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The role of Innate Immunity in the Host Defense Against intestinal Bacterial Pathogens

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Abstract

Eradication of infectious disease is our global health challenge. After encountering intestinal infection with a bacterial pathogen, the host defense program is initiated by local antigen-presenting cells (APCs) that eliminate invading pathogens by phagocytosis and establish localized inflammation by secreting cytokines and chemokines. These pathogen-experienced APCs migrate to the mesenteric lymph nodes, where host immune responses are precisely orchestrated. Initiation and regulation of this defense program appear to be largely dependent on innate immunity which is antigen non-specific and provides a rapid defense against broader targets. On the other hand, many bacterial enteropathogens have evoked abilities to modify the host defense program to their advantage. Therefore, better understanding of the host-pathogen interactions is essential to establish effective eradication strategies for enteric infectious diseases. In this review, we will discuss the current understanding of innate immune regulation of the host defense mechanisms against intestinal infection by bacterial pathogens.

Keywords

innate immunity; bacterial pathogens; host defense; macrophages; enteropathogen; Gram-negative; toll-like receptors; Nod-like receptors; mucosal defense; protective immunity; antimicrobial peptide; secretory IgA; commensals

Introduction

Intestinal bacterial infection is a common health problem and could lead to a major impact on public health and the global economy [1]. Many pathogenic bacteria cause foodborne illness resulting in epidemics. Outbreaks often occur following natural disasters (e.g., recent outbreaks of *Vibrio cholera* in Haiti) making already dire situations catastrophic [2–4]. Recent advances of our knowledge have led to the idea that intestinal mucosa maintains physiological homeostasis through host-commensal interactions, and that gastrointestinal infections occur through host-pathogen interactions. However, it is largely unknown how the host-pathogen interactions lead to intestinal pathology and systemic diseases.

The gastrointestinal mucosa is under continuous exposure to a milliard of microorganisms that comprise the commensal flora. Mucosal immunity maintains tolerance to commensal flora while inducing effector immunity to pathogens via a fine interplay between innate and adaptive immune responses. In this process, innate immunity is responsible for recognition

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of microorganisms and initiation of effector and/or regulatory immune responses. In addition, innate immunity triggers mucosal restitution programs following epithelial injury or inflammation. Therefore, innate immunity is crucial for regulation of host-commensal interactions in the maintenance of intestinal homeostasis.

How the host immunity induces effector responses only to harmful pathogens amongst diverse commensal flora in the intestine is an essential question. Upon pathogenic infection, both virulence factors of the pathogen and the host defense mechanisms determine the consequence of intestinal immune responses. Many commensal bacteria that colonize the intestine have diminished virulence [5]. By contrast, most clinically significant bacterial enteropathogens have evolutionarily acquired an ability to evade host immune defenses with individually unique virulence factors [6–8]. The strategies used by these pathogens for immune evasion mainly target innate immunity, highlighting the importance of innate immunity in intestinal defense mechanisms.

On the host side, identification of the pathogens and selective induction of immune responses require utilization of innate immunity, which is initiated by pattern recognition receptors (PRRs) on APCs including macrophages, dendritic cells (DCs), and intestinal epithelial cells (IECs). Recognition of specific pathogen-associated molecular patterns (PAMPs) by PRRs initiates an innate immune response inducing the production of antimicrobial peptides, phagocytic microbial killing, and expression of cytokines, chemokines, and reactive oxygen species. These processes lead to the recruitment of acute inflammatory cells in order to establish localized inflammation. Diverse pathogen patterns are precisely recognized by toll-like receptors (TLRs) and nucleotide-binding oligomerization domain (NOD) like receptors (NLRs). Simultaneously, activated APCs initiate adaptive immunity, which has the ability to terminate the infection and inflammation.

Intestinal Innate Immune System Forms Host-Pathogen Interactions

Most enteropathogens invade our body through microfold cells (M-cells) within the follicle-associated epithelium (FAE) that covers intestinal Peyer's patches (PPs). Pathogens can easily attach to the surface of M-cells because the surface has a poorly formed glycocalyx layer. M-cells in turn actively uptake the attached pathogens by transcytosis, a process involving TLR signaling [9]. When pathogens are delivered to PPs, they are phagocytosed by APCs; i.e., macrophages and DCs. Pathogens that have an ability to evade phagocytic elimination by APCs establish an infection and colonize PPs. The initial colonization by the pathogen induces localized inflammation in PPs characterized by recruitment of neutrophils, DCs and monocytes, leading to activation of CD4+ T cells and production of secretory IgA [10,11]. The existence of an M-cell-independent route of infection has also been suggested [12]. The CX3CR1+ subset of DCs is thought to sample luminal contents through a dendrite extended between the epithelial cells, and may initiate the host immune response [13]. This transepithelial dendrite formation is known to increase with oral *Salmonella* infection, and this process also involves TLR signaling [14]. However, the roles of these DCs in host defense against in vivo pathogenic infection have been controversial [15].

Pathogens that survive primary host responses in PPs or intraepithelial DCs travel to and colonize the mesenteric lymph nodes (MLNs). In this process, pathogens (especially intracellular pathogens) may utilize phagocytes as a carrier to transport them to the MLNs. In particular, TLR5 signaling in DCs seems to play a role in the transfer of *Salmonella* to the MLNs because TLR5−/− mice show a reduced number of *Salmonella*-harbored DCs in the MLNs after mucosal infection [16]. Taking the risk of MLN colonization underlines the importance of transferring the pathogen-experienced APCs to the MLNs for the host to

establish an organized immune response against bacterial dissemination [17,18]. In fact, mice with surgically removed MLNs demonstrate increased bacterial dissemination and severe immunopathology in peripheral organs [19].

We have discussed that innate immunity is responsible for induction of localized inflammation in intestinal mucosa, PPs, and MLNs after enteric infection with bacterial pathogens. Although it may result in severe enterocolitis and lymphadenitis, regional inflammation is required for prevention of systemic dissemination of the pathogen. TLR4−/− mice are unable to recruit neutrophils in response to chemically induced mucosal injury and have enhanced bacterial translocation to the spleen [20]. Therefore, prevention of systemic dissemination of the infected pathogen is one of the important tasks of the innate immune defense in the face of bacterial infections in the intestine.

Mouse Models of Intestinal Bacterial Infection

One of the obstacles in investigating the role of intestinal defense mechanisms against enteropathogens is that experimental mice are resistant to many pathogens that cause serious diseases in humans. Nevertheless, some mouse models of intestinal bacterial infection have been established (Table 1). Individual models have unique features that are useful to investigate specific aspects of host immune responses as well as *in vivo* function of the virulence factors of the pathogens.

Salmonella enterica serovar typhimurium infection of mice is a well-established model of intestinal as well as systemic *Salmonella* infection in humans. Except for infection with *typhi*, *paratyphi*, and *sendai* serovars, natural infection with *Salmonella* manifests enterocolitis in most human cases [21]. In mice, however, *S. typhimurium* causes a systemic disease resembling human typhoid fever but neither colonizes the intestine nor manifests as enterocolitis after oral infection [22]. Interestingly, pretreatment of mice with antibiotics (e.g., streptomycin) leads to acute intestinal inflammation in response to *S. typhimurium* infection, highlighting the importance of commensals in the establishment of intestinal versus systemic pathology by this pathogen [22].

Yersinia enterocolitica orally infects mice without the elimination of commensals and the mouse model of *Y. enterocolitica* infection reproduces the manifestations of human disease [23]. Once *Y. enterocolitica* establishes an infection, it forms microcolonies, microabscesses, and granulomatous lesions in PPs and the MLNs, and may disseminate to the liver and spleen. Therefore, together with *S. typhimurium*, the mouse model of *Y. enterocolitica* infection is useful to investigate intestinal immune mechanisms and defense against systemic dissemination of invading pathogens.

Citrobacter rodentium is an enteric pathogen that causes natural infection in mice and is considered a good model for human enteropathogenic *Escherichia coli* (EPEC) and enterohomorrhagic *E. coli* (EHEC), because it carries similar virulence factors as EPEC and EHEC. In stark contrast to *S. typhimurium* and *Y. enterocolitica*, *C. rodentium* is not an invasive pathogen. *C. rodentium* colonizes the surface of the cecal lymphoid patches a few hours post oral infection and spreads toward the distal colon within a few days [24]. After attaching to colonocytes, *C. rodentium* breaks epithelial barrier integrity through tight junction disruption by its virulence factors, which manifests diarrhea with colitis and epithelial hyperplasia [25].

Helicobacter hepaticus naturally infects mice and can induce intestinal pathology and chronic active hepatitis in immune deficient mice [26]. *H. hepaticus* does not manifest enterocolitis in normal mice, but infection with this pathogen exacerbates colonic inflammation in mouse colitis models [26]. A major component of this colitis is mediated by

innate immunity, as evidenced by the severe colitis induced by oral infection of lymphopenic RAG2−/− mice with *H. hepaticus* [27]. In addition, adoptively transferred regulatory T cells inhibit induction of colitis in *H. hepaticus* infected RAG2−/− mice. Accordingly, this model is advantageous to study how bacterial signaling influences intestinal inflammation in the context of both innate and adaptive immune responses.

TLRs and NLRs in Intestinal Defense against Bacterial Pathogens

Recognition of PAMPs by specific PRRs triggers innate immunity, which induces a variety of gene expression via distinct intracellular signaling pathways. Figure 1 shows the complex interactions of intracellular signaling pathways under the activation of PRRs. TLRs are expressed on the plasma membrane or endosomes, and NLRs are expressed within the cytosol of most cell types in intestinal mucosa. Each pathogen induces a unique pattern of signaling pathways as different PRRs may simultaneously recognize more than one PAMP on each pathogen. In addition, different cell types induce different responses to the same pathogen. This combination of cellular and molecular diversity increases the host capacity to establish organized and directed immune responses to a variety of pathogens. Expression of most TLRs in IECs and resident APCs in the lamina propria seems to be down-regulated presumably to avoid excessive immune responses to the commensals [28–30]. However, the *in vivo* functional consequences of these TLRs in individual cell types in the intestine are yet to be fully determined.

Most bacterial pathogens that cause intestinal pathology in humans, such as *Yersinia*, *Salmonella*, *Vibrio* or *Shigella*, are Gram-negative species. TLR4 is highly suspected to be involved in the host defense mechanisms against these pathogens, as lipopolysaccharide (LPS) of the outer membrane of the Gram-negative bacteria is the major ligand for TLR4. In fact, TLR4 deficient mice are highly susceptible to oral and systemic infection with *S. typhimurium* as well as *Y. enterocolitica* due to impaired bacterial killing by macrophages and defective cytokine production [31–33]. Invasive enteropathogens that carry flagella can be recognized by TLR5 or NLRC4 at the host cell plasma membrane or cytosol, respectively. Phagocytes infected with *S. typhimurium in vitro* activate NLRC4, which induces cellular production of IL-1β and IL-18 via caspase-1 activation [34–36]. This process appears to be important for host defense as mice deficient in caspase-1, IL-1β, and IL-18 are individually susceptible to *S. typhimurium* and rapidly succumb to infection [37,38]. Bacterial DNA can be recognized by TLR9 within the cytoplasm. However, it is likely that these PRRs are dispensable for establishing an intestinal immune defense against *S. typhimurium in vivo*, because none of the mice deficient in TLR5, TLR9, or NLRC4 shows increased susceptibility to oral *S. typhimurium* infection [16,37,39●●]. The discrepancy between these results implies involvement of multiple cell types and upstream pathways that are responsible for caspase-1 activation and IL-1β, and IL-18 production in response to *S. typhimurium* infection. Moreover, the contribution of TLR signaling to virulence of the pathogens has been suggested [16,33,39●●].

TLR signaling can be a driving force of mucosal inflammation in the setting of enteric infection with non-invasive pathogens. For example, absence of TLR4 reduces intestinal inflammation and morbidity in *C. rodentium* infection [40]. In the *H. hepaticus* infection model, RAG2×MyD88 double knockout mice as well as RAG2−/− chimeric mice that carry MyD88-deficient bone marrow demonstrate no intestinal inflammation, while MyD88 sufficient RAG2−/− counterparts show chronic colitis [41●]. These findings suggest that MyD88-dependent TLR signaling in myeloid cells during the infection with non-invasive bacterial pathogens dominantly mediates intestinal inflammation.

Secretory IgA and Antimicrobial Peptides

Secretory IgA (sIgA) and antimicrobial peptides are crucial component of host immune defense against enteric pathogens. It has been suggested that sIgA prevents adherence and invasion by enteric pathogens [42,43]. Antimicrobial peptides are also known to inhibit colonization of microorganisms on epithelial surfaces [44]. Signaling through TLRs and NLRs appears to play a central role in regulation of sIgA induction and antimicrobial peptides and thus contributes to the maintenance of commensals as well as primary defense against intestinal pathogens [45].

PRR signaling seems to be involved in multiple steps in intestinal IgA secretion. In the intestine, follicular B cells in PPs are activated by direct contact with activated T cells. The activated B cells then undergo terminal differentiation to plasma cells by activation of the transcriptional factors Bimp-1 and IRF-4 and travel to the lamina propria to secrete IgA [46]. On the other hand, recruitment of B cells to the lamina propria requires expression of specific chemokines from IECs that may be induced by several types of PRR signaling [47]. This T cell-dependent intestinal sIgA secretion takes five to seven days. To compensate for this time lag, lamina propria B cells can rapidly undergo class switch recombination in a T cell-independent manner through induction of B cell-activating factors, APRIL (A proliferation-inducing ligand) and BAFF (B cell-activating factor of the TNF family) [48,49]. DCs and IECs have been shown to express these B cell-activating factors in response to bacterial recognition through TLRs [50,51]. This sIgA has been considered to have multiple cross-reactions and contributes to host defense against a variety of pathogens [52,53]. Constitutively-active expression of TLR4 in IECs results in increased lamina propria B cell number and sIgA secretion along with higher expression of mucosal APRIL [54]. Activation of TLR3 and TLR4 has been shown to induce expression of the polymeric Ig receptor (pIgR), an epithelial immunoglobulin transporter, by IECs that enhances luminal IgA secretion [55,56]. The pIgR−/− mice have more *S. typhimurium* colonization in PPs than wild-type (WT) mice, and succumb to infection with a lower infective dose [57]. Since the sIgA-mediated intestinal defense mechanism is associated with a reduction in local bacterial burden in the intestine, sIgA is also suggested to contribute to prevention of epidemics with enteropathogens [57].

Defensins are one of the major antimicrobial peptides in the intestine. Depending on the molecular structure, defensins are classified into two major forms i.e., α-defensins (cryptdins in mice) and β-defensins [58]. In contrast to α-defensins that are specific to intestinal Paneth cells, β-defensins are expressed by a variety of cell types including IECs. Defensins mediate non-oxidative microbial killing by inducing membrane disruption of the microorganisms. Mice deficient in the metalloprotease matrilysin (MMP7), an enzyme required for maturation of α-defensins, are highly susceptible to oral *S. typhimurium* infection [59]. Conversely, transgenic expression of human α-defensin HD5 by mice confers greater resistance to oral *S. typhimurium* infection [60]. Although some defensins are expressed constitutively, α-defensins and β-defensins may be induced by bacterial stimuli suggesting a contribution of PRR signaling. Therefore, defensins are an important component of intestinal defense against pathogenic infection which may be regulated by PRR signaling.

Conventionally raised MyD88−/− mice have been found to have an increased bacterial translocation to the MLNs due to a significant defect in production of multiple antimicrobial peptides in Paneth cells [61]. Paneth cell specific transgenic expression of MyD88 restores the bacterial burden in the MLNs to the WT levels. In addition to α-defensins, MyD88 signaling regulates the expression of another type of antimicrobial Paneth cell product, c-type lectins such as RegIIIγ and RegIIIβ [61]. TLR9−/− and NOD2−/− mice have impaired expression of Paneth cell cryptdins compared to WT mice [62,63]. Expression of functional

NOD2 in IECs inhibits invasion and growth of *S. typhimurium in vitro* [64]. NOD2−/− mice are susceptible to oral but not systemic infection with *L. monocytogenes* due to defective expression of Paneth cell cryptdin-4 [62]. These NOD2−/− mice also show increased colonization of *H. hepaticus* compared to WT mice after oral inoculation [65]. In addition, signaling through TLR2, TLR3, TLR4, TLR5, NOD1, NOD2 and NLR (NLRP3) have all been implicated with the expression of β-defensins in IECs [16,66,67]. Therefore, multiple PRR signaling may induce several sets of antimicrobial peptides that allows the host to prevent the colonization of pathogens.

Macrophage Phagocytosis: Bacterial Killing vs. Pathogen Carrier

Elimination of infectious agents by macrophage phagocytosis is an important part of host defense. There are several phagocytic receptors that are required to initiate the uptake of pathogens into endosomes. Signaling from phagocytic receptors further facilitates phagosome maturation. Emerging evidence has demonstrated a significant contribution of TLR signaling in multiple steps of phagocytosis indicating TLRs as potent phagocytic receptors [68–70]. During the maturation process, phagosomes fuse with lysosomes to degrade internal pathogens. Formation of phagolysosomes activates NADPH oxidase and inducible nitric oxide synthase that catalyzes acidification and oxidative burst resulting in bacterial killing. Digestion of pathogens produces other PAMPs in the phagosomes that further allow activation of other PRRs, which in turn induce cytokines and chemokines forming organized innate immune responses. Secreted cytokines such as TNF-α and IFN-γ help activate other phagocytic cells preparing against further invasion of the pathogens. Finally, phagocytes that digest pathogens initiate adaptive immunity to generate pathogen-specific immune defense programs.

Another aspect of phagocytosis is that macrophage phagocytosis can be used by the pathogens to disseminate to multiple organs of the host. Many pathogens have evolved a variety of strategies to survive macrophage phagocytosis, which include avoidance of phagocytosis, disruption of phagosome trafficking, promotion of cell apoptosis, dampening of inflammation, and alteration of intercellular signaling [71]. Several pathogens have shown their ability to utilize PRR signaling for their survival in the host [16,71,72]. Pathogens surviving intracellularly may be carried to the MLNs or the spleen by macrophages, which may cause a systemic infection. Therefore, limitation of pathogens by macrophage versus virulence of pathogens that utilize phagocytosis for dissemination may determine the outcome of enteric infections.

Natural Killer Cells and Innate Lymphoid Cells (ILCs) in Intestinal Defense Mechanism

Natural Killer (NK) cells are one of the major innate immune cells that are indispensable for early host defense against pathogenic infection. Their defense mechanisms are comprised of the strong cytotoxicity and cytokine secretion, particularly IFN-γ [73]. Absence of NK cells increases colonization of *S. typhimurium* in the intestine and enhances dissemination to the peripheral organs after oral infection [74]. Interestingly, depletion of NK cells results in reduced susceptibility of intestinal inflammation during oral *S. typhimurium* infection in streptomycin pre-treated mice due to reduced IFN-γ expression in the intestine [75]. These results indicate that NK cells are important not only to block pathogen invasion but also to strongly induce inflammatory responses in the intestinal interface.

ILCs are a recently identified NK cell-related cell type that is composed of phenotypically heterogenous populations. Emerging evidence has highlighted the importance of ILCs in immuno-surveillance of intestinal mucosa [76,77]. The precise mechanism of ILCs-

mediated intestinal defense against bacterial pathogens is still largely unknown, but the effector component appears to be associated with a rapid secretion of IL-17, IFN-γ, and IL-22. The cytokine phenotypes of ILCs are mainly regulated by the expression of a transcriptional factor, retinoic acid receptor-related orphan receptorγt (RORγt) [78]. IL-17 is known to contribute to host defense against enteropathogens through induction of neutrophil recruitment and antimicrobial peptides such as β-defensins in the intestine [79,80]. On the other hand, IL-22 deficient mice are highly susceptible to intestinal *C. rodentium* infection [81]. IL-22-mediated host defense seems to be associated with induction of RegIIIβ and RegIIIγ [77]. However, the role of IL-22 in intestinal defense may be pathogen specific as IL-22 is dispensable for the clearance of oral *L. monocytogenes* infection [82].

Conclusions

The recent globalization of the food supply increases the chance for wide-spread exposure to food-borne pathogens and the risk of outbreaks. Although antibiotic therapy may be effective in treating enteric pathogens, the rapid onset of host systemic inflammatory responses, the acquisition of antimicrobial resistance, and potential induction of chronic inflammatory disorders mediated by enteric pathogens have become serious concerns. In contrast to our evolutionally conserved PRRs, the diverse virulence profiles of enteric pathogens threaten to overcome host defense mechanisms. Recent studies continue to elucidate the mechanism by which host innate immunity interacts with pathogens during intestinal bacterial infection. Innate immunity is a strong host defense program yet opportunistic modification of this mechanism by pathogen seems to be associated with intestinal pathology as well as progression to systemic disease. Targeting of this mechanism will help develop effective strategies to protect host against multiple types of pathogens that infect through intestinal mucosa.

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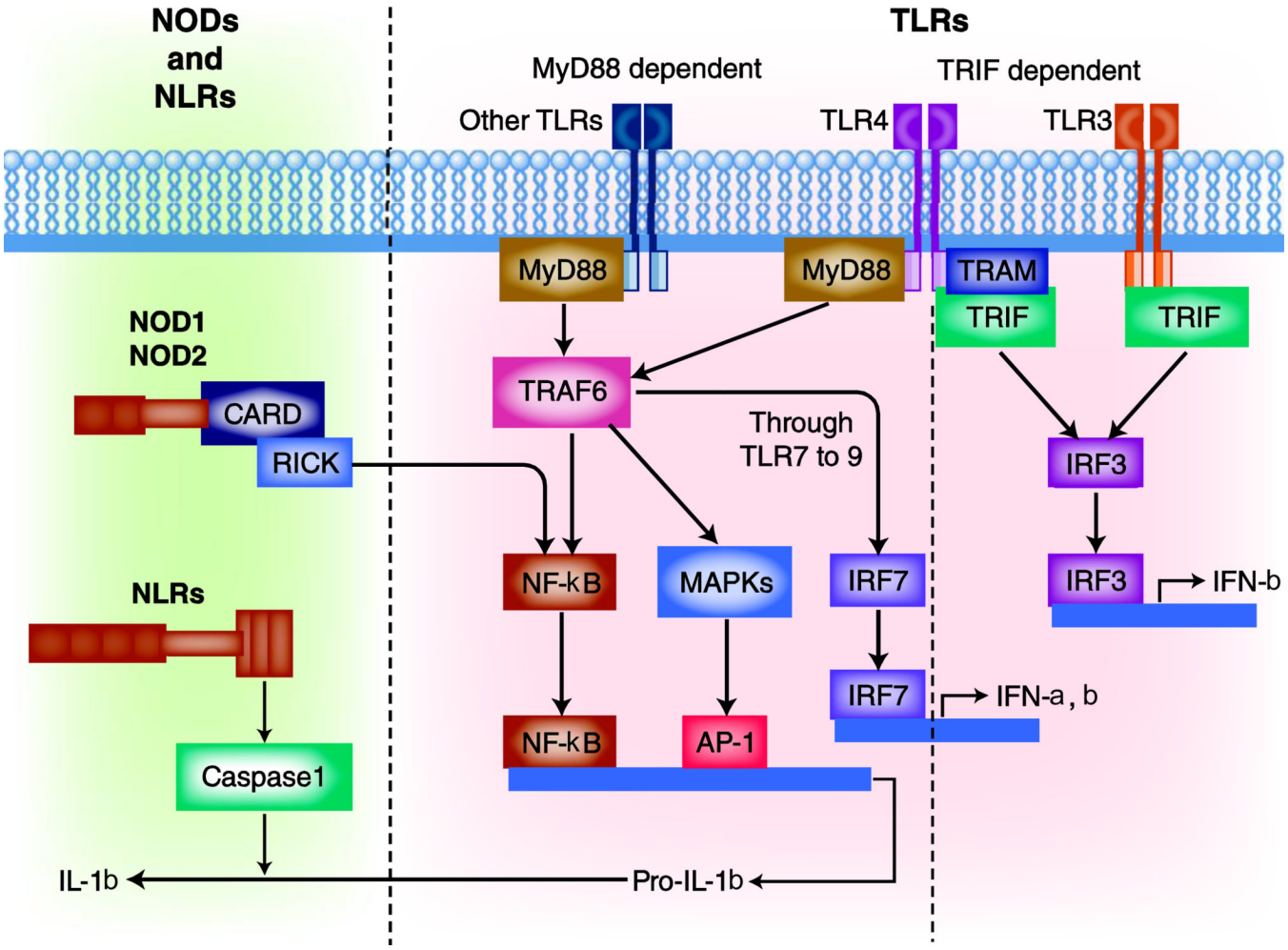

Figure 1. Signal transduction pathways of TLR and NLR

Pathogens are recognized by the host APCs through TLRs, which induce intracellular signaling mainly through two adapter molecules, myeloid differentiation factor 88 (MyD88) or Toll/interleukin-1 receptor domain-containing adapter inducing IFN-β (TRIF). While MyD88 is used by most TLRs, the TRIF pathway can be exclusively induced by TLR4 and TLR3. The MyD88 pathway strongly induces NF-κB activation and pro-inflammatory cytokine secretion associated with pathogen clearance [83]. The TRIF pathway, on the other hand, induces type I IFNs and slower NF-κB activation [84,85]. On the other hand, pathogens can be recognized by NLRs in the cytosol, which assemble a protein complex named inflammasome to activate caspase-1. Activated caspase-1 induces cellular apoptosis and promotes secretion of IL-1β and IL-18.

Table 1

Well-established mouse models of intestinal bacterial infection.

Pathogens	Type	Gram staining	Pathology
S. typhimurium	Invasive	Negative	Oral infection leads to systemic dissemination of *S. typhimurium* demonstrating typhoid-like disease. Infection with *S. typhimurium* after pretreatment of mice with streptomycin (elimination of commensals) demonstrates intestinal pathology (enterocolitis).
Y. enterocolitica	Invasive	Negative	Oral infection demonstrates intestinal inflammation, microabscesses in the PPs and MLNs. Infection eventually disseminates to the spleen and the liver.
C. rodentium	Non-invasive	Negative	A model pathogen of EPEC and EHEC in human disease. Oral infection leads to colonization of the large intestine. Induces diarrhea disease with colitis.
H. hepatices	Non-invasive	Negative	Natural infection leads to life-long colonization of this pathogen in mice. Inoculation of normal mice with this pathogen does not demonstrate pathology, but influences the course of colitis models. Infection to lymphopenic mice demonstrates severe colitis.