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Innate immune relationship between commensal flora and the mammalian intestinal epithelium

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Abstract. Commensal bacteria in the lumen of the intestine exist in a mutually advantageous relationship with the mammalian host, providing benefits such as increased metabolic/digestive capabilities and exclusion of harmful microbes, and in turn receiving a nutrient-rich environment. However, in the context of a dysfunctional intestinal epithelial barrier, commensal bacteria may elicit an immune inflammatory response similar to what occurs during infection by a pathogen. Recent work has established that most eukaryotic cells possess families of receptors that can detect the structural signatures of prokaryotic life. Cells may respond to the perception of microbes by activating distinct cytoplasmic signaling cascades that ultimately result in the transcriptional activation of genes needed for proinflammatory and anti-apoptotic functions, as well as for a pro-apoptotic response. Collectively, these responses generally suffice to eliminate microbial threats and may be integral to normal intestinal homeostasis. An understanding of these mechanisms, as well as those by which microbes themselves influence intestinal epithelial responses, may help provide a new perspective on the pathogenesis of intestinal diseases.

Key words. Epithelium; bacteria; Toll-like receptor; NF-κB, Nod.

Introduction

Prokaryotic organisms can exist in intimate and continuous contact with members of the eukaryotic kingdom. The implications of this statement reflect an emerging theme in the life sciences that has recently come to the forefront of our general view of multicellular plants and animals – that microbes may affect our biology in profound and perhaps previously unsuspected ways. Cooperative interactions between eukaryotes and prokaryotes are well known. In these symbiotic relationships, the microbe profits by acquisition of a stable temperature, oxygen and nutrient supply. Eukaryotic hosts may gain extended metabolic/digestive ability and benefit from competitive exclusion of harmful microbes. Of course, the relationship of eukaryotes and prokaryotes is not always benign. Either overt pathogens or typical commensals (when in a genetically susceptible or clinically compromised host) can be harmful to their hosts. Eukaryotic tissues must be able to respond and defend against/manage microbial presence. However, an immunological paradox presents itself when specifically considering the intestine; it too is threatened by microbial invaders and can respond in a typical fashion – with acute inflammation – but virtually unique among tissues, it must tolerate the close proximity of staggering quantities of microbes and their products. In humans, the mucosal lining of the intestine has a surface area of greater than 100 m^2 [1]. This vast epithelial expanse coexists without clinically significant inflammation while in intimate contact with greater than 10^{12} resident bacteria [2]. Thus, the intestine can and must be able to respond to microbial threats, but also tolerate microbial bystanders, and perhaps even encourage bacterial commensals.

This review will discuss the mechanisms by which intestinal epithelial cells recognize the presence of bacteria, necessarily both potential pathogens and commensals, how that recognition is translated into signaling events and what initial effector mechanisms are used by cells and tissues to respond to microbial interactions. We will

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discuss mechanisms by which bacteria can manipulate the epithelial responses and the potential consequences for both parties.

Epithelial monitoring of bacteria: PRRs and PAMPs

Eukaryotes have evolved mechanisms to constantly survey their surroundings for the telltale presence of microbes. They must remain vigilant against potential threats both from the outside and from within. To accomplish this task, virtually all metazoans scan their environment with pattern recognition receptors (PRRs), an operational term for transmembrane or intracytoplasmic receptors that are defined by their ability to specifically bind distinctive microbial ligands designated pathogen-associated molecular patterns (PAMPs) [3]. PAMPs represent structural motifs that are restricted to, and definitive of, microbial organisms, both pathogenic and commensal. For example, lipopolysaccharide (LPS) is a component of Gram-negative bacterial walls, peptidoglycan (PGN) is part of the Gram-negative and Gram-positive cell wall structure and double stranded RNA (dsRNA) is a nucleic acid intermediate typically occurring during viral replication. Because of the efficiency of eukaryotic recognition of PAMPs, bacteria have predictably evolved significant ability to modify and conceal these structures, including such mechanisms as flagellar phase variation, LPS chain modification and encapsulation [4].

In the intestine, epithelial cells line the lumen and are in actual physical contact with the normal flora and their products. In addition to their well-known barrier function, the enterocytes are poised as sentinels and likely play a key role in scanning the gut environment for microbial threats. Using complementary DNA (cDNA) microarray expression profiling and other assays of proinflammatory function, our and other laboratories have shown that bacterial flagellin is a major proinflammatory PAMP detected by polarized epithelia [5–7]. An aflagellate *Salmonella* strain did not elicit the classical proinflammatory signaling pathways characteristically induced by enteropathogenic *Salmonella* [8, 9]. A wide variety of bacterial strains are flagellated, and it is likely that the structural constraints necessary for the motility function of flagella permit recognition of flagellin from many different organisms [10]. Flagellin is known to be a potent activator of systemic inflammation in murine models [11], and in humans, serum levels of the protein correlate with clinical severity of bacteremic shock syndromes [12]. Interestingly, studies of circulating antibodies in the serum of human Crohn's disease patients and in murine colitis models identified flagellin as a dominant antigen, suggesting a role for this bacterial protein in the immunopathogenesis of inflammatory bowel disease [13].

Table 1. Pattern recognition receptors and their ligands.

TLR1:	lipopeptides
TLR2:	lipoprotein, lipoteichoic acid, others
TLR3:	double-stranded RNA
TLR4:	lipopolysaccharide
TLR5:	flagellin
TLR6:	unknown
TLR7:	unknown
TLR8:	unknown
TLR9:	unmethylated CpG-containing DNA
TLR10:	unknown
TLR11:	uropathogenic *E. coli*
Nod1:	Gram-negative peptidoglycan
Nod2:	Gram-positive and negative peptidoglycan

The best-studied mammalian PRRs are the Toll-like receptors (TLRs). The designation Toll-like receptor reflects the homology to the Toll receptor in *Drosophila melanogaster*, a protein involved in early embryonic patterning, which also plays a critical role in the insect innate immune system [14]. The human genome contains at least 11 known TLRs (table 1) [3, 15]. The gene products are transmembrane receptors defined by the presence of two ancient and highly conserved structural motifs. The first is the leucine-rich repeat (LRR) in the extracellular portion of the molecule that functions in selective ligand (PAMP) recognition. The second is the TIR (Toll/interleukin-1 receptor) motif in the cytoplasmic domain of the TLR, which interacts with and activates signal-transducing proteins that will be discussed in the next section.

An unanswered question is how the luminal epithelial cells distinguish between pathogenic versus commensal bacteria. Commensal microbes or their by-products must not continually or excessively activate TLRs, for to do so would seem to result in a constant state of gut inflammation. No doubt physical and mechanical barriers, such as mucus layers, an apical epithelial glycocalyx and impermeable intercellular junctions play an important role in this tolerance [16, 17]. In addition, TLRs may be strategically deployed; clearly, a circulating cell such as a macrophage would be expected to express receptors circumferentially around the cell. In contrast, epithelial cells – whose apical surfaces must interface with the gut lumen and whose basal aspect with the interior of the organism – may spatially restrict TLR expression. Our laboratory has found that TLR5 is present along basolateral epithelial cell membranes and only signals when flagellin is applied to this aspect of the cells [8, 9], although apical TLR5 and signaling has been reported by other groups [5, 18]. These observed differences in flagellin response and subcellular location might reflect in vitro differences in cell culture models or experimental thresholds for detection of proinflammatory signaling. Clearly, commensal bacteria must not elicit as intense an inflammatory response as a pathogen; however, recent evidence in trans-

genic mice that are unable to transduce TLR signaling suggests that a degree of constitutive TLR stimulation may be necessary for intestinal health [17]. Interestingly, commensal and non-invasive *Escherichia coli* strains have been reported to activate flagellin-dependent proinflammatory signaling upon apical incubation with murine intestinal tissue mounted ex vivo in Ussing chambers [19]. This experimental design utilizes a histologically complete mucosa harboring microfold (M) cells and dendritic cells that may be able to sample the apical compartment to a greater degree than in cultured epithelial monolayers.

PRRs are also capable of detecting intracytoplasmic PAMPs [20]. Certain bacteria exhibit an intracytoplasmic stage during infection. The intracellular LRR – containing proteins of the nucleotide-binding oligomerization domain (Nod) family may act to monitor the cytoplasm of cells for PAMPs present on intracellular pathogens, thus providing a means of detecting internalized bacteria not perceived by surface receptors. The Nod proteins have a modular structure similar to that of TLRs. The LRR ligand-binding motifs interact with specific PAMPs, to date identified as specific peptide components of larger PGN macromolecules. The N-termini of Nod1 and 2 proteins contain CARDs, or caspase activation and recruitment domains, which are functionally analogous to the TIR domain (of TLRs) that mediates second-messenger activation necessary for subsequent signaling pathways [21].

In summary, eukaryotic cells possess PRRs capable of detecting microbial 'signatures'. These PRRs have a wide range of specificity and spatial distribution to allow efficient monitoring of the local environment. Upon perception of a PAMP, the PRR transmits an 'alarm' signal to the interior of the cell – a process we now consider.

Epithelial reaction to bacteria: activation of signaling pathways

The interaction of a PRR with its cognate PAMP results in activation of cytoplasmic signaling relays. As signaling events are usually transient and reversible, they are prime candidates for manipulation – both by bacteria seeking to counter defenses and by humans seeking points of therapeutic intervention. Such relays may be controlled by the regulated transfer of covalent modifications along a series of cytoplasmic protein intermediates or by sequential proteolytic cascades. An example of the former is the proinflammatory Rel/nuclear factor kappa B (NF-κB) family pathway. The terminal result of activating this pathway is the nuclear appearance of Rel/NF-κB factors, which bind specific promoter elements and stimulate *de novo* transcription of genes involved in responses to microbes (fig. 1). An example of a controlled regulated series of proteolytic cleavages initiated by PRRs is the activation of 'extrinsic' pro-apoptotic signaling [22]. This signaling cascade culminates in the appearance of activated effector caspases that mediate the controlled physiological demolition of the infected or otherwise damaged cell (fig. 1).

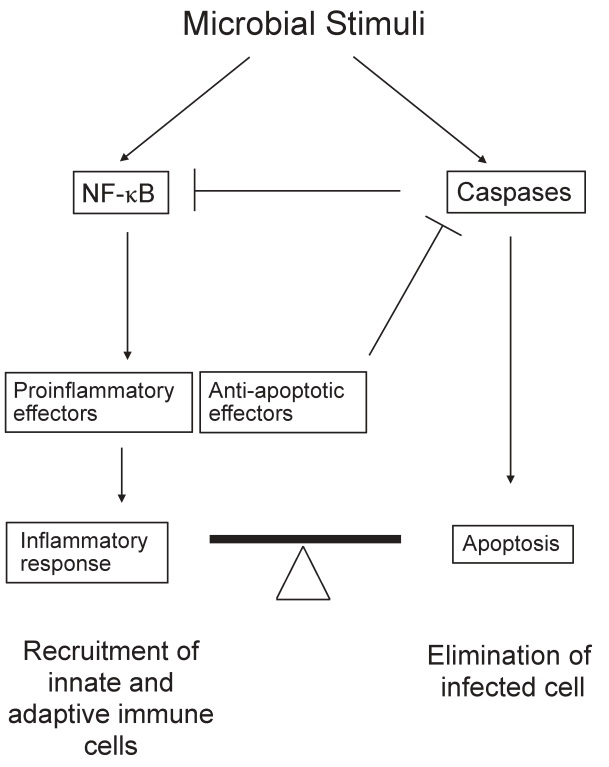

Figure 1. Microbial activation of signaling pathways. Activating interactions are indicated with arrows, while inhibitory interactions are indicated with bars.

The molecular pathways that are activated by bacteria, particularly via TLRs and Nod proteins, are beginning to be unraveled (fig. 2) [23, 24]. As currently understood, binding of a PAMP to a TLR and consequent TLR dimerization results in the formation of a TIR domain competent to bind a family of adaptor proteins. The original member of this family is known as MyD88, and additional adaptors have been described in recent years, including TRAM, MAL/TIRAP and TRIF/TICAM [25]. Different adaptor proteins (or combinations of them) may preferentially interact with homodimeric or heterodimeric TLRs and presumably have a role in orchestrating the most appropriate response (i.e. cellular inflammation or apoptosis) to a given PAMP/TLR interaction. Biochemical analyses have demonstrated that classical proinflammatory signaling pathways such as that elicited by tumor necrosis factor (TNF) can potently activate both inflammation and apoptosis [26]. Similarly, with the binding of an adaptor protein to the TIR of a PAMP-stimulated TLR, a bifurcation of signaling may occur along proinflammatory and/or pro-apoptotic pathways.

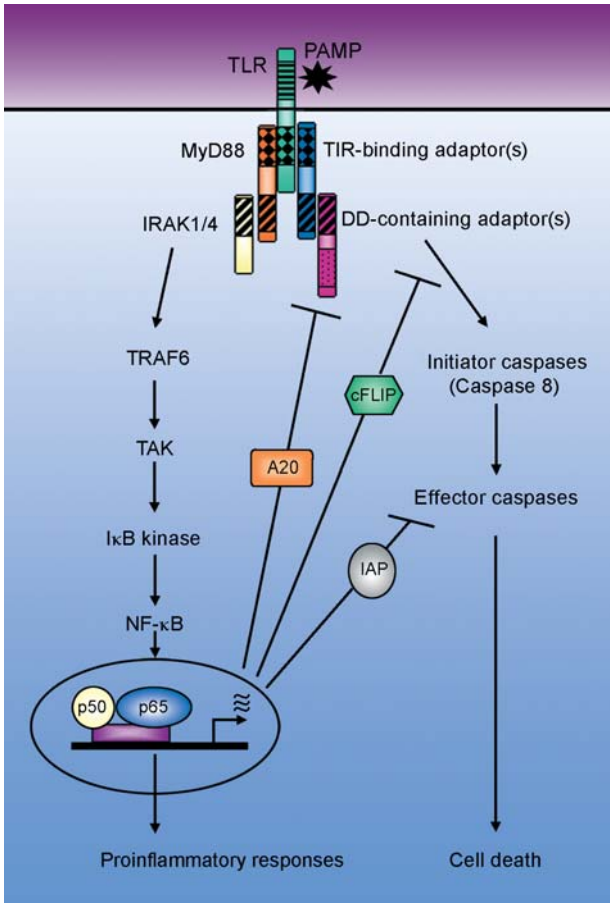

Figure 2. Proinflammatory and pro-apoptotic pathways. The LRR region of a TLR binds to a PAMP and transmits signals via cytoplasmic intermediates (TIR-binding adaptor proteins, i.e. MyD88, TRIF; DD-containing adaptor proteins, i.e. IRAK, FADD, RIP) that can have proinflammatory or pro-apoptotic outcomes. Activating interactions are indicated with arrows, while inhibitory interactions are indicated with bars. Domain patterns: LRR, horizontal bars; TIR, checkered; DD, cross-hatched; DED, spotted.

Proinflammatory signaling is largely mediated by activation of the NF-κB pathway, with involvement of other signaling systems of the mitogen-activated protein kinase (MAPK) and interferon regulatory factor (IRF) families [27]. NF-κB is a collective term for members of the Rel family of DNA-binding transcription factors. Active NF-κB is a dimer that recognizes characteristic sequence motifs present in the promoters of genes involved in immune, inflammatory and anti-apoptotic responses. During steady-state conditions, NF-κB is sequestered in the cytoplasm by the action of a third protein, IκB or inhibitor of κB. NF-κB activation occurs by a rapid post-translational modification pathway that allows phosphorylation, ubiquitination and subsequent degradation of IκB [28] (fig. 2). Free NF-κB dimers can then translocate to the nucleus and activate transcription.

NF-κB activation in response to TLR-mediated signals occurs when a cytoplasmic adaptor molecule is recruited to a dimerized TIR domain (fig. 2). The MyD88 adaptor and other signaling adaptors/receptors, including the TNF receptor or Nod family receptors, possess a protein-protein interaction motif termed the death domain (DD), so-called due to its common presence in proteins involved in pro-apoptotic signaling. The DD of MyD88 interacts with a DD of a second family of adaptor molecules, known as IRAK. The IRAK serine kinases then activate the cytoplasmic signaling intermediate TRAF6 by promoting ubiquitination [29]. Ubiquitin is a highly conserved 76-amino acid peptide employed as a common covalent modification of cellular proteins, usually on lysine residues [30]. Originally, ubiquitination of proteins was assumed to target the modified protein for regulated destruction by the cellular proteasome organelle. Recent discoveries of alternative ubiquitin chain linkages, as well as families of related molecules such as SUMO and NEDD8, have revealed that these modifications play a role in diverse processes such as intracellular trafficking and, as in the case of TRAF6, enzymatic activation [31]. In any event, once activated by ubiquitination, TRAF6 in turn activates TAK1, in complex with TAB1 and TAB2. The TAK1/TAB1/TAB2 complex functions as an IKK kinase [32].

IKK is the signaling nexus, receiving and integrating signals from multiple proinflammatory signal transduction pathways (fig. 3) [33]. The catalytic subunits of the IKK complex, IKK-α and IKK-β, recognize a conserved 6-amino acid motif on IκB and phosphorylate two serine residues within the motif [34]. The phosphorylated IκB isoforms are subjected to polyubiquitination by the IκB ubiquitin ligase, a multicomponent enzymatic complex designated β-TrCP-SCF. The 18S regulatory subunit of the proteasome recognizes polyubiquitinated IκB and induces the proteolysis of the IκB molecule. Following IκB degradation, the NF-κB nuclear localization signal is exposed, allowing regulated translocation across the nuclear membrane.

Pro-apoptotic signaling also utilizes TIR-binding adaptor molecules (fig. 2). The DD of the adaptor molecules can interact with other DD-containing proteins that also encode a death effector domain (DED). The DED can directly interact with the inactive zymogen forms of initiator caspases, such as caspase 8, setting into motion the proteolytic cascades that ultimately lead to programmed cell death (PCD) [35]. For example, MyD88 can directly interact with the DED-containing adaptor Fas associated death domain (FADD) that subsequently activates procaspase 8. This pro-apoptotic action has been demonstrated for TLR2, but presumably any TLRs that bind MyD88 could mediate a similar outcome [22]. Ligand-induced dimerization of Nod receptors may result in the similar recruitment of DED-bearing intermediates and caspase activation by CARD domains [36]. Dominant negative forms of FADD [37], MyD88 and

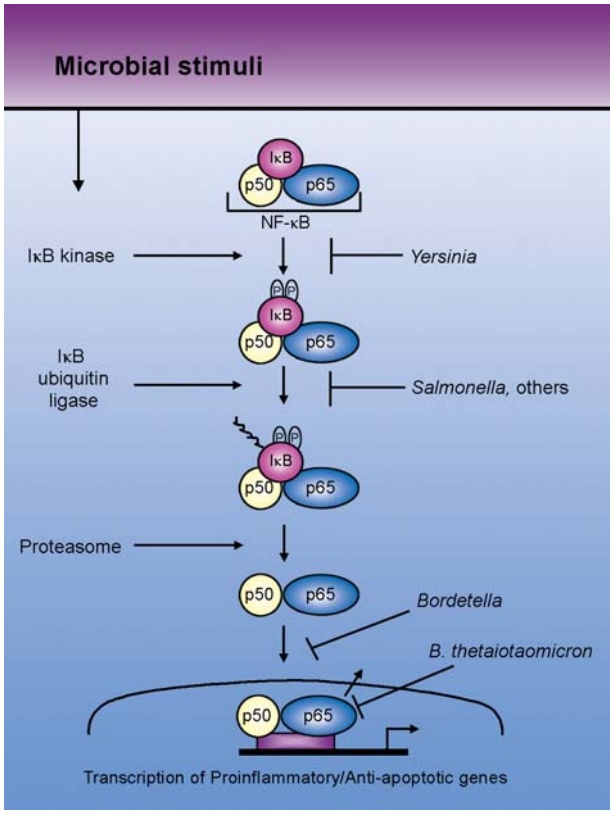

Figure 3. Microbial inhibition of the NF-κB pathway. Inhibitory interactions are indicated with bars.

the downstream signaling intermediate IRAK2 [38] have been shown to suppress TLR4-mediated apoptosis in transfected macrophages, indicating that these proteins are involved in transducing TLR-mediated pro-apoptotic signals. Interestingly, in this system, dominant negative IRAK1 and TRAF6 augmented TLR4-meditated apoptosis, suggesting that these proteins act selectively on the transmission of proinflammatory 'survival' signaling [38]. Consistently, in macrophages derived from mice null for candidate signaling intermediates, TRAF6 –/– and MyD88 –/– macrophages exhibited markedly increased apoptosis on LPS stimulation [39]. A second group reported that MyD88 –/– macrophages did not show differences when infected with live *Yersinia* or treated with LPS, but a TRIF –/– macrophage line did show a marked reduction in apoptosis, suggesting a role for this adaptor in transducing apoptotic signals [40]. It seems likely that TLR ligand binding induces parallel and simultaneous activation of proinflammatory (survival) pathways and pro-apoptotic caspase cascades. The switching of the signals is apparently mediated most proximally by differential utilization of MyD88 family adaptor proteins. Much work remains to clarify the role of these proteins and to identify which TLR they interact with. The consequences of pro-apoptotic activation will be discussed in the next section.

In summary, eukaryotic cells have independently evolved two pathways to respond to microbial threats: proinflammatory (exemplified by the NF-κB pathway) and pro-apoptotic (i.e. the extrinsic caspase cascade). Both pathways seem to be activated concurrently and in parallel, and the end goal of either pathway is elimination of the microbial presence. At the cellular level, acute inflammation allows cellular integrity to be preserved, while apoptosis acts to eliminate the infected cell.

Epithelial response to bacteria: inflammation or apoptosis

The result of signaling pathways is the activation of effector molecules. As we have implied, animal cells seem to have two options to respond to a microbial threat. First, activation of NF-κB and associated pathways results in inflammation. Broadly defined, inflammation is a cellular influx of immune effector cells that serve to eliminate the inciting microbial threat, generally by phagocytosis and subsequent intracellular lysis. Alternatively, or in parallel, apoptosis is initiated. Again broadly defined, apoptosis is a controlled, programmed autodigestion of a selected cell. In the sense of responding to microbial threats, apoptosis of an infected cell can accomplish precisely the same thing as phagocytosis. Loss of individual infected cells, especially in a population of interchangeable or highly turned-over cells such as epithelia, would have no deleterious effects on the whole organism.

Inflammatory response

Details of the inflammatory response in vertebrates have been well discussed elsewhere [41]. To briefly review, the usual paradigm holds that activation of NF-κB and other proinflammatory signal transduction pathways result in de novo transcription of a battery of effector molecules. Studies utilizing large-scale expression profiling techniques indicate that these molecules include antibacterial peptides, metabolic enzymes (with roles in bacterial killing, cell protection and wound healing processes), chemotactic messengers, anti-apoptotic proteins, cytokines, adhesion molecules and mediators of adaptive immunity (major histocompatibility complex (MHC) and co-stimulatory receptors) [8, 42, 43].

Typically, inflamed vertebrate tissue exhibits increased numbers of phagocytic and immunomodulatory leukocytes, which busily phagocytose microbes and cellular debris. The process can involve any tissue and appears temporally as distinct acute and chronic phases, which usually resolve with complete restitution of function. Inflammation is a classic two-edged sword in that the

oxidants and proteases released by neutrophils, although aiding in eradication of the inciting microbe, can cause lasting tissue damage and likely play a role in the early development of neoplasia. Therefore, it is extremely important for the host to activate the inflammatory process sparingly.

Apoptosis

Apoptosis, or PCD, is a genetically defined mechanism by which individual cells can eliminate themselves while largely preserving the surrounding cells and overall tissue architecture [35, 44]. It mediates a variety of physiological and adaptive events, such as developmental tissue remodeling, elimination of self-reactive lymphocytes or senescent neutrophils and culling of continually proliferating cell populations (e.g. intestinal epithelial crypts). The process of apoptosis is mediated by an arsenal of effector cysteinyl aspartate-specific proteases (caspases) that in the active state carry out limited proteolysis on apparently dozens of cellular structural and regulatory proteins, and thus effectively dismantle the cell without injury in adjacent tissues [35]. Understandably, this process is tightly regulated. The effector caspases exist in an inactive zymogen form until they are processed by an amplifying cascade of upstream initiator caspases. Commencing caspase activation can occur by the 'intrinsic' pathway. In this case, a variety of cellular stresses, such as physical injury, DNA damage or withdrawal of growth factors/hormones, can result in the leakage of mitochondrial cytochrome C into the cytosol, eliciting the formation of a scaffold complex, termed the apoptosome, that serves to activate the initiator caspase 9 and the subsequent effector caspases. Alternatively, as discussed earlier in the 'extrinsic' pathway, extracellular ligand-binding events result in activation of receptor complexes mediating assembly of DED-bearing proteins, and ultimately in activation of separate initiator caspases, such as caspase 8.

The primordial function of PRRs may have been to activate cellular self-destruction. Just as highly turned-over or interchangeable tissue such as epithelia can tolerate individual cell death without significant compromise of physiological tissue function, early metazoans with a colonial cellular organization may have employed PCD to eliminate individual infected cells and thus spare the entire organism from overwhelming infection. This manner of antimicrobial defense is known in nematodes [45] and is common in plants [46]. Given the parallel and concurrent activation of the proinflammatory and pro-apoptotic pathways by microbial signals, the individual cell is given a choice whether to recruit inflammatory cells or undergo controlled demolition. Recent work has demonstrated a complex and interrelated cross-talk of checks and balances during the execution phases of either inflammation and/or apoptosis in epithelial tissues.

Proinflammatory inhibition of apoptosis

Mouse mutants deficient in components of the NF-κB pathway have allowed discovery of a vital role for this system as an anti-apoptotic control. Mice null in the p65 subunit of NF-κB show embryonic lethality secondary to massive apoptosis in the liver [47]. A mouse strain harboring null alleles of IKK-β solely in intestinal epithelium exhibits no abnormalities under normal conditions, but responds to a systemic stress (transient intestinal ischemia) with massive apoptosis of the enterocytes [48]. Expression-profiling studies have identified a subset of anti-apoptotic effectors consistently induced by bacterial (and other proinflammatory) stimuli [49, 50]. These observations have led to the hypothesis that NF-κB and other proinflammatory pathways act to suppress apoptosis by the upregulation of anti-apoptotic effector proteins. These proteins include the inducible NF-κB-dependent IAP (inhibitor of apoptosis) family (cIAP1, cIAP2 and XIAP). These anti-apoptotic effectors act by binding to and inhibiting the activity of individual caspases, thus inactivating either intrinsic or extrinsic pathways. Interestingly, these proteins also possess C-terminal RING (Really Interesting New Gene) domains, indicating that they may function as ubiquitin ligases and thus target caspases for proteasomal-mediated degradation [51].

Other NF-κB-dependent, highly inducible anti-apoptotic proteins include A20, which possesses both ubiquitin ligase and de-ubiquitinating activity and inhibits the adaptor protein RIP associated with the DD of death receptors [52, 53]. Yet another similarly regulated protein is cFLIP. This apoptotic inhibitor contains both a DED and a catalytically inactive caspase-like domain. It interacts with the DED of FADD and/or procaspase 8, apparently acting as a dominant negative inhibitor of caspase 8 activation [54]. Thus, a battery of inducible anti-apoptotic genes are present in vertebrates and activated by proinflammatory stimuli (fig. 1, 2). It has been commented that these proteins interrupt multiple rate-limiting steps of the pro-apoptotic pathway, acting as a redundant fail-safe system to allow proinflammatory signaling to proceed unimpeded by caspase activation [50]. Thus, activation of the NF-κB pathway or, perhaps more accurately, increased activation of the NF-κB pathway relative to pro-apoptotic pathways serves to squelch whatever pro-apoptotic activation has been induced by threatening stimuli.

Both pro-apoptotic and proinflammatory effectors are activated by the perception of microbes by PRRs. It is intriguing that these sentinel receptors utilize much of the same signaling circuitry to initiate both pathways. The upstream components of the proinflammatory NF-κB pathway may have co-evolved with the apoptotic machin-

ery to ensure that activation of NF-κB proceeds in parallel with initiation of the apoptotic death program. It has been suggested that the PRR-mediated stimulation of PCD programs may represent a primordial immune reaction [55]. Once a PRR-dependent system for activating an early version of inflammation appeared, the organism must have required a negative feedback mechanism to neutralize PCD pathways. This arrangement may also serve to protect the whole organism from microbial-mediated inhibition of proinflammatory pathways by committing individual infected cells to default apoptotic destruction. Such a hypothesis also suggests that microbes with immunomodulatory functions could have wide-ranging effects on the host.

Pro-apoptotic inhibition of proinflammatory signaling

Conversely, activation of apoptotic pathways can inactivate NF-κB by caspase-mediated destruction of NF-κB signaling intermediates. IKK-β, p65 and IκB have all been identified as physiological substrates of activated effector caspases [56, 57]. Partial cleavage products of some of these proteins have also been shown to act as dominant negative inhibitors of NF-κB signaling [50]. Other proteins act as inhibitors of the IAPs, providing another biochemical activity augmenting pro-apoptotic activity [58, 59]. For example, Smac/DIABLO is a mitochondrial protein that seems to specifically target IAPs for proteasomal degradation either by stimulating an IAP auto-ubiquitination function or by acting as an ubiquitin ligase in its own right. Overall, proteolytic-mediated inhibition of proinflammatory and anti-apoptotic components apparently ensures that caspase-mediated dismantling of the cell can occur unimpeded.

Natural infection can vary in multiplicity of infection, duration, associated organisms, concurrent state of innate or adaptive immune activation and undoubtedly many other variables. It is probably accurate to say that both proinflammatory and pro-apoptotic activation both occur rapidly and reliably during the initial interaction of a bacterial organism with a potential host. The cellular endpoints of these biochemical events are the result of whether proinflammatory or pro-apoptotic signaling gains the upper hand and is able to abort the sequence of events that mediates the contrary pathway.

Microbial influences on eukaryotic signaling

It should come as no surprise that microbes have evolved myriad mechanisms to evade, inhibit or usurp the various detection, signaling and effector mechanisms deployed against them, a topic well reviewed elsewhere [60, 61]. One form of microbial management of eukaryotic defenses that is gaining increasing attention is active repression of innate immune signaling [62]. A spectrum of bacterial pathogens and nonpathogens exhibits mechanisms to directly inhibit the NF-κB pathway or reduce synthesis of inflammatory mediators in infected host cells (fig. 3). *Mycobacterium avium* blocks IL-8 secretion in cultured epithelia exposed to *S. typhimurium* [63]. *Bordetella bronchiseptica* colonization of cultured respiratory epithelial cells inhibits the cytoplasmic to nuclear translocation of the NF-κB subunit p65 in response to TNF, potently activating apoptosis [64]. Members of the genus *Yersinia* repress MAPK and NF-κB in in vitro co-culture systems at the level of IκB phosphorylation and are potently pro-apoptotic to the macrophages they infect [65]. *Yersinia* utilizes a translocated Type III effector, YopJ, which can inhibit NF-κB and MAPK signaling via a cysteine protease activity [66]. *Yersinia* mutants lacking YopJ fail to elicit macrophage apoptosis; however, TLR4-deficient macrophages show reduced apoptosis when infected by wild-type *Yersinia,* indicating that the pro-apoptotic effect of YopJ is linked to its ability to suppress proinflammatory survival pathways [37, 67]. It has also been reported that overexpression of YopJ is only weakly pro-apoptotic in transfected macrophages but apoptosis could be greatly augmented by addition of a proinflammatory stimulant (LPS) [68]. Furthermore, the homolog of YopJ that is found in enteropathogenic *Salmonella*, AvrA, can also inhibit the NF-κB pathway and elicit apoptosis in epithelial cells [69, 70]. While these examples are all from pathogenic bacterial strains, our laboratory has described several strains of nonpathogenic *Salmonellae* that not only inhibit the NF-κB pathway in infected epithelial cells, but also block subsequent proinflammatory responses elicited by endogenous cytokines [71]. In contrast to the inhibitory mechanisms associated with *Yersinia* infection, nonpathogenic *Salmonella* block the polyubiquitination of IκB without interfering with phosphorylation. These bacteria are also pro-apoptotic [70]. Experiments in our laboratory suggest that bacterial interactions may suppress activity of the IκB ubiquitin ligase, β-TrCP-SCF [unpublished data]. Collectively, such observations suggest that microbe-mediated active blockade of proinflammatory pathways could act at the cellular level by blunting cellular innate immune responses and/or by activating apoptosis.

Mammalian intestinal commensal bacteria have been observed to inhibit inflammatory pathways as well. In vitro co-culture experiments with lactic acid bacteria have shown dampening of inflammatory responses [72], and *Lactobacillus* sp. have been shown to prevent colitis in spontaneous murine models [73]. Furthermore, the mammalian intestinal commensal *Bacteroides thetaiotaomicron* has been demonstrated to inhibit NF-κB pathways by the novel mechanism of enhancing nuclear export of NF-κB [74]. These prokaryotic regulatory controls over

key eukaryotic signaling pathways are likely to have profound effects on many aspects of normal intestinal function and are likely involved in pathogenesis of inflammatory disease.

PRR recognition of commensal bacteria is also influential in health and disease. The Nod proteins include Nod2, certain mutant alleles of which are associated with the debilitating human inflammatory condition Crohn's disease [75, 76]. This disorder is thought to represent abnormal host inflammatory responses to normal intestinal flora. The mutant Nod alleles are an 'experiment of nature' that illustrates the role of PRRs in monitoring a microbial presence generally recognized as commensal rather than overtly pathogenic, though how the mutated Nod leads to chronic inflammation is unknown. Other PRRs may play a significant role in negotiating cellular interactions with commensal/symbiotic organisms. Experiments with mice null for MyD88, a key intermediate of proinflammatory TLR signaling, revealed that intestinal tissues were hypersensitive to local injury. Furthermore, this hypersensitivity could be replicated by depletion of normal flora and remedied by luminal administration of PAMPs [17]. Thus, a certain level of 'tonic' TLR recognition of the commensal flora may be necessary for normal intestinal function, plausibly by activating cellular survival or cytoprotective genes. Observations of flagellin-dependent NF-κB activation by commensal *E. coli* may be relevant in this model [19]. Thus, appropriate microbial recognition by PRRs may represent a balance between too little perception and response, with consequent hypersensitivity to injury, and too much, with resultant overt inflammation.

It would not be an overstatement to conclude that our own bacterial flora may affect our biology and health in ways that we have only begun to consider.

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