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Detection of cytosolic bacteria by inflammatory caspases

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The sanctity of the cytosolic compartment is rigorously maintained by a number of innate immune mechanisms. Inflammasomes detect signatures of microbial infection and trigger caspase-1 or caspase-11 activation, culminating in cytokine secretion and obliteration of the replicative niche via pyroptosis. Recent studies have examined inflammatory caspase responses to cytosolic bacteria, including *Burkholderia*, *Shigella*, *Listeria*, *Francisella*, and *Mycobacterium* species. For example, caspase-11 responds to LPS introduced into the cytosol after Gram-negative bacteria escape the vacuole. Not surprisingly, bacteria antagonize these responses; for example, *Shigella* delivers OspC3 to inhibit caspase-4, a potential human homolog of murine caspase-11. These findings underscore bacterial coevolution with the innate immune system, which has resulted in few, but highly specialized cytosolic pathogens.

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Introduction

The immune defenses of the extracellular environment are severe, as are those of the phagolysosome. The prospect of refuge from these insults therefore makes the cytosolic compartment a theoretically attractive refuge for potential bacterial pathogens. However, the fact that bona fide cytosolic bacteria can be counted on one's fingers (see Table 1 for a summary of these pathogens, their cell tropisms, and their mechanisms for invading the cytosol) highlights the success of immune defenses employed to maintain the sterility of the cytosolic niche. A number of cytosolic sensors detect signatures of infection, initiating potent inflammatory responses and/or host cell death. The importance of inflammatory caspases in this regard is underscored by the extreme susceptibility of mice deficient in these enzymes to infection by cytosolic pathogens. Interestingly, the few cytosolic specialist bacteria are among the most virulent pathogens known. Herein, we discuss the role of inflammatory caspases in the innate immune response to cytosolic bacteria, focusing on recent advances in our understanding of how cells detect intruders and trigger caspase activation, and how caspases mediate containment of an infection.

The inflammatory caspases

Caspases are ancient and evolutionarily conserved proteases that are integral to development, homeostasis, and immunity. Some caspases are involved in apoptosis, an immunologically silent form of programmed cell death. In contrast, the inflammatory caspases, caspase-11 (or the presumed human homologs caspase-4 and caspase-5) and caspase-1, initiate a form of lytic cell death termed pyroptosis following their activation, which releases inflammatory mediators, removes the replicative niche of cytosolic bacteria, and exposes intruders to extracellular defenses and neutrophils [1] (reviewed in [2]). In addition, caspase-1 mediates the maturation and secretion of pro-IL-1β and pro-IL-18, two pleiotropic inflammatory cytokines best known for inducing fever and interferon (IFN)-γ secretion, respectively [3].

The inflammasomes

The inflammatory caspases are expressed as inactive zymogens. The canonical inflammasomes, a class of cytosolic pattern recognition receptors (PRR), activate caspase-1 in response to specific signatures of infection. A theorized non-canonical inflammasome(s) is proposed to activate caspase-11 [4••]. Relevant inflammasomes and their agonists are detailed in Table 2; for in-depth review, see [2,3].

Burkholderia

Burkholderia pseudomallei and *Burkholderia thailandensis* have served as models for studying the interaction of inflammatory caspases and cytosolic bacteria. These Gram-negative bacteria exist ubiquitously in the soil of southeast Asia and sporadically elsewhere [5]. Although closely related, only *B. pseudomallei* causes severe human and murine disease; however, *B. thailandensis* can infect macrophages and epithelial cells both *in vitro* and *in vivo*. *B. pseudomallei* and *B. thailandensis* rapidly escape the vacuole via their type III secretion system (T3SS) [6,7]. NLRC4 is positioned to detect signatures of T3SS activity, alerting the immune system to pathogens that reprogram and parasitize host cells. Not surprisingly, we and others found that macrophage infection triggers NLRC4 activation [8•,9••]. Mediating this activation, we showed that the T3SS rod protein BsaK is detected through NLRC4 [10], and Zhao and colleagues demonstrated that NAIP2

Table 1

Cell tropism and vacuolar escape determinants of cytosolic bacteria

Genus	Gram +/−	Cell tropism	Vacuolar escape determinants, bacterial
Burkholderia	−	Mφ, PMN, epithelial cells	$T3SS_{BSA}$
Shigella	−	Mφ, DC, intestinal epithelial cells	Mxi-Spa T3SS, IpaB
Francisella	−	Mφ, PMN, DC, epithelial cells, hepatocytes	IglC, MglA, FTT11103
Listeria	+	Mφ, intestinal epithelial	LLO, phospholipase C
Rickettsia	−	Vascular endothelial, Mφ	Phospholipases, hemolysin
Mycobacterium	Acid-fast +	Mφ	ESX-1 T7SS, ESAT-6

Table 2

Interaction of inflammatory caspases and cytosolic bacteria

Bacteria	Caspase-1			Caspase-11 or Caspase-4
	NLRC4	NLRP3	AIM2	
Stimulus/sensor				
Burkholderia	BsaK/NAIP2	Infection		LPS/casp11
Shigella	Needle/NAIP1 and human NAIP Rod/NAIP2	Infection		LPS/casp11 ?/casp4
Francisella		Infection (human)	DNA	
Listeria	Flagellin/NAIP5	Infection, LLO	DNA	
Rickettsia				
Mycobacterium		Infection, ESAT-6	DNA	
Antagonism				
Burkholderia				
Shigella				OspC3 inhibits casp4
Francisella			Possible	Tetra-acyl LPS
Listeria	Represses flagellin at host temperatures	pH dependent LLO activity		
Rickettsia				
Mycobacterium		Zmp1 metalloprotease		

is the sensor upstream of NLRC4 [11•]. Later the T3SS needle protein BsaL, as well as needle proteins from a variety of other bacteria, was found to be detected by murine NAIP1 and human NAIP, both signaling through NLRC4 downstream [11•,12,13]. By an ill-defined mechanism, *Burkholderia* species also activate NLRP3 [8•,9••]. Together, NLRC4 and NLRP3 are critical for mice to resist intranasal *B. pseudomallei* challenge [8•]. In this model, IL-18 is central to this resistance, coordinating bacterial clearance, whereas IL-1β secretion mediates immune pathology driven by neutrophil recruitment.

Recently, we determined that caspase-11 is critical for mice to resist infection by both virulent *B. pseudomallei* as well as avirulent *B. thailandensis* [9••]. Caspase-11 functions independently of all known inflammasomes, instead working in parallel with caspase-1 to mediate protection against ubiquitous environmental bacteria. We discovered that caspase-11 responds specifically to Gram-negative cytosolic bacteria, where normally vacuolar bacteria such as *Legionella pneumophila* and *Salmonella enterica* serovar *typhimurium* (*S. typhimurium*) rapidly induce caspase-11 dependent pyroptosis only after aberrant translocation to the cytosol. In complementary studies, we and Kayagaki and colleagues determined that cytoplasmic translocation of penta-acylated and hexa-acylated LPS, but not tetra-acylated LPS, triggers caspase-11 activation [14••,15••]. Although enhanced by TLR4 signaling, this pathway can proceed independently of extracellular LPS signaling. Thus, $Tlr4^{-/-}$ mice primed with a TLR3 agonist succumb to secondary LPS challenge in a model of endotoxic shock. Previous studies indicate that during prolonged infections, caspase-11 activates in response to all Gram-negative bacteria [4••,16,17,18]. We speculate that such activation may reflect vacuole leakage events that accumulate over 16 h, which may have relevance in the setting of Gram-negative septic shock. In contrast, caspase-11 rapidly responds to *L. pneumophila* infection in pre-activated macrophages [19,20]; whether vacuolar integrity is compromised under these conditions remains to be examined. Taken together, the recent literature suggest that the physiologic role of caspase-11 during infection is to combat cytosolic bacteria. The upstream sensor that detects cytosolic LPS remains unknown.

Shigella

Members of the Gram-negative *Shigella* genus are exquisitely adapted to cause human gastrointestinal disease. *Shigella flexneri* infects a variety of cell types, such as intestinal epithelial cells and macrophages. Following