

Review

Systemic features of immune recognition in the gut

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Abstract

The immune system, to protect the body, must discriminate between the pathogenic and non-pathogenic microbes and respond to them in different ways. How the mucosal immune system manages to make this distinction is poorly understood. We suggest here that the distinction between pathogenic and non-pathogenic microbes is made by an integrated system rather than by single types of cells or single types of receptors; a systems biology approach is needed to understand immune recognition.

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1. Introduction

The healthy gut houses about 100 trillion (10^{14}) microorganisms. Commensal gut microbes help digest complex polysaccharides, stimulate the development of the mucosal immune system and protect the body from pathogens. However, when confronted with disease-causing microbes, the gut immune system can distinguish them from the commensals and mount a response that protects the host. Defects in the capacity of the mucosal immune system to discriminate between pathogenic and commensal microorganisms can cause pathology: On the one hand, non-inflammatory responses to pathogenic microbes can foster infection; on the other hand, inflammatory responses to non-pathogenic agents can damage the gut and lead to inflammatory bowel disease (IBD). The question of how the immune system manages to recognize pathogenic microbes is one of the most important issues of mucosal immunology.

According to the classical view, single types of cells and molecules can recognize pathogens and trigger inflammatory responses [1]. This classical view is changing now [2]. There is accumulating evidence suggesting that discrimination between pathogenic and non-pathogenic microbes is the outcome of a complex exchange of information between various types of immune-system cells and non-immune cells.

2. Single types of cells and molecules cannot distinguish between pathogenic and non-pathogenic microbes

Two classes of innate receptors crucially involved in intestinal immune responses are Toll-like receptors (TLRs) and Nucleotide-binding oligomerization domain-like receptors (NOD-like receptors) [3]. Pattern recognition receptors, sometimes referred to as pathogen recognition receptors (PRRs), are often considered as sensors of pathogens [4].

Initially, it was suggested that PRRs can recognize pathogens by binding structures unique to them: “The receptors of the innate immune system,... are specific for structures found exclusively in microbial pathogens (pathogen-associated molecular patterns), which is why they function to signal the presence of infection” [5]. However, later studies revealed that

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PRRs can become activated by motifs common to pathogenic and non-pathogenic microbes. There are at least 11 kinds of TLRs and all of them can become activated by motifs common to pathogenic and non-pathogenic microbes. For example, the TLR2 ligand, lipoprotein, TLR4 ligand, lipopolysaccharide (LPS), TLR5 ligand, flagellin are present on bacteria independently of their power to cause damage to the host. Another important class of innate immune receptors active in the intestine are NOD-like receptors (NLR). But they too appear to lack the power to discriminate between pathogenic and commensal microbes [6].

It quickly became clear that PRRs recognize microbe-associated molecular patterns (MAMPs) rather than “pathogen-associated molecular patterns” (PAMPs); nevertheless, it has been maintained that PRRs can distinguish self from microbial non-self: “Recognition of PAMPs by the innate immune system allows the distinction between ‘self’ and ‘microbial non-self’” [7]. It has been suggested that this self/non-self discrimination could be sufficient for pathogen detection if, for example, TLRs were compartmentalized in the tissue so that only invasive agents had access to these receptors [7]. However, further experiments revealed that PRRs are not so sequestered and can recognize and become activated by self as much as by non-self motifs [8]. TLR4 alone can bind microbial structures like LPS, and fimbriae but also self-derived molecules like hyaluronan, β -defensin and heat-shock protein 60 (HSP60) [9]. Similarly, TLR9, once considered a sensor of unmethylated repeats of the dinucleotide CpG, has turned out to recognize DNA in a sequence-independent manner [10]. All in all, it has been shown that PRRs can distinguish neither between pathogenic and non-pathogenic nor between self and non-self.

In the face of data showing that PRRs recognize microbial as well as host motifs it has been proposed that the primary role of PRRs is to signal danger. Indeed, many endogenous ligands bound by PRRs are molecules released during stress or tissue damage. Thus, it was suggested that the primary role of PRRs is to recognize endogenous and exogenous (microbe-derived) damage-associated molecular patterns (DAMPs) [9,11]. Apart from cases of sterile inflammation [12], damage signals recognized by PRRs seem to indicate infection events and thus also, indirectly, the presence of a pathogen. However, recent studies show that PRRs can promote down-regulatory type responses as well as proinflammatory responses. For example, it has been shown that TLR2 is required for the induction of IL-10-producing T-cells by capsular polysaccharide A from one commensal species [13]. TLR9 is known to protect mice from experimental colitis by inducing IFN α [14]. A TLR4 agonist, LPS has been shown to enhance survival and proliferation of CD4⁺CD25⁺ T-cells [15]. Finally, PRRs are known to be expressed on the apical surface of IECs where they are constantly stimulated by segmented filamentous bacteria (SFB) strongly attached to the epithelium. This stimulation does not signal danger nor does it initiate inflammatory responses; “A problem with TLR agonists that has not been fully appreciated is that they can generate suppressive as well as proinflammatory responses in innate immune cells and can promote the induction of regulatory as

well as effector T-cells” [16]. These observations do not undermine the concept that PRRs do participate in danger signaling, rather they suggest that these receptors have no power of their own to signal danger and their activation is not sufficient for pathogen detection or self/non-self discrimination.

In the face of empirical data demonstrating that single types of immune receptors cannot distinguish between pathogenic and commensal microbes, some researchers have turned to the idea that immune recognition is performed by single type of cells. For example, Aderem formulated a “barcode model of immune recognition”. He argues that different types of TLRs expressed by an immune cell jointly read a “barcode” on a pathogen and induce immune responses accordingly: “For example, if microbe 1 activates TLR4 and TLR5, it is likely to be a flagellated gram-negative organism, whereas TLR2 and TLR6 together with TLR5 will detect a flagellated gram-positive bacterium” [17]. In addition, to the “barcode model” there are several other attempts to explain immune recognition in terms of collective activation of many receptors on a single cell (or a single type of cell) [18].

IECs, for example, have been proposed to be able to recognize pathogens, acting as sentinels for certain types of microbes [19]. The crucial feature enabling pathogen detection by IECs seems to be cell polarization [20]: The apical and basolateral surfaces of IECs feature different types of TLR molecules [21]. For example, it has been suggested that IECs do not express TLR5 on the apical surface thus making it impossible for commensal agents resident in the lumen, to activate this receptor [22]. However, further experiments demonstrated that TLR5 as well as many other PRRs are expressed on the apical surface and luminal agents can engage these receptors without triggering inflammatory responses [23–25].

Dendritic cells (DCs) are another class of cells that was believed to be decisive regarding the phenotype of immune responses. It has often been asked explicitly how DCs can distinguish between pathogenic and non-pathogenic agents; “An unresolved question fundamental to the field of mucosal immunity is how mucosal DCs distinguish commensal flora from pathogenic bacteria and only mount protective immunity against the latter” [26]. It is becoming clear now that DCs do not have power to distinguish between pathogenic and non-pathogenic agents. The course of immune responses induced by DC depends on conditioning by IEC-derived factors [27–29]. Depending on conditioning by cytokines, intestinal DCs can promote the differentiation of T-cells into regulatory T-cells or effector T-cells.

In addition, to DCs and IECs, macrophages and neutrophils have been proposed to be able to distinguish between pathogenic and non-pathogenic microbes. However, there is evidence suggesting that the type of response induced by a macrophage or a neutrophil depends on detection of common microbial constituents and environmental conditions more than the actual status of the recognized microorganism as pathogenic or safe. Collectively, these data suggest that single types of immune cells cannot recognize microbes as pathogenic or safe.

3. Single types of cells and molecules, in principle, cannot recognize pathogens because pathogens lack structures unique to them

Immune cells sense their environment by means of their receptors. These receptors recognize molecular motifs in the microenvironment. Taking this into account, we can say that an immune cell would be able to discriminate between pathogenic and non-pathogenic microbes only if pathogenic and non-pathogenic (commensal) microbes would have molecular motifs unique to each type.

Do microbes really express such discriminatory molecular motifs? The question, however, is hypothetical because the same microorganism can be pathogenic in one circumstance and commensal or beneficial in another [30]. For example, *Helicobacter pylori* colonizes the gastric mucosa of 80–90% of people in less developed countries [31], but it causes symptomatic disease in only 15–20% of infected individuals [32]. The same strains of uropathogenic *Escherichia coli* clones can be commensal in one condition and pathogenic in another [33]. One could provide a very large number of examples of microbes whose pathogenic potential is context-dependent. Obviously, the pathogenic potential of a microbe varies with the colonization site and depends on the state of the host immune system. “The question ‘what is a pathogen’ cannot be separated from the question ‘what is a host’” [34].

As we have mentioned, many PRR ligands are expressed by both pathogenic and non-pathogenic agents: LPS, flagellin, CpG, peptidoglycan (PGN), all can be found on disease-causing agents as well as on commensal microbes. However there is a class of features that might be exclusive domains of pathogenic agents. These are the products of the so-called “pathogenicity genes”; “Genetic analyses have shown that bacterial pathogens differ from their non-pathogenic relatives or commensal bacteria by the presence of specific pathogenicity genes” [35]. Toxins, polysaccharide capsules, secretion systems and IgA proteases seem to be exclusive to pathogenic agents. The fact that immunosuppression of the host can lead to a change in host–parasite relationship from symbiosis to pathogenesis challenges the pathogenicity gene concept [36]. Surprisingly, the opposite can also happen; an initially pathogenic microorganism can become symbiotic if the immune system succeeds in controlling microbial expansion and tissue damage [37]. If the status of a microbe depends on the immune competence of the host, expression of a virulence gene by the microbe cannot serve as a reliable marker of its pathogenicity. If the potential to produce damage to the host is host-dependent, the host may have to “self-reflect” in order to learn whether a microbe is pathogenic [38].

Indeed, no clear-cut distinction can be made between virulence factors and symbiosis factors. A standard example of a virulence gene that belongs to a pathogenicity island is a gene coding for the type III secretion system (T3SS). T3SS is a microhomologue of a syringe that is used by many gram-negative bacteria such as *Salmonella* to transfer into the cytosol of the host proteases that can interfere with the physiology of the host cell [39]. One might think that T3SS would serve as a dependable context-independent marker of

pathogenicity. However, it has been demonstrated that, depending on the context, T3SS can be used by the same bacterial species as a virulence factor or as a symbiosis factor [40,41]. Moreover, type III and type IV secretion systems have been shown to be expressed by many commensal bacteria [42,43]. Tracheal cytotoxin (TCT), a fragment of a bacterial PGN, is known to be a powerful tissue-damaging factor [44]. However, it has also been shown to be an important symbiotic factor enhancing tissue development [45]. Apart from genes that allow subjugation of host cell machinery, other genes enable pathogenic agents to adhere to the epithelial cell wall. Enterohemorrhagic *E. coli* has been found to express an adherence factor called the *E. coli* common pilus (ECP). Pili are traditionally considered as virulence factors [46]; however, it has also been demonstrated that ECP is critical for colonization by both pathogenic and commensal *E. coli* [47].

There are several reasons why the products of the so-called “pathogenicity” genes cannot be reliable markers of pathogenicity. The most important reason is that these genes are usually encoded by “pathogenicity islands” that are prone to mutations and can be transferred from one organism to another. Since these genes are not conserved, their products cannot serve as universal indicators of pathogenicity [7]. The second reason is that there are non-pathogenic microbes that express these genes and there are microbes that are pathogenic despite lacking them. However, the most unexpected support for the thesis that the property of being pathogenic is not just a matter of having certain unique structural or biochemical features comes from studies showing that, in certain conditions, persistent bacterial infections have long-lasting beneficial consequences for the host [31]. For example, it has been pointed out that the coordinated balance between *H. pylori* infection and the host immune response has beneficial effects for the host [48]. Thus, from a wider perspective, a putative pathogen can establish its role as a symbiont.

As one can see, cytotoxins, secretion systems, fimbriae and others can be virulence factors in some conditions, colonization factors in other conditions and symbiosis factors in yet other conditions. Pathogenic and commensal microorganisms appear to employ similar or even identical molecular mechanisms to express their pathogenic or symbiotic potential [37]. In particular, both pathogenic and symbiotic bacteria must actively “manipulate” the host immune system to make it possible for them to colonize the body. In short, no microorganism is *just* pathogenic or *just* commensal. Being pathogenic or commensal is not a pre-established, context-independent and host-independent property. Any microorganism is pathogenic or commensal in a *given* context, under *given* conditions. It is the interplay between the context and the intrinsic features of a microbe that make it pathogenic or safe. Thus, in addition to the genetic predilections of a microorganism, environmental factors play an important role in defining a microorganism as pathogenic or not [49]. Since the identity of a microbe as pathogenic or safe does not depend on its structural features exclusively, a single type of receptor or a single type of cell is not in the position to recognize the microbe as pathogenic or safe in principle.

4. Return to basics: what makes a pathogen a pathogen?

Structure is an important factor influencing the pathogenic or commensal function of a given microbe. However, as we have seen, structurally identical agents can be pathogenic or commensal depending on the context. This implies that structure is not the only factor. In the gastrointestinal tract, another important pathogenicity-making factor is the composition of the commensal flora.

There are at least four ways in which commensal-bacterial communities can influence the status of a microorganism as pathogenic or commensal. First, luminal bacteria can provide a degree of protection by occupying environmental niches needed by pathogens or by producing antimicrobial peptides [50]. The damaging potential of a microorganism can thus be modulated by competition with other microbes. Secondly, bacteria are equipped with quorum-sensing mechanisms whose activation can promote expression of genes that mediate attachment, invasion, dissemination and survival in the host. Induction or inhibition of these genes can affect the capacity of a microorganism to invade the body [51,52]. Thirdly, commensal-derived metabolites such as butyrate have been shown to inhibit the production of proinflammatory cytokines and promote secretion of immunoregulatory mediators such as IL-10 [53]. This IL-10 production can in turn indirectly affect the pathogenic properties of microbes. Finally, alterations in the composition of commensal communities can affect the balance between immunity and tolerance and thus facilitate or impede the power of bacteria to establish a site of infection. For example, one commensal species, *Faecalibacterium prausnitzii*, has been shown to induce the production of IL-10 and downregulate secretion of TNF- α and IL-12 by an epithelial cell line [54]. This suggests that *F. prausnitzii* has the capacity to attenuate immune responsiveness to other bacterial species in the intestine. This down-regulation of responsiveness can assure integrity of the mucosal tissues and enhance the non-pathogenic properties of some agents.

Studies by Ivanov and colleagues provide another example of how alterations in the composition of gut microflora can influence pathogenic or commensal properties of intestinal microbes. The specific composition of commensal communities has been shown to regulate the balance between interleukin-17 producing CD4⁺ T-cells (Th17 cells) and Foxp3⁺ regulatory T-cells (Tregs) [55]. Disregulation of the balance between these two classes of cells can influence the pathogenic potential of many kinds of intestinal microorganisms [56]. Everything being taken into account, it is becoming clear that the microbiota of the human gut produce complex and powerful mechanisms for shaping the pathogenic or symbiotic potential of any intestinal microbe [57,58]. Therefore, regulated control of the composition of the microflora has emerged as a promising therapeutic opportunity for the treatment of acute and chronic intestinal diseases [59].

In addition to the biochemical and structural composition of the gut and the microbial environment, the very act of immune recognition is another important determinant of the

pathogenicity of a given intestinal agent. Sometimes, the recognition of a microbe as pathogenic makes it pathogenic and the recognition of the microbe as safe makes it safe. For example, *Shigella* is an opportunistic bacterium responsible for dysentery that invades the colonic and rectal mucosa. As a gram-negative bacterium, *Shigella* expresses LPS. Fernandez et al. reported that dimeric IgA produced by B-cells in subepithelial tissues colocalizes to LPS in the apical recycling endosome compartment of the IEC, thereby preventing LPS-induced NF κ B translocation and a subsequent proinflammatory response. This colocalization makes it possible for the immune system to shut itself down and so avoid recognizing *Shigella* as pathogenic. This natural down-regulation of the response assures that the microbe remains non-pathogenic. On the other hand, in conditions of IgA deficiency, bacterial-derived LPS can successfully induce secretion of TNF- α by IECs and promote proinflammatory responses. The inflammation, in turn, can lead to mucosal damage, enabling *Shigella* to colonize the subepithelial tissues and so become pathogenic. As one can see, in the case of IgA deficiency, it is the recognition of the bacterium as pathogenic that makes it pathogenic [60]. However, even this pathogenicity-making factor does not determine the disease-causing potential of a microbe in isolation. In fact, patients with selective IgA deficiency often do not have a clinical phenotype. On the other hand, experimental studies demonstrate that IgA specific to LPS protects from *Shigella* infection [61]. The protective role of LPS-specific IgA is also taken for granted in human volunteer studies of Shigellosis [62]. All of this suggests that a change in one pathogenicity-making factor can often reverse the overall potential of an intestinal agent to damage the host even if this factor is not the only one that promotes the invasive potential of the microbe.

Apart from cases where recognition of a microorganism as pathogenic or safe makes it pathogenic or safe respectively, there are also opposite examples. Recognition of a microbe as safe can sometimes grant it the power to invade the body and become pathogenic. Many microorganisms have developed strategies to hijack host cellular mechanisms to assure that non-inflammatory responses will be induced against them. The most straightforward example is that of *Salmonella typhimurium*. *Salmonella* expresses a type III secretion system that allows it to transfer effector proteins into host cells. One of these effector proteins is a deubiquitinase that prevents polyubiquitination of I κ B. This mechanism inhibits NF κ B-mediated TNF- α production [63,64].

As one can see, the property of being pathogenic is not a pre-established feature of a microorganism. The property of being pathogenic or commensal is complex, context-dependent and host-dependent. There are many environmental and structural factors influencing the pathogenic behavior of a microorganism, and we have mentioned only a few of them. Fig. 1A enumerates some additional pathogenicity-making factors. It is very important to note that, regardless of their mode of action and number, pathogenicity-making factors do not act *in isolation*; there is always a *causal interplay* between them. In other words, the potential to produce damage by

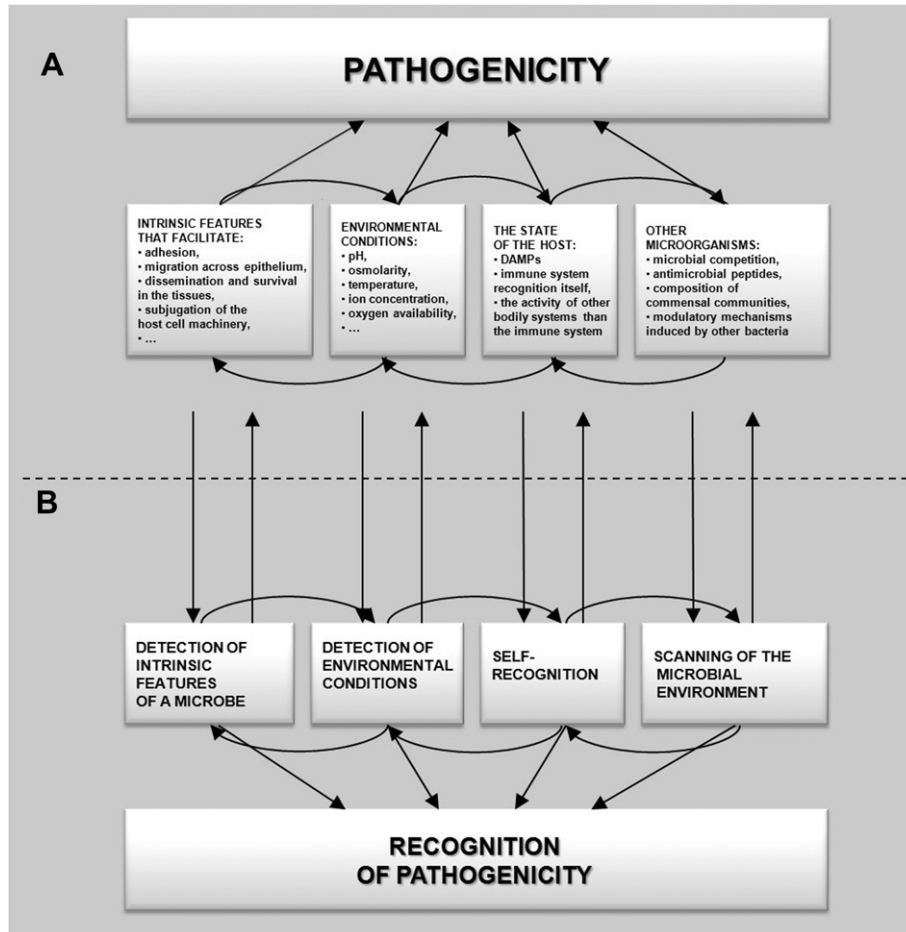


Fig. 1. Immune recognition of intestinal microbes as a complex decision-making process at the systems level. (A) The potential to produce damage to the host (pathogenicity) is constituted by various intrinsic and extrinsic features of a given microbe. It is crucial to note that the property of being pathogenic is constituted by these features *jointly*. In other words, the status of a microorganism as pathogenic (or safe) results from an interplay between pathogenicity-making factors and not from their collective impact. Perhaps the simplest example of the interplay is the interaction between environmental conditions and the expression of colonization factors. Some “virulence” factors are believed to be expressed only in certain pH, temperature, ion concentration or oxygen concentration. For example, the expression of type III secretion system by *Shigella flexneri* is controlled by local concentration of O_2 [80]. On the other hand, the expression of the colonization factors by a microorganism can change environmental conditions directly or indirectly. For example, *Helicobacter pylori* secrete urease that increases pH in its microenvironment [81]. (B) There are many factors that jointly determine the status of a microorganism as pathogenic or commensal. The immune system has evolved detection modules specializing in recognition of these factors. Intrinsic features, environmental conditions, state of the host and other microorganisms influence pathogenic or commensal properties of a given microbe. Therefore there are detection modules specializing in recognition of the intrinsic features, environmental conditions, state of the host and the composition of commensal communities respectively. All these detection modules have to be integrated to give rise to immune recognition of a certain kind. It is interesting to note that one of the detection modules is self-recognition. If the property of being pathogenic is host-dependent, the immune system has to be able to recognize itself in order to produce suitably tailored immune responses [38,76].

a given microbe is realized by *causal interactions* between pathogenicity-making factors, and not by the *collective sum of individual factors*. The same pathogenicity-making factors interlinked by different kinds of causal connections can determine the host-damaging potential of a microorganism in various ways.

It is impossible to make a list of *all* the causal connections between pathogenicity-making factors and explain how their mutual interactions might jointly affect the status of a microorganism as pathogenic or safe. However, a simple example can help us understand this causal interplay. For example, the composition of commensal communities and their immune recognition have been cited here as examples of pathogenicity-making factors. There is a strong interaction between them, so they can be said to determine pathogenic or commensal

properties of a given microorganism *jointly*. For example, it has been proposed recently that *Lactobacillus paracasei* can downregulate the production of proinflammatory cytokines by DCs. By inhibiting these cytokines, *L. paracasei* can affect the recognition of other bacteria by the immune system. Indeed, it has been demonstrated that this probiotic microbe can inhibit differentiation of T helper 1 cells (Th1) in response to *S. typhimurium*. Thus, a modified immune recognition of *Salmonella* can restrain the damaging potential of this bacterium [65].

There are also studies showing that immune recognition of intestinal microbes can be modulated by a combination of specific microbial species. For example, specific microbial compositions can induce regulatory responses to *S. typhimurium* by inhibiting proinflammatory NF κ B activation following

infection. This inhibition increases the frequency of CD4⁺CD25⁺FoxP3⁺ regulatory T-cells (Tregs) in the intestinal lamina propria. Thus, induced regulatory type responses prevent *S. typhimurium*-derived inflammatory tissue damage, thereby limiting the potential of these bacteria to invade the tissues [66]. In other words, the specific composition of commensal communities has been shown to make *S. typhimurium* safe and this limits the pathogenic potential of the bacterium. As one can see, immune recognition and commensal composition jointly influence the status of a microorganism as pathogenic or safe. However, one should be aware that the ultimate status of a microbe does not depend on the joint action of just *two* factors but on a complex causal interplay between a *number* of factors. Some of these causal interactions are shown in Fig. 1A.

5. How does pathogen/non-pathogen discrimination take place?

If a single type of receptor or a single type of cell is not able to tell whether a given microorganism is pathogenic or safe, then how is recognition of the difference performed by the immune system? If the status of a microorganism depends on a number of proximal and distant environmental factors, then reliable recognition of the microorganism as pathogenic or safe requires recognition of all of these factors. Therefore, we propose here that immune recognition of a microorganism as pathogenic or safe requires the engagement of multiple *detection modules* specialized in the detection of individual pathogenicity-making factors.

One such detection module is a mechanism dedicated to the identification of the exact anatomical localization of a given microbe. Accurate evaluation of the potential danger inherent in an intestinal bacterium requires detecting where the bacterium is located in the host. For example, in the context of the gut associated lymphoid tissue (GALT), if a bacterium is located in the lamina propria, the bacterium is invasive. If, in contrast, the bacterium is retained in the intestinal lumen by a mucus layer, IgAs and tight junctions between IECs, it is likely to be harmless.

There are a number of adaptations in the intestinal immune system that help to determine the exact localization of a microorganism, one of which is polarization of IECs that has been mentioned above. The kind of signaling pathway initiated by a given type of immune receptor may depend on its apical, cytosolic or basolateral localization. However, information about the apical or basolateral localization of a microorganism alone is not sufficient to determine its status as pathogenic or safe. For example, *H. pylori* do not normally penetrate the epithelium, but rather express their pathogenic potential by altering epithelial cell functions [32]. Moreover, commensal non-pathogenic bacteria may find themselves in the submucosal tissues by chance. Intestinal macrophages, in contrast to other kinds of macrophages, have been shown to be resistant to the induction of inflammatory responses despite retaining their phagocytic and bactericidal functions [67]. This resistance helps prevent induction of active responses against

non-pathogenic bacteria that may enter the subepithelial tissues because of tissue damage. If the anatomical localization of a microorganism would be a reliable marker of its status as a pathogen or commensal, it would be advantageous for the host to express PRRs exclusively on the basolateral surface of the epithelium. This possibility was investigated extensively but turned out to be wrong [68].

Another important piece of information about the status of a microorganism as pathogenic or safe comes from a detection module specializing in the recognition of microbial structural features. These features are sensed directly by antigen-sampling DCs and IECs. Recognition of such structural features is translated into signals that can influence the type of induced response.

Detecting structural motifs and topology provides very important information to help the immune system identify a microorganism as pathogenic or safe. However, as discussed above, the potential to produce damage to the host by a given agent does not only depend on its localization and structure, but also on the composition of the intestinal flora (cf. Fig. 1A). Consequently, there is a specialized detection module whose role is to recognize the composition of commensal communities; this module involves receptors on the apical surface of the intestinal epithelium. Activation of these receptors is translated into signals that can modulate immune responses accordingly. Different bacterial products can activate different signaling cascades that are translated into secreted cytokines, chemokines and other mediators. Apart from being able to activate various intracellular signaling pathways, commensal bacteria are able to suppress signaling cascades at different levels of their activation. For example, it has been shown that *Yersinia* spp. blocks the NFκB pathway at the level of IκB phosphorylation, whereas *Bacteroides* spp. can inhibit transcriptional activity of NFκB by activating peroxisome-proliferation-activated receptor-γ pathways [69,70]. Inhibition of specific signaling pathways at different levels of activation results in transcriptional responses correlated with the presence of specific bacterial products in the intestine. IECs do not have to detect the structure of each individual microbe to acquire information about the composition of the luminal flora. Apart from SFBs that directly interact with IECs, there are also microbes separated from the intestinal epithelium by a mucus layer. The latter, however, have only mild effects on immune activity [71].

As one can see, no single detection module alone has the capacity to distinguish between pathogenic and non-pathogenic microbes. Instead, each module provides important information about the damaging potential of a given microorganism. All these pieces of information have to be integrated to empower the immune system to decide about the type of response is appropriate for dealing with the microbe. The immune system cannot achieve this object by behaving as a passive collector of cues about various aspects of a microorganism; the system has to *integrate* all the collected information. Just as pathogenicity is the outcome of a multiplicity of pathogen and host factors, the recognition of pathogenicity requires the integrated recognition of a multiplicity of factors

(Fig. 1A). In other words, the immune system must be able to recognize the *interactions of pathogenicity-making factors, and not only their mere presence or absence.*

This “holistic” recognition requires an exchange of information between detection modules for individual pathogenicity-making factors. How is this exchange of information possible? It is difficult to answer this question because of the complexity of the integration processes and the scarcity of knowledge about them. However, one can get some idea of how bits of information about pathogenicity-making factors are integrated by looking at the relationship between *two* well-characterized detection modules for microbial structure and for the composition of the environment. These two cues have to be integrated. DCs detect information about the structure of the microbe, and IECs detect information about the composition of the luminal flora. The molecular basis of detection is slightly different in each case because IECs are polarized and DCs are not. This polarization of IECs tells whether a microbe is located in the lumen or in the lamina propria. Integration of the information results from a constant dialogue between these two classes of cells. IEC-derived products have been shown to condition DCs to promote certain types of immune responses [49]. Thus, immune responses induced by activated DCs depend on the sampled antigens as well as on IEC conditioning mediators [27–29]. For example, it has been demonstrated that luminal bacterial products can activate IECs to produce thymic stromal lymphopoietin (TSLP). TSLP, in turn, has the capacity to induce the production of IL-10 and downregulate the production of IL-12 by DCs [72]. Activation of naïve T-cells in the presence of IL-10 promotes their conversion into IL-10-producing T-cells. IL-10-producing T-cells, in turn, have been shown to be able to cure and protect from colitis in a T-cell transfer model. In addition to TSLP, there are many other mediators produced by IECs in response to different kinds of luminal content, including TGF- β , prostaglandin E2 (PGE2), B-cell activating factor (BAFF) and a proliferation-inducing ligand (APRIL) [73]. Another example of IEC-mediated conditioning of DCs involves prostaglandin E2 (PGE2). PGE2 can induce expression of indoleamine 2,3-dioxygenase (IDO) in DCs [74], an enzyme with potent immunosuppressive effects [75]. Production of these factors (TSLP, PGE2 and many others) by IECs depends on gene expression. Gene expression in IECs, in turn, is influenced by the composition of commensal-bacterial communities. As one can see, informational crosstalk between a module dedicated to recognition of commensal-bacterial composition and a module specializing in detection of microbial structure expresses itself in the conditioning of dendritic cells.

Fig. 1B lists observations indicating that recognition of a microorganism as pathogenic or safe is mediated by detection of factors that influence the status of a given microorganism as pathogenic or safe. It also illustrates the idea that fragments of information about various properties of a microorganism must be *put together* to produce a unified and properly adjusted immune response. This unified representation of a microorganism in the immune system is what we call

immune recognition. The mere binding of a ligand to a receptor may activate the receptor, but it does not constitute functional recognition, which, as we have discussed here, depends on the integration of information collected from various sources. In order to reflect the dynamic interaction between pathogenicity-making factors, dynamic crosstalk between individual detection mechanisms has to take place. We refer to this crosstalk as “co-response” [76]. Co-response is a complex exchange of signals between cells and molecules leading to a decision at the level of the interacting population. In our case, it is an inter-cellular and inter-molecular “dialogue” whose outcome is recognition of a microorganism as pathogenic or commensal [77]. Since there is no information center in a strict topological sense (no brain-like controller), “co-response” itself should be regarded as a central processing unit integrating pieces of information about pathogenicity-making factors. Representation of a microorganism as pathogenic or safe is not encoded in a single type of receptor activation or cell activation, but is a distributed image unfolding over time and involving the dynamic actions of a great number of signals [76].

6. Concluding remarks

The theory that single types of cells and molecules act as sentinels, when originally formulated, was a good representation of the available data. However, more recent experimental findings challenge this paradigm; discrimination between pathogens and non-pathogens is the outcome of a complex exchange of signals between cells and molecules, both of the host and the bacteria. Why has this paradigm been maintained?

One possible reason is historical. The idea of PAMPs has been developed in the context of the paradigm according to which innate immune receptors can distinguish between self and non-self. It was taken for granted that microbes must be recognized as foreign regardless of their status. Another reason is methodological. Contemporary immunology tends to favor molecular and cellular methods of analysis. Such reduction allows one to study single types of cells and their local interactions, and permits tracing a linear sequence of consecutive events from cause to effect. However, immune recognition is barely visible at this reduced level of analysis. Discrimination between pathogenic and non-pathogenic microbes does not involve single types of cells but many types of cells constituting a complex causal network (Fig. 1B). Most importantly, immune recognition requires *integrated exchange* of signals and it is the *exchange* that represents the greatest obstacle to the classical paradigm of the discriminating sentinel.

Biologic processes that go beyond direct signaling within or between cells can be termed systemic processes. Here, we show that immune recognition is not only a cellular and molecular process but is also a systemic process performed by the system as a system. A bird’s eye view is needed to grasp systemic processes. One has to zoom out to larger scales in order to see and understand how the system makes its

decisions about how to respond to a microorganism [78]. Systems biology, the study of a system as a whole, is a new field that promises to explain higher level biological processes. We propose that immune recognition is a property of the system domain [79].

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References

- [1] T.H. Mogensen, Pathogen recognition and inflammatory signaling in innate immune defenses, *Clin. Microbiol. Rev.* 22 (2009) 240–273.
- [2] G. Eberl, A new vision of immunity: homeostasis of the superorganism, *Mucosal Immunol.* 3 (2010) 450–460.
- [3] J.H. Fritz, S.E. Girardin, How Toll-like receptors and Nod-like receptors contribute to innate immunity in mammals, *J. Endotoxin Res.* 11 (2005) 390–394.
- [4] F. Martinon, J. Tschopp, NLRs join TLRs as innate sensors of pathogens, *Trends Immunol.* 26 (2005) 447–454.
- [5] R. Medzhitov, C. Janeway, Innate immunity, *N. Engl. J. Med.* 343 (2000) 338–344.
- [6] T.A. Kufer, D.J. Banks, D.J. Philpott, Innate immune sensing of microbes by Nod proteins, *Ann. N. Y. Acad. Sci.* 1072 (2006) 19–27.
- [7] R. Medzhitov, Toll-like receptors and innate immunity, *Nat. Rev. Immunol.* 1 (2001) 135–145.
- [8] M.T. Abreu, Toll-like receptor signalling in the intestinal epithelium: how bacterial recognition shapes intestinal function, *Nat. Rev. Immunol.* 10 (2010) 131–144.
- [9] S.Y. Seong, P. Matzinger, Hydrophobicity: an ancient damage-associated molecular pattern that initiates innate immune responses, *Nat. Rev. Immunol.* 4 (2004) 469–478.
- [10] T. Haas, J. Metzger, F. Schmitz, A. Heit, T. Müller, E. Latz, H. Wagner, The DNA sugar backbone 2' deoxyribose determines toll-like receptor 9 activation, *Immunity* 28 (2008) 315–323.
- [11] M.E. Bianchi, DAMPs, PAMPs and alarmins: all we need to know about danger, *J. Leukoc. Biol.* 81 (2007) 1–5.
- [12] G.Y. Chen, G. Nuñez, Sterile inflammation: sensing and reacting to damage, *Nat. Rev. Immunol.* 10 (2010) 826–837.
- [13] J.L. Round, S.K. Mazmanian, Inducible Foxp3⁺ regulatory T-cell development by a commensal bacterium of the intestinal microbiota, *Proc. Natl. Acad. Sci. U.S.A.* 107 (2010) 12204–12209.
- [14] K. Katakura, J. Lee, D. Rachmilewitz, G. Li, L. Eckmann, E. Raz, Toll-like receptor 9-induced type I IFN protects mice from experimental colitis, *J. Clin. Invest.* 115 (2005) 695–702.
- [15] I. Caramalho, T. Lopes-Carvalho, D. Ostler, S. Zelenay, M. Haury, J. Demengeot, Regulatory T cells selectively express toll-like receptors and are activated by lipopolysaccharide, *J. Exp. Med.* 197 (2003) 403–411.
- [16] H. Conroy, N.A. Marshall, K.H. Mills, TLR ligand suppression or enhancement of Treg cells? A double-edged sword in immunity to tumours, *Oncogene* 27 (2008) 168–180.
- [17] A. Aderem, Phagocytosis and the inflammatory response, *J. Infect. Dis.* 187 (2003) S340–S345.
- [18] R.V. Vance, R.R. Isberg, D.A. Portnoy, Patterns of pathogenesis: discrimination of pathogenic and nonpathogenic microbes by the immune system, *Cell Host Microbe* 6 (2009) 10–21.
- [19] P.J. Sansonetti, War and peace at mucosal surfaces, *Nat. Rev. Immunol.* 4 (2004) 953–964.
- [20] J. Lee, J.M. Gonzales-Navajas, E. Raz, The “polarizing-tolerizing” mechanism of intestinal epithelium: its relevance to colonic homeostasis, *Semin. Immunopath.* 30 (2008) 3–9.
- [21] B. Schmausser, M. Andrulis, S. Endrich, S.K. Lee, C. Josenhans, H.K. Müller-Hermelink, M. Eck, Expression and subcellular distribution of toll-like receptors TLR4, TLR5 and TLR9 on the gastric epithelium in *Helicobacter pylori* infection, *Clin. Exp. Immunol.* 13 (2004) 521–526.
- [22] A.T. Gewirtz, T.A. Navas, S. Lyons, P.J. Godowski, J.L. Madara, Cutting edge: bacterial flagellin activates basolaterally expressed TLR5 to induce epithelial proinflammatory gene expression, *J. Immunol.* 167 (2001) 1882–1885.
- [23] J.C. Bambou, A. Giraud, S. Menard, B. Begue, S. Rakotobe, M. Heyman, F. Taddei, N. Cerf-Bensussan, V. Gaboriau-Routhiau, In vitro and ex vivo activation of the TLR5 signaling pathway in intestinal epithelial cells by a commensal *Escherichia coli* strain, *J. Biol. Chem.* 279 (2004) 42984–42992.
- [24] J. Lee, J.H. Mo, K. Katakura, I. Alkalay, A.N. Rucker, Y.T. Liu, H.K. Lee, C. Shen, G. Cojocaru, S. Shenouda, M. Kagnoff, L. Eckmann, Y. Ben-Neriah, E. Raz, Maintenance of colonic homeostasis by distinctive apical TLR9 signalling in intestinal epithelial cells, *Nat. Cell. Biol.* 8 (2006) 1327–1336.
- [25] M. Rimoldi, M. Chieppa, P. Larghi, M. Vulcano, P. Allavena, M. Rescigno, Monocyte-derived dendritic cells activated by bacteria or by bacteria-stimulated epithelial cells are functionally different, *Blood* 106 (2005) 2818–2826.
- [26] A. Iwasaki, Mucosal dendritic cells, *Annu. Rev. Immunol.* 25 (2007) 381–418.
- [27] I.D. Iliev, G. Matteoli, M. Rescigno, The yin and yang of intestinal epithelial cells in controlling dendritic cell function, *J. Exp. Med.* 204 (2007) 2253–2257.
- [28] I.D. Iliev, E. Mileti, G. Matteoli, M. Chieppa, M. Rescigno, Intestinal epithelial cells promote colitis-protective regulatory T-cell differentiation through dendritic cell conditioning, *Mucosal Immunol.* 2 (2009) 340–350.
- [29] I.D. Iliev, I. Spadoni, E. Mileti, G. Matteoli, A. Sonzogni, G.M. Sampietro, D. Foschi, F. Caprioli, G. Viale, M. Rescigno, Human intestinal epithelial cells promote the differentiation of tolerogenic dendritic cells, *Gut* 58 (2009) 1481–1489.
- [30] A. Casadevall, L. Pirofski, Host-pathogen interactions: the attributes of virulence, *J. Infect. Dis.* 184 (2001) 337–344.
- [31] S. Falkow, Is persistent bacterial infection good for your health? *Cell* 124 (2006) 699–702.
- [32] R.L. Ferrero, Innate immune recognition of the extracellular mucosal pathogen, *Helicobacter pylori*, *Mol. Immunol.* 42 (2005) 879–885.
- [33] P. Klemm, V. Hancock, M.A. Schembri, Mellowing out: adaptation to commensalism by *Escherichia coli* asymptomatic bacteriuria strain 83972, *Infect. Immun.* 75 (2007) 3688–3695.
- [34] A. Casadevall, L. Pirofski, What is a pathogen? *Ann. Med.* 34 (2002) 2–4.
- [35] J.G. Magalhaes, I. Tattoli, S.E. Girardin, The intestinal epithelial barrier: how to distinguish between the microbial flora and pathogens, *Semin. Immunol.* 19 (2007) 106–115.
- [36] R.H. Rubin, Fungal and bacterial infections in the immunocompromised host, *Eur. J. Clin. Microbiol. Infect. Dis.* 12 (Suppl. 1) (1993) S42–S48.
- [37] U. Hentschel, M. Steinert, J. Hacker, Common molecular mechanisms of symbiosis and pathogenesis, *Trends Microbiol.* 8 (2000) 226–231.
- [38] I.R. Cohen, Biomarkers, self-antigens and the immunological humunculus, *J. Autoimmun.* 29 (2007) 246–249.
- [39] J.E. Galán, A. Collmer, Type III secretion machines: bacterial devices for protein delivery into host cells, *Science* 284 (1999) 1322–1328.
- [40] J.E. Galán, H. Wolf-Watz, Protein delivery into eukaryotic cells by type III secretion machines, *Nature* 444 (2006) 567–573.
- [41] A.C. Silver, Y. Kikuchi, A.A. Fadl, J. Sha, A.K. Chopra, J. Graf, Interaction between innate immune cells and a bacterial type III secretion system in mutualistic and pathogenic associations, *Proc. Natl. Acad. Sci. U.S.A.* 104 (2007) 9481–9486.
- [42] H. Nagai, C.R. Roy, Show me the substrates: modulation of host cell function by type IV secretion systems, *Cell. Microbiol.* 5 (2003) 373–383.
- [43] A.P. Tampakaki, V.E. Fadoulglou, A.D. Gazi, N.J. Panopoulos, M. Kokkinidis, Conserved features of type III secretion, *Cell. Microbiol.* 6 (2004) 805–816.

- [44] W.E. Goldman, D.G. Klapper, J.B. Baseman, Detection, isolation, and analysis of a released *Bordetella pertussis* product toxic to cultured tracheal cells, *Infect. Immun.* 36 (1982) 782–794.
- [45] T.A. Koropatnick, J.T. Engle, M.A. Apicella, E.V. Stabb, W.E. Goldman, M.J. McFall-Ngai, Microbial factor-mediated development in a host-bacterial mutualism, *Science* 306 (2004) 1186–1188.
- [46] A. Jonson, S. Normark, M. Rhen, Fimbriae, pili, flagella and bacterial virulence. in: W. Russell, H. Herwald (Eds.), *Concepts in bacterial virulence*. Karger, Basel, 2005, pp. 67–89.
- [47] M.A. Rendón, Z. Saldaña, A.L. Erdem, V. Monteiro-Neto, A. Vázquez, J. B. Kaper, J.L. Puente, J.A. Girón, Commensal and pathogenic *Escherichia coli* use a common pilus adherence factor for epithelial cell colonization, *Proc. Natl. Acad. Sci. U.S.A.* 104 (2007) 10637–10642.
- [48] M.J. Blaser, J.C. Atherton, *Helicobacter pylori* persistence: biology and disease, *J. Clin. Invest.* 113 (2004) 321–333.
- [49] M. Rescigno, U. Lopatin, M. Chieppa, Interactions among dendritic cells, macrophages, and epithelial cells in the gut: implications for immune tolerance, *Curr. Opin. Immunol.* 20 (2008) 669–675.
- [50] S.C. Corr, Y. Li, C.U. Riedel, P.W. O'Toole, C. Hill, C.G.M. Gahan, Bacteriocin production as a mechanism for the antiinfective activity of *Lactobacillus salivarius* UCC118, *Proc. Natl. Acad. Sci. U.S.A.* 104 (2007) 7617–7621.
- [51] V. Sperandio, A.G. Torres, B. Jarvis, J.P. Nataro, J.B. Kaper, Bacteria–host communication: the language of hormones, *Proc. Natl. Acad. Sci. U.S.A.* 100 (2003) 8951–8956.
- [52] M. Walters, V. Sperandio, Quorum sensing in *Escherichia coli* and *Salmonella*, *Int. J. Med. Microbiol.* 296 (2006) 125–131.
- [53] M.D. Saemann, G.A. Bohmig, C.H. Osterreicher, H. Burtscher, O. Parolini, C. Diakos, J. Stöckl, W.H. Hörl, G.J. Zlabinger, Anti-inflammatory effects of sodium butyrate on human monocytes: potent inhibition of IL-12 and up-regulation of IL-10 production, *FASEB J.* 14 (2000) 2380–2382.
- [54] H. Sokol, B. Pigneur, L. Watterlot, O. Lakhdari, L.G. Bermúdez-Humarán, J.J. Gratadoux, S. Blugeon, C. Bridonneau, J.P. Furet, G. Corthier, C. Grangette, N. Vasquez, P. Pochart, G. Trugnan, G. Thomas, H.M. Blottière, J. Doré, P. Marteau, P. Seksik, P. Langella, *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients, *Proc. Natl. Acad. Sci. U.S.A.* 105 (2008) 16731–16736.
- [55] I.I. Ivanov, R.L. Frutos, N. Manel, K. Yoshinaga, D.B. Rifkin, R.B. Sartor, B.B. Finlay, D.R. Littman, Specific microbiota direct the differentiation of IL-17-producing T-helper cells in the mucosa of the small intestine, *Cell Host Microbe* 4 (2008) 337–349.
- [56] Y. Belkaid, K. Tarbell, Regulatory T cells in the control of host-microorganism interactions, *Annu. Rev. Immunol.* 27 (2009) 551–589.
- [57] B. Foxman, D. Goldberg, C. Murdock, C. Xi, J.R. Gilsdorf, Conceptualizing human microbiota: from multicelled organ to ecological community, *Interdiscip. Perspect. Infect. Dis.* (2008) 1–5.
- [58] A.M. O'Hara, F. Shanahan, The gut flora as a forgotten organ, *EMBO Rep.* 7 (2006) 688–693.
- [59] A.T. Borchers, C. Selmi, F.J. Meyers, C.L. Keen, M.E. Gershwin, Probiotics and immunity, *J. Gastroenterol.* 44 (2009) 26–46.
- [60] M. Fernandez, T. Pedron, R. Tournebise, J. Olivo-Marin, P. Sansonetti, A. Phalipon, Anti-inflammatory role for intracellular dimeric immunoglobulin A by neutralization of lipopolysaccharide in epithelial cells, *Immunity* 18 (2003) 739–749.
- [61] S. Boullier, M. Tanguy, K.A. Kadaoui, C. Caubet, P. Sansonetti, B. Corthésy, A. Phalipon, Secretory IgA-mediated neutralization of *Shigella flexneri* prevents intestinal tissue destruction by down-regulating inflammatory circuits, *J. Immunol.* 183 (2009) 5879–5885.
- [62] J.K. Simon, M. Maciel, E.D. Weld, R. Wahid, M.F. Pasetti, W.L. Picking, K.L. Kotloff, M.M. Levine, M.B. Sztein, Antigen-specific IgA B memory cell responses to *Shigella* antigens elicited in volunteers immunized with live attenuated *Shigella flexneri* 2a oral vaccine candidates, *Clin. Immunol.* 139 (2011) 185–192.
- [63] G. Le Negrate, B. Faustin, K. Welsh, M. Loeffler, M. Krajewska, P. Hasegawa, S. Mukherjee, K. Orth, S. Krajewski, A. Godzik, D.G. Guiney, J.C. Reed, *Salmonella* secreted factor L deubiquitinase of *Salmonella typhimurium* inhibits NF-kappaB, suppresses I kappa Balpha ubiquitination and modulates innate immune responses, *J. Immunol.* 180 (2008) 5045–5056.
- [64] Z. Ye, E.O. Petrof, D. Boone, E.C. Claud, J. Sun, *Salmonella* effector AvrA regulation of colonic epithelial cell inflammation by deubiquitination, *Am. J. Pathol.* 171 (2007) 882–892.
- [65] E. Mileti, G. Matteoli, I.D. Iliiev, M. Rescigno, Comparison of the immunomodulatory properties of three probiotic strains of *Lactobacilli* using complex culture systems: prediction for in vivo efficacy, *PLoS One* 4 (2009) e7056.
- [66] C. O'Mahony, P. Scully, D. O'Mahony, S. Murphy, F. O'Brien, A. Lyons, G. Sherlock, J. MacSharry, B. Kiely, F. Shanahan, L. O'Mahony, Commensal-induced regulatory T cells mediate protection against pathogen-stimulated NF-kappaB activation, *PLoS Pathog.* 4 (2008) e1000112.
- [67] L.E. Smythies, M. Sellers, R.H. Clements, M. Mosteller-Barnum, G. Meng, W.H. Benjamin, J.M. Orenstein, P.D. Smith, Human intestinal macrophages display profound inflammatory anergy despite avid phagocytic and bacteriocidal activity, *J. Clin. Invest.* 115 (2005) 66–75.
- [68] J.L. Coombes, K.J. Maloy, Control of intestinal homeostasis by regulatory T cells and dendritic cells, *Semin. Immunol.* 19 (2007) 116–126.
- [69] D. Artis, Epithelial-cell recognition of commensal bacteria and maintenance of immune homeostasis in the gut, *Nat. Rev. Immunol.* 8 (2008) 411–420.
- [70] M. Rescigno, Gut commensal flora: tolerance and homeostasis, *F1000 Biol. Rep.* 1 (2009) 1–6.
- [71] N. Cerf-Bensussan, V. Gaboriau-Routhiau, The immune system and the gut microbiota: friends or foes? *Nat. Rev. Immunol.* 10 (2010) 735–744.
- [72] M. Rimoldi, M. Chieppa, V. Salucci, F. Avogadri, A. Sonzogni, G.M. Sampietro, A. Nespoli, G. Viale, P. Allavena, M. Rescigno, Intestinal immune homeostasis is regulated by the crosstalk between epithelial cells and dendritic cells, *Nat. Immunol.* 6 (2005) 507–514.
- [73] B. He, W. Xu, P.A. Santini, A.D. Polydorides, A. Chiu, J. Estrella, M. Shan, A. Chaddburn, V. Villanacci, A. Plebani, D.M. Knowles, M. Rescigno, A. Cerutti, Intestinal bacteria trigger T cell-independent immunoglobulin A(2) class switching by inducing epithelial-cell secretion of the cytokine APRIL, *Immunity* 26 (2007) 812–826.
- [74] D. Braun, R.S. Longman, M.L. Albert, A two-step induction of indoleamine 2,3 dioxygenase (IDO) activity during dendritic-cell maturation, *Blood* 106 (2005) 2375–2381.
- [75] A. Heitger, Regulation of expression and function of IDO in human dendritic cells, *Curr. Med. Chem.* 18 (2011) 2222–2233.
- [76] I.R. Cohen, *Tending Adam's garden: evolving the cognitive immune self*. Academic Press, London, San Diego, 2000.
- [77] I.R. Cohen, Discrimination and dialogue in the immune system, *Semin. Immunol.* 12 (2000) 215–219.
- [78] I.R. Cohen, D. Harel, Explaining a complex living system: dynamics, multi-scaling and emergence, *J. R. Soc. Interface* 4 (2006) 175–182.
- [79] J.L. Gardy, D.J. Lynn, F.S. Brinkman, R.E. Hancock, Enabling a systems biology approach to immunology: focus on innate immunity, *Trends Immunol.* 30 (2009) 249–262.
- [80] B. Marteyn, N.P. West, D.F. Browning, J.A. Cole, J.G. Shaw, F. Palm, J. Mounier, M.C. Prévost, P. Sansonetti, C.M. Tang, Modulation of *Shigella* virulence in response to available oxygen in vivo, *Nature* 465 (2010) 355–358.
- [81] J.P. Celli, B.S. Turner, N.H. Afdhal, S. Keates, I. Ghiran, C.P. Kelly, R. H. Ewoldt, G.H. McKinley, P. So, S. Erramilli, R. Bansil, *Helicobacter pylori* moves through mucus by reducing mucin viscoelasticity, *Proc. Natl. Acad. Sci. U.S.A.* 106 (2009) 14321–14326.