Microbiology Press, Washington DC, 2003).
6. Turner, J. R. *et al*. Physiological regulation of epithelial junctions is associated with myosin light-chain phosphorylation. *Am. J. Physiol.* **273**, C1378–C1385 (1997).
7. Furuse, M., Sasaki, H., Fujimoto, K. & Tsukita, S. A single gene product, claudin-1 or -2, reconstitutes tight junction strands and recruits occludin in fibroblasts. *J. Cell Biol.* **143**, 391–401 (1998).
8. Furuse, M., Fujimoto, K., Sato, N., Hirase, T. & Tsukita, S. Overexpression of occludin, a tight junction-associated integral membrane protein, induces the formation of intracellular multilamellar bodies bearing tight junction-like structures. *J. Cell Sci.* **109**, 429–435 (1996).
9. Specian, R. D. & Neutra, M. R. Mechanisms of rapid mucus secretion in goblet cells stimulated by acetylcholine. *J. Cell Biol.* **85**, 626–640 (1980).
10. Kindon, H., Pothoulakis, C., Thim, L., Lynch-Devaney, K. & Podolsky, D. K. Trefoil peptide protection of intestinal epithelial barrier function: cooperative interaction with mucin glycoprotein. *Gastroenterology* **109**, 516–523 (1995).
11. Wadolkowsky, E. A., Laux, D. C. & Cohen, P. S. Colonization of the streptomycin-treated mouse large intestine by a human fecal *Escherichia coli* strain: role of growth in mucus. *Infect. Immun.* **56**, 1030–1035 (1988).
12. Schneider, D. R. & Parker, C. D. Purification and characterization of the mucinase of *Vibrio cholerae*. *J. Infect. Dis.* **145**, 474–482 (1982).
13. Vallance, B. A., Chan, C. & Finlay, B. B. in *Microbial Pathogenesis and the Intestinal Epithelial Cell* (ed. Hecht, G. A.) 423–437 (American Society for Microbiology Press, Washington DC, 2003).
14. Hecht, G. A. in *Microbial Pathogenesis and the Intestinal Epithelial Cell* (ed. Hecht, G. A.) 285–300 (American Society for Microbiology Press, Washington DC, 2003).
15. White, S. H., Wimley, W. C. & Selsted, M. E. Structure, function and membrane integration of defensins. *Curr. Opin. Struct. Biol.* **5**, 521–527 (1995).
16. Lehrer, R. I. & Ganz, T. Defensins of vertebrate animals. *Curr. Opin. Immunol.* **14**, 96–102 (2002).
An excellent review of defensins.
17. Ouelette, A. J. & Bevins, C. L. Paneth cell defensins and innate immunity of the small bowel. *Inflamm. Bowel Dis.* **7**, 43–50 (2001).
18. O'Neil, D. A. *et al*. Expression and regulation of the human β-defensins hBD1 and hBD2 in intestinal epithelium. *J. Immunol.* **163**, 6718–6724 (1999).
19. Lehrer, R. I. & Ganz, T. Cathelicidins: a family of endogenous antimicrobial peptides. *Curr. Opin. Hematol.* **9**, 18–22 (2002).
20. McCormick, B. A., Parkos, C. A., Colgan, S. P., Carnes, D. K. & Madara, J. L. Apical secretion of a pathogen-elicited epithelial chemoattractant (PEEC) activity in response to surface colonization of intestinal epithelia by *Salmonella typhimurium*. *J. Immunol.* **160**, 455–456 (1998).
21. Tato, C. M. & Hunter, C. A. Host-pathogen interactions: subversion and utilization of the NF-κB pathway during infection. *Infect. Immun.* **70**, 3311–3317 (2002).
22. Neutra, M. R., Sansonetti, P. J. & Kraehenbuhl, J. P. in *Microbial Pathogenesis and the Intestinal Epithelial Cell* (ed. Hecht, G. A.) 23–42 (American Society for Microbiology Press, Washington DC, 2003).
23. Owen, R. Uptake and transport of intestinal macromolecules and microorganisms by M cells in Peyer's patches — a personal and historical perspective. *Semin. Immunol.* **11**, 1–7 (1999).
24. Fujimura, Y., Hosobe, M. & Kihara, T. Ultrastructural study of M cells from colonic lymphoid nodules obtained by colonoscopic biopsy. *Dig. Dis. Sci.* **37**, 1089–1098 (1992).
25. MacPherson, A. J. & Uhr, T. Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria. *Science* **303**, 1662–1665 (2004).
A research article about T-cell independent generation of IgA specificity.
26. Lode, M. J. *et al*. Bacterial flagellin is a dominant antigen in Crohn disease. *J. Clin. Invest.* **113**, 1296–1306 (2004).
27. Macpherson, A. J. & Harris, N. L. Interactions between commensal intestinal bacteria and the immune system. *Nature Rev. Immunol.* **4**, 478–485 (2004).
28. Rescigno, M. *et al*. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nature Immunol*. **2**, 361–367 (2001).
Unravels an original pathway of crossing the epithelium.
29. Pickard, K. M., Bremner, A. R., Gordon, J. N. & MacDonald, T. T. Immune responses. *Best Pract. Res. Clin. Gastroenterol.* **18**, 271–285 (2004).
30. Sansonetti, P. J. & Phalipon, A. M cells as ports of entry for enteroinvasive pathogens: mechanisms of interaction, consequences for the disease process. *Semin. Immunol.* **11**, 193–203 (1999).
31. Didierlaurent, A., Sirard, J.-C., Kraehenbuhl, J.-P. & Neutra, M. R. How the gut senses its content. *Cell. Microbiol*. **4**, 61–72 (2002).
32. Ming, S. & Goldman, H. *Pathology of the Gastrointestinal Tract* (W. B. Saunders, Philadelphia, 1992).
33. Medzhitov, R. & Janeway, C. A. Decoding the patterns of self and non-self by the innate immune system. *Science* **296**, 298–300 (2002).
An outstanding introduction to the concept of innate immunity.
34. Aderem, A. & Ulevitch, R. J. Toll-like receptors in the induction of the innate immune response. *Nature* **406**, 782–787 (2000).
35. Rahoff-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* **118**, 229–241 (2004).
36. Schwandner, R., Dziarski, R., Wesche, H., Rothe, M. & Kirschning, C. J. Peptidoglycan- and lipoteichoic acid-induced cell activation is mediated by Toll-like receptor 2. *J. Biol. Chem.* **274**, 17406–17409 (1999).
37. Poltorak, A. *et al*. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in *Tlr4* gene. *Science* **282**, 2085–2088 (2001).
38. Gewirtz, A. T., Navas, T. A., Lyons, S., Godowski, P. J. & Madara, J. L. Bacterial flagellin activates baso-laterally expressed TLR5 to induce epithelial proinflammatory gene expression. *J. Immunol.* **167**, 1882–1885 (2001).
39. Bauer, S. *et al*. Human TLR9 confers responsiveness to bacterial DNA via species-specific CpG motif recognition. *Proc. Natl Acad. Sci. USA* **98**, 9237–9242 (2001).
40. Girardin, S. *et al*. Nod1 detects a unique muropeptide from Gram-negative bacterial peptidoglycan. *Science* **300**, 1584–1587 (2003).
41. Chamaillard, M. *et al*. An essential role for Nod1 in host recognition of bacterial peptidoglycan containing diaminopimelic acid. *Nature Immunol.* **4**, 702–707 (2003).
References 40 and 41 identify NOD1 as an intracellular sensor for bacteria that allows discrimination between Gram-positive and Gram-negative bacteria on the basis of differences in their PGN.
42. Girardin, S. *et al*. Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. *J. Biol. Chem.* **278**, 8869–8872 (2003).
43. Inohara, N. *et al*. An induced proximity model for NF-κB activation of the Nod1/RICK and RIP signaling pathways. *J. Biol. Chem.* **275**, 27823–27831 (2000).
44. Girardin, S. E. *et al*. CARD4/Nod1 mediates NF-κB and JNK activation by invasive *Shigella flexneri*. *EMBO Rep.* **2**, 736–742 (2001).
45. Hornef, M. W., Frisan, T., Vandewalle, A., Normark, S. & Richter-Dahlfors, A. Toll-like receptor 4 resides in the Golgi apparatus and colocalizes with internalized lipopolysaccharide in intestinal epithelial cells. *J. Exp. Med.* **195**, 559–570 (2002).
46. Ortega-Cava, C. F. *et al*. Strategic compartmentalization of Toll-like receptor 4 in the mouse gut. *J. Immunol.* **170**, 3977–3985 (2003).
47. Hugot J. P. *et al.* Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* **411**, 599–603 (2001).
48. Ogura, Y. *et al*. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* **411**, 603–606 (2001).
49. Lala, S. *et al*. Crohn's disease and the *NOD2* gene: a role for Paneth cells. *Gastroenterology* **125**, 47–57 (2003).
50. Berg, R. D. The indigenous gastrointestinal microflora. *Trends Microbiol.* **4**, 430–435 (1996).
51. Hooper, L. V., Midtvedt, T. & Gordon, J. I. How host–microbial interactions shape the nutrient environment of the mammalian intestine. *Annu. Rev. Nutr.* **22**, 283–307 (2002).
52. Weintraub, A., Zahninger, U., Wollenweber, H. W., Seydel, U. & Rietschel, E. Structural characterization of the lipid A component of *Bacteroides fragilis* strain NCTC 9343 lipopolysaccharide. *Eur. J. Biochem.* **183**, 425–431 (1989).
53. Golenbock, D. T., Hampton, R. Y., Qureshi, N., Takayama, K. & Raetz, C. R. Lipid A-like molecules that antagonize the effects of endotoxins on human monocytes. *J. Biol. Chem.* **266**, 19490–19498 (1991).
54. Smith, P. D. *et al*. Intestinal macrophages lack CD14 and CD89 and consequently are down-regulated for LPS- and IgA-mediated activities. *J. Immunol.* **167**, 2651–2656 (2001).
55. Abreu, M. T. *et al*. 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.* **167**, 1609–1616 (2001).
56. Naik, S., Kelly, E. J., Meijer, L., Petterson, S. & Sanderson, I. R. Absence of Toll-like receptor-4 explains endotoxin hyporesponsiveness in human intestinal epithelium. *J. Pediatr. Gastroenterol. Nutr.* **32**, 449–453 (2001).
57. Hayashi, F. *et al*. The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. *Nature* **410**, 1099–1103 (2001).
58. Gewirtz, A. T. *et al*. *Salmonella typhimurium* translocates flagellin across intestinal epithelia, inducing a pro-inflammatory response. *J. Clin. Invest.* **107**, 99–109 (2001).
59. Sierro, F. *et al*. Flagellin stimulation of intestinal epithelial cells triggers CCL20-mediated migration of dendritic cells. *Proc. Natl Acad. Sci. USA* **98**, 13722–13727 (2001).
An important paper about transepithelial signalling by bacteria.
60. Lee, S. K. *et al*. *Helicobacter pylori* flagellins have very low intrinsic activity to stimulate human gastric epithelial cells via TLR5. *Microbes Infect.* **5**, 1345–1356 (2003).
61. Fernandez, M. I. *et al*. Anti-inflammatory role for intracellular dimeric immunoglobulin A by neutralization of lipopolysaccharide in epithelial cells. *Immunity* **18**, 739–749 (2003).
62. Odenbreit, S. *et al*. Translocation of *Helicobacter pylori* CagA into gastric epithelial cells by type IV secretion. *Science* **287**, 1497–1500 (2000).
63. Janssens, S., Burns, K., Tschopp, R. & Beyaert, R. Regulation of interleukin-1 and NF-κB activation by alternative splicing of MyD88. *Curr. Biol.* **12**, 467–471 (2002).
64. Kobayashi, K. *et al*. IRAK-M is a negative regulator of Toll-like receptor signalling. *Cell* **110**, 191–202 (2002).
65. Su, C. G. *et al*. A novel therapy for colitis utilizing PPAR-γ ligands to inhibit the epithelial inflammatory response. *J. Clin. Invest.* **104**, 383–389 (1999).
66. Desreumaux, P. *et al*. Attenuation of colon inflammation through activators of the retinoid X receptor (RXR)/peroxisome proliferator-activated receptor γ (PPARγ) heterodimer. A basis for new therapeutic strategies. *J. Exp. Med.* **193**, 827–838 (2001).
67. Kelly, D. *et al*. Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear–cytoplasmic shuttling of PPAR-γ and RelA. *Nature Immunol.* **5**, 104–112 (2004).
68. Neish, A. S. Prokaryotic regulation of epithelial responses by inhibition of IκB-α ubiquitination. *Science* **289**, 1560–1563 (2000).
Coins the idea that non-pathogenic bacteria could mediate anti-inflammatory signals.
69. Jijon, H. *et al*. DNA from probiotic bacteria modulates murine and human epithelial and immune functions. *Gastroenterology* **126**, 1358–1373 (2004).
70. Duchmann, R., Schmitt, E., Knolle, P., Meyer zum Büschenfelde, K. H. & Neurath, M. Tolerance towards resident intestinal flora in mice is abrogated in experimental colitis and restored by treatment with interleukin-10 or antibodies to interleukin-12. *Eur. J. Immunol.* **26**, 934–938 (1996).
71. Chen, Y. *et al*. Oral tolerance in myelin basic protein T cell receptor transgenic mice: suppression of auto-immune encephalomyelitis and dose-dependent induction of regulatory cells. *Proc. Natl Acad. Sci. USA* **93**, 388–391 (1996).
72. MacDonald, T. M. Breakdown of tolerance to the intestinal bacterial flora in inflammatory bowel disease (IBD). *Clin. Exp. Immunol.* **102**, 445–447 (1995).
73. Hacker, J. in *Molecular Infection Biology: Interactions Between Microorganisms and Cells* (eds Hacker, J. & Heesemann, J.) 225–270 (Wiley-Liss, Heidelberg, 2002).
74. Cossart, P. & Sansonetti P. J. Bacterial invasion: the paradigms of enteroinvasive pathogens. *Science* **304**, 242–248 (2004).
75. Sansonetti, P. J., Kopecko, D. J. & Formal, S. B. Involvement of a large plasmid in the invasive ability of *Shigella flexneri*. *Infect. Immun.* **35**, 852–860 (1982).
76. Isberg, R. R. Discrimination between intracellular uptake and surface adhesion of bacterial pathogens. *Science* **252**, 934–938 (1991).

77. Galan, J. E. *Salmonella* entry into mammalian cells: different yet converging signal transduction pathways? *Trends Cell Biol.* **4**, 196–199 (1994).
78. Hueck, C. Type III protein secretion systems in bacterial pathogens of animals and plants. *Microbiol. Mol. Biol. Rev.* **62**, 379–433 (1998).
79. Baumler, A. J., Tsolis, R. M. & Heffron, F. The *lpf* fimbrial operon mediates adhesion of *Salmonella typhimurium* to murine Peyer's patches. *Proc. Natl Acad. Sci. USA* **93**, 279–283 (1996).
80. Marra, A. & Isberg, R. R. Invasin-dependent and invasin-independent pathways for translocarion of *Yersinia pseudotuberculosis* across the Peyer's patch intestinal epithelium. *Infect. Immun.* **65**, 3412–3421 (1997).
81. Sansonetti, P. J., Arondel, J., Cantey, J. R., Prévost, M. C. & Huerre, M. Infection of rabbit Peyer's patches by *Shigella flexneri*: effect of adhesive or invasive bacterial phenotypes on follicle-associated epithelium. *Infect. Immun.* **64**, 2752–2764 (1996).
82. Darwin, K. H. & Miller, V. L. Molecular basis of the interaction of *Salmonella* with the intestinal mucosa. *Clin. Microbiol. Rev.* **12**, 405–428 (1999).
83. Kelsall, B. L. & Strober, W. Distinct populations of dendritic cells are present in the subepithelial dome and T cell regions of murine Peyer's patches. *J. Exp. Med.* **183**, 237–247 (1996).
84. Cornelis, G. R. The *Yersinia* Ysc–Yop 'type III' weaponry. *Nature Rev. Mol. Cell Biol.* **3**, 742–752 (2002).
85. Grassl, G. A., Bohn, E., Müller, Y., Bühler, O. T. & Autenrieth, I. B. Interaction of *Yersinia enterocolitica* with epithelial cells: invasin beyond invasion. *Int. J. Med. Microbiol.* **293**, 41–54 (2003).
86. Fernandez, M. I. & Sansonetti, P. J. in *Microbial Pathogenesis and the Intestinal Epithelial Cell* (ed. Hecht, G. A.) 455–479 (American Society for Microbiology Press, Washington DC, 2003).
87. Zychlinsky, A. *et al*. *In vivo* apoptosis in *Shigella flexneri* infections. *Infect. Immun.* **64**, 5357–5365 (1996).
88. Raqib, R. *et al*. Apoptosis in acute shigellosis is associated with increased production of Fas/Fas ligand, perforin, caspase-1, and caspase-3, but reduced production of Bcl-2 and interleukin-2. *Infect. Immun.* **70**, 3199–3207 (2002).
89. Zychlinsky, A., Prevost, M. C. & Sansonetti, P. J. *Shigella flexneri* induces apoptosis in infected macrophages. *Nature* **358**, 167–169 (1992).
90. Hilby, H. *et al*. *Shigella*-induced apoptosis is dependent on caspase-1 which binds to IpaB. *J. Biol. Chem.* **273**, 32895–32900 (1998).
91. Perdomo, J. J., Gounon, P. & Sansonetti, P. J. Polymorphonuclear leukocyte transmigration promotes invasion of colonic epithelial monolayer by *Shigella flexneri*. *J. Clin. Invest.* **93**, 633–643 (1994).
92. Perdomo, J. J. *et al*. Acute inflammation causes epithelial invasion and mucosal destruction in experimental shigellosis. *J. Exp. Med.* **180**, 1307–1319 (1994).
First evidence that inflammation disrupts the epithelial barrier and promotes bacterial invasion.
93. Sansonetti, P. J., Arondel, J., Cavaillon, J. M. & Huerre, M. Role of IL-1 in the pathogenesis of experimental shigellosis. *J. Clin. Invest.* **96**, 884–892 (1995).
94. Sansonetti, P. J. *et al*. Caspase-1 activation of IL-1β and IL-18 are essential for *Shigella flexneri* induced inflammation. *Immunity* **12**, 581–590 (2000).
95. Way, S. S., Borczuk, A. C., Dominitz, R. & Goldberg, M. An essential role for γ interferon in innate resistance to *Shigella flexneri* infection. *Infect. Immun.* **66**, 1342–1348 (1998).
96. Weinrauch, Y., Drujan, D., Shapiro, S. D., Weiss, J. & Zychlinsky, A. Neutrophil elastase targets virulence factors of enterobacteria. *Nature* **417**, 91–94 (2002).
Shows that neutrophil elastase is essential for the degradation of bacterial virulence factors.
97. Brinkmann, V. *et al*. Neutrophil extracellular traps kill bacteria. *Science* **303**, 1532–1535 (2004).
98. Sansonetti, P. J. Rupture, invasion and inflammatory destruction of the intestinal barrier by *Shigella*, making sense of prokaryote–eukaryote cross-talk. *FEMS Microbiol. Rev.* **25**, 3–14 (2001).
99. Ingersoll, M. A. *et al*. The ShiA protein encoded by the *Shigella flexneri* SHI-2 pathogenicity island attenuates inflammation. *Cell. Microbiol.* **5**, 797–807 (2003).
100. Islam, D. *et al*. Downregulation of bactericidal peptides in enteric infections: a novel immune escape mechanism with bacterial DNA as a potential regulator. *Nature Med.* **7**, 180–185 (2001).
101. Monack, D. M. *et al*. *Salmonella* exploits caspase-1 to colonize Peyer's patches in a murine typhoid model. *J. Exp. Med.* **192**, 249–258 (2000).
102. Holden, D. W. Trafficking of the *Salmonella* vacuole in macrophages. *Traffic* **3**, 161–169 (2002).
103. Hensel, M., Shea, J. E. & Holden, D. Genes encoding putative effector proteins of the type III secretion system of *Salmonella* pathogenicity island 2 are required for bacterial virulence and proliferation in macrophages. *Mol. Microbiol.* **30**, 163–174 (1998).
104. Meresse, S., Steele-Mortimer, O., Finlay, B. B. & Gorvel, J.-P. The rab7 GTPase controls the maturation of *Salmonella typhimurium*-containing vacuoles in HeLa cells. *EMBO J.* **18**, 4394–4403 (1999).
105. Steele-Mortimer, O., Méresse, S., Gorvel, J.-P., Toh, B. H. & Finlay, B. B. Biogenesis of *Salmonella typhimurium*-containing vacuoles in epithelial cells involves interactions with the early endocytic pathway. *Cell. Microbiol.* **1**, 33–49 (1999).
106. Salcedo, S. P. & Holden, D. SseG, a virulence protein that targets *Salmonella* to the Golgi network. *EMBO J.* **22**, 5003–5014 (2003).
107. Gaillard, J.-L., Berche, P., Frehel, C., Gouin, E. & Cossart, P. Entry of *Listeria monocytogenes* into cells is mediated by internalin, a repeat protein reminiscent of surface antigens from Gram-positive cocci. *Cell* **65**, 1127–1141 (1991).
108. Pron, B. *et al*. Comprehensive study of the intestinal stage of listeriosis in a rat ligated ileal-loop system. *Infect. Immun.* **66**, 747–755 (1998).
109. Lecuit, M. *et al*. A transgenic model for listeriosis: role of internalin in crossing the intestinal barrier. *Science* **292**, 1722–1725 (2001).
Example of a rational switch of species specificity that allows the modelling of a human disease in mice.
110. Tang, P., Rosenshine, I., Cossart, P. & Finlay, B. B. Listeriolysin O activates mitogen-activated protein kinase in eukaryotic cells. *Infect. Immun.* **6**, 2359–2361 (1996).
111. Izadpanah, A., Dwinnel, M. B., Eckmann, L., Varki, N. M. & Kagnoff, M. F. Regulated MIP-3α/CCL20 production by human intestinal epithelium: mechanisms for modulating mucosal immunity. *Am. J. Physiol.* **280**, G710–G719 (2001).
112. Mariathasan, S. *et al*. Differential activation of the inflammasome by caspase-1 adaptors ASC and Ipaf. *Nature* **430**, 213–218 (2004).
113. McCormick, B. A., Colgan, S. P., Archer, C. D., Miller, S. I. & Madara, J. L. *Salmonella typhimurium* attachment to human intestinal monolayers; transcellular signaling to subepithelial neutrophils. *J. Cell Biol.* **123**, 895–907 (1993).
114. Mrsny, R. J. *et al*. Identification of hepoxilin A3 in inflammatory events: a required role in neutrophil migration across intestinal epithelia. *Proc. Natl Acad. Sci. USA* **101**, 7421–7426 (2004).
Identifies the first transepithelial chemoattractant.
115. McCormick, B. A. in *Microbial Pathogenesis and the Intestinal Epithelial Cell* (ed. Hecht, G. A.) 439–453 (American Society for Microbiology Press, Washington DC, 2003).
116. Takeuchi, A., Spring, H., Labrec, E. H. & Formal, S. B. Experimental bacillary dysentery: an electron microscopic study of the response of intestinal mucosa to bacterial invasion. *Am. J. Pathol.* **4**, 1011–1044 (1965).
117. Singer, M. & Sansonetti, P. J. IL-8 is a key chemokine regulating neutrophil recruitment in a new mouse model of *Shigella*-induced colitis. *J. Immunol.* **173**, 4197–4206 (2004).
118. Sansonetti, P. J., Arondel, J., Huerre, M., Harada, A. & Katsushima, K. Interleukin-8 controls bacterial transepithelial translocation at the cost of epithelial destruction in experimental shigellosis. *Infect. Immun.* **67**, 1471–1480 (1999).
119. Vazquez-Torres, A. *et al*. Extraintestinal dissemination of *Salmonella* by CD18-expressing phagocytes. *Nature* **401**, 804–808 (1999).
120. Sundquist, M., Rydstrom, A. & Wick, M. J. Immunity to *Salmonella* from a dendritic point of view. *Cell. Microbiol.* **6**, 1–11 (2004).
121. Girardin, S. E., Hugot, J.-P. & Sansonetti, P. J. Lessons from Nod2 studies: towards a link between Crohn's disease and bacterial sensing. *Trends Immunol.* **24**, 652–658 (2003).

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The author declares no competing financial interests.

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