



Innate immune recognition and inflammasome activation in *Listeria monocytogenes* infection

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Listeria monocytogenes is an intracellular, Gram-positive bacterium that can cause life-threatening illness especially in immunocompromised individuals and newborns. The pathogen propagates within the cytosol of various host cells after escaping from the phagosomal compartment depending on the cytolysin listeriolysin O. While *L. monocytogenes* can manipulate the endocytic and many host-cell signaling cascades to its advantage, host cells are however capable of detecting *Listeria* infection at different cellular compartments by expressing innate immune receptors that trigger antibacterial defense pathways. These receptors include the Toll-like receptors, NOD-like receptors (NLRs), and cytosolic DNA sensors. Some NLRs as well as the DNA sensor AIM2 form multiprotein complexes called inflammasomes. Inflammasomes regulate caspase-1-dependent production of the key inflammatory cytokines IL-1 β and IL-18 as well as pyroptotic cell death in *L. monocytogenes*-infected cells. This review describes the current knowledge about innate immune sensing and inflammasome activation in *Listeria* infection.

Keywords: *Listeria*, innate immunity, NOD-like receptor, inflammasome, AIM2

LISTERIA MONOCYTOGENES – INFECTION AND INTRACELLULAR LIFE-STYLE

Listeria monocytogenes is a flagellated Gram-positive bacterium that can cause life-threatening illness characterized by gastroenteritis, meningitis, encephalitis, materno-fetal, and perinatal infections. Infection with *L. monocytogenes* occurs through ingestion of contaminated food, such as unpasteurized dairy products and undercooked meats (Allerberger and Wagner, 2010). *Listeria* cross the intestinal barrier by invading intestinal epithelial cells, reaching the liver as well as spleen via the lymphoid system and the blood, where they are internalized by splenic and hepatic macrophages. During severe infections, the bacteria disseminate via the blood and cross the blood–brain barrier resulting in infections of the meninges and the brain. Furthermore it can cross the fetoplacental barrier in pregnant women which leads to infection of the fetus. *L. monocytogenes* is able to invade different non-phagocytic cells and is resistant to intracellular killing by macrophages after phagocytosis (Hamon et al., 2006; Barbuddhe and Chakraborty, 2009). In the intestinal tract, *L. monocytogenes* invades epithelial cells via interaction of its virulence protein internalin A (InlA) with epithelial cadherin (E-cadherin), leading to bacterial internalization within a membrane-bound vacuole (Mengaud et al., 1996; Schubert et al., 2002). In contrast, internalin B (InlB) binds to c-Met, a receptor tyrosine kinase and the natural receptor for hepatocyte growth factor (HGF) and thus promotes invasion of multiple mammalian cell types (Shen et al., 2000; Veiga and Cossart, 2005). InlB has been implicated in murine liver colonization after intravenous infection (Dramsi et al., 1995; Shen et al., 2000). In the vacuole, a decreased pH activates the pore-forming toxin listeriolysin O (LLO) that destroys the phagosomal membrane and the bacterium subsequently escapes into the cytosol (Bielecki et al., 1990). This allows the bacterium to replicate in the cytosol to high numbers. Cytosolic *Listeria* express ActA to

induce host-cell actin polymerization and direct spread from cell to cell, thereby escaping immune detection and defense at the extracellular compartment (Tilney and Portnoy, 1989; Domann et al., 1992; Kocks et al., 1992). In addition to the cytosolic replication, a vacuolar growth of some bacteria might play a role for persistent infection in mice (Birmingham et al., 2008). Overall, *L. monocytogenes* manipulates the endocytic and many host-cell signaling cascades in order to replicate. On the other hand, host cells possess surveillance systems at different cellular compartments capable of detecting *Listeria* infection and activating defense pathways which in most cases might control infection.

SENSING OF *L. MONOCYTOGENES* BY PATTERN RECOGNITION RECEPTORS OF THE INNATE IMMUNE SYSTEM

Upon infection with *L. monocytogenes*, innate immune responses are rapidly triggered and are essential for host survival (Pfeffer et al., 1993; Krull et al., 1997; Unanue, 1997). However, the activation of innate immunity by pattern recognition receptors (PRRs) in response to infection with *L. monocytogenes* is still not completely understood. In general, the membrane-bound Toll-like receptors (TLRs), as well as the cytosolic nuclear oligomerization domain (NOD)-like receptors (NLRs) and DNA sensors are critical for innate defense by recognizing conserved structures of microorganisms (Corr and O'Neill, 2009; Opitz et al., 2009; Takeuchi and Akira, 2010). Some of these PRRs activate signaling pathways leading to activation of transcription factors such as NF- κ B and/or IFN regulatory factor 3 (IRF3) which direct upregulation of proinflammatory genes such as TNF α , IL-8 and pro-IL-1 β , or type I IFNs, respectively. Other PRRs form protein complexes called inflammasomes that regulate production of IL-1 β and IL-18 at a post-translational level and trigger the caspase-1-dependent inflammatory cell death (pyroptosis; Bergsbaken et al., 2009; Schroder and Tschopp, 2010).

Lipoproteins of *L. monocytogenes* are recognized by TLR2 at the cell surface (Machata et al., 2008). Some studies showed that mice deficient in TLR2 were more susceptible to *L. monocytogenes* infection with increased bacterial loads and reduced activation of macrophages, compared to wild-type mice (Torres et al., 2004). Other studies, however, did not reveal differences in susceptibility of TLR2-knockout and wild-type mice to wild-type *Listeria* (Edelson and Unanue, 2002; Gekara et al., 2009). The adapter molecule MyD88, that signals downstream of most TLRs and of the IL-1 as well as IL-18 receptors, has been shown to be essential for innate immunity to *L. monocytogenes* (Seki et al., 2002). Mice deficient in MyD88 displayed a higher susceptibility to *L. monocytogenes* infection than mice lacking either IFN- γ or both IL-12 and IL-18 (Edelson and Unanue, 2002; Seki et al., 2002). Furthermore, *L. monocytogenes* flagellin activates TLR5. However, since some *L. monocytogenes* strains do not express flagellin at 37°C, and bacteria mutants deficient in flagellin show an unaltered virulence, the role of TLR5 in recognition of *Listeria* remains unclear (Hayashi et al., 2001; Way et al., 2004).

In the cytosol, peptidoglycan fragments of *L. monocytogenes* are sensed by NOD1 and NOD2 leading to expression of proinflammatory genes and antimicrobial peptides (Kobayashi et al., 2005; Opitz et al., 2006; Park et al., 2007; Mosa et al., 2009). This NOD1/2-stimulated gene expression is dependent on the receptor interacting protein-2 (Rip-2) as well as NF- κ B and p38 mitogen-activated protein kinase (Chin et al., 2002; Kobayashi et al., 2002). Accordingly, mice deficient in NOD1 or Rip-2 show increased susceptibility toward *Listeria* infection (Chin et al., 2002; Mosa et al., 2009).

Another surveillance mechanism that detects intracellular *L. monocytogenes* is mediated by a yet-to-be-identified cytosolic PRR that triggers a type I IFN response (O'Riordan et al., 2002; Stockinger et al., 2002; McCaffrey et al., 2004). This PRR possibly detects *Listeria* DNA, although known DNA sensors such as AIM2 or DAI/ZBP1 are most likely not involved (Auerbuch et al., 2004; Carrero et al., 2004; O'Connell et al., 2004; Stetson and Medzhitov, 2006; Leber et al., 2008; Lippmann et al., 2008; Rathinam et al., 2010). The *Listeria*-mediated type I IFN production occurs through a pathway dependent on the adapter molecule STING, the serine threonine kinase TBK1 and the transcription factor IRF3 (Stockinger et al., 2004; O'Connell et al., 2005; Ishikawa et al., 2009). Of note, this pathway appears to be detrimental for *L. monocytogenes* infections *in vivo* since mice deficient in IRF3 or the type I IFN receptor are more resistant than wild-type mice toward the bacterial infection (Auerbuch et al., 2004; Carrero et al., 2004; O'Connell et al., 2004).

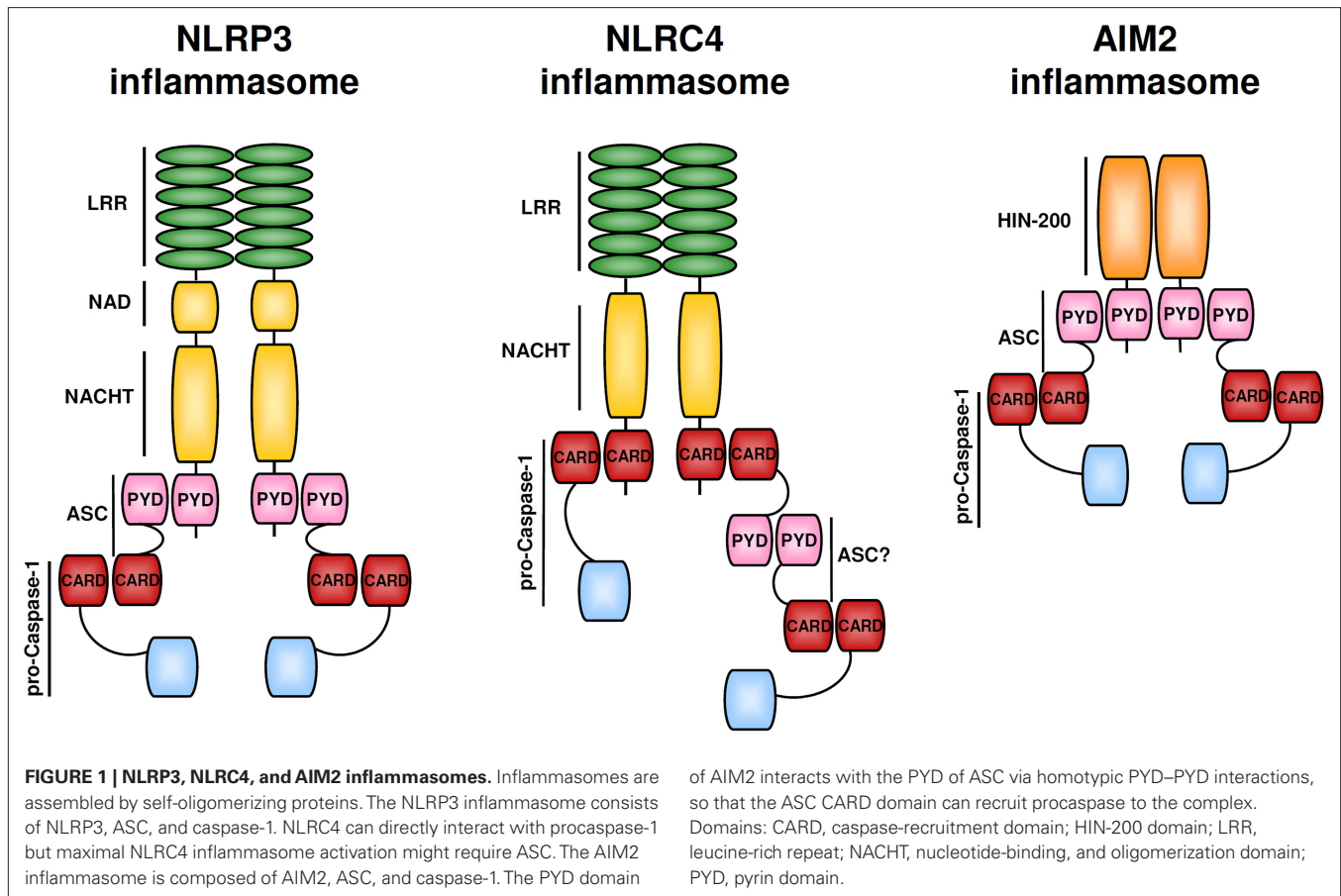
In addition to the above mentioned PRRs that mainly control immune responses to *Listeria* infection via transcriptional upregulation of inflammatory genes, some NLRs as well as other cytosolic receptors regulate production of the key proinflammatory cytokines IL-1 β and IL-18 at a post-translational level and stimulate pyroptosis in *L. monocytogenes*-infected cells (see below).

ROLE OF INFLAMMASOME PATHWAYS IN *L. MONOCYTOGENES* INFECTION

The NLR protein family consists of over 20 members in mammals. They are all composed of a C-terminal leucine-rich repeat domain, a central nucleotide-binding domain, and of an N-terminal effec-

tor domain (Franchi et al., 2009). This effector domain is either a caspase-recruitment domain (CARD) or pyrin domain (PYD; **Figure 1**). Some NLR members that contain a PYD (this NLR subgroup is called NLRP), and the NLR member NLRC4 which contains a CARD are able to form inflammasomes (Schroder and Tschopp, 2010). These protein complexes also include the CARD-bearing caspase-1 and, in most cases, the CARD- and PYD-containing adapter molecule ASC. Homophilic interactions between respective domains in the NLRs, ASC, and caspase-1 are necessary for inflammasome activation. The NLRP protein NLRP3 plays a central role in caspase-1 activation in response to a variety of exogenous and endogenous stimuli, such as bacterial pore-forming toxins, ATP, uric acid crystals, cholesterol crystals, and alum (Martinon et al., 2002, 2006; Kanneganti et al., 2006; Mariathasan et al., 2006; Duewell et al., 2010). While it appears unlikely that these NLRP3 activators directly bind to NLRP3, the exact signal(s) that stimulates NLRP3 itself is still a matter of debate. Some studies suggest that lysosomal damage and the accompanying release of lysosomal cathepsins lead to the activation of the NLRP3 pathway (Halle et al., 2008; Hornung et al., 2008). Another model proposes that the generation of reactive oxygen species by mitochondria is an event upstream of NLRP3 activation (Dostert et al., 2008; Zhou et al., 2010). Future studies are required to clarify the underlying mechanism of NLRP3 activation (see also Stutz et al., 2009; Schroder and Tschopp, 2010). The cytosolic NLRC4 detects bacterial flagellin and the presence of type III secretion systems (Franchi et al., 2006; Miao et al., 2006, 2010). Upon activation, NLRs oligomerize and recruit procaspase-1 via the CARD domain, directly or indirectly via the adaptor protein ASC (Mariathasan et al., 2004). The assembled inflammasome then mediates caspase-1 activation. Activated caspase-1 post-translationally processes pro-IL-1 β as well as pro-IL-18 to their mature forms and stimulates pyroptosis (Martinon et al., 2002; Bergsbaken et al., 2009). Recently, the HIN-200 family member AIM2 has been identified as a cytosolic double-stranded DNA (dsDNA) sensor that induces caspase-1-dependent IL-1 β maturation and thus is the first non-NLR family member forming an inflammasome (Burckstummer et al., 2009; Fernandes-Alnemri et al., 2009; Hornung et al., 2009; Roberts et al., 2009). In contrast to the NLRs, oligomerization of the AIM2 complex presumably is mediated by clustering upon direct binding to the ligand dsDNA, to which AIM2 binds via its C-terminal HIN-domain (Burckstummer et al., 2009; Fernandes-Alnemri et al., 2009; Hornung et al., 2009). The AIM inflammasome is composed of AIM2, ASC, and caspase-1.

In addition to the above-mentioned stimuli, *L. monocytogenes* infection also leads to a strong activation of caspase-1, production of IL-1 β as well as IL-18, and to caspase-1-dependent cell death (Tsuiji et al., 2004; Ozoren et al., 2006; Cervantes et al., 2008). *Listeria* eradication in the early phase of infection was impaired in caspase-1-deficient mice. These mutant mice showed a prominent decrease in production of IL-18, and as a consequence, of IFN γ (Tsuiji et al., 2004). The first inflammasome identified to be activated by *L. monocytogenes* was the NLRP3 inflammasome (Mariathasan et al., 2006). In mouse macrophages infected with *L. monocytogenes*, NLRP3 as well as the adapter ASC were essential for caspase-1 activation and secretion of IL-1 β and IL-18 (Mariathasan et al., 2006; Ozoren et al., 2006; **Figure 2**). Furthermore, mouse macrophages

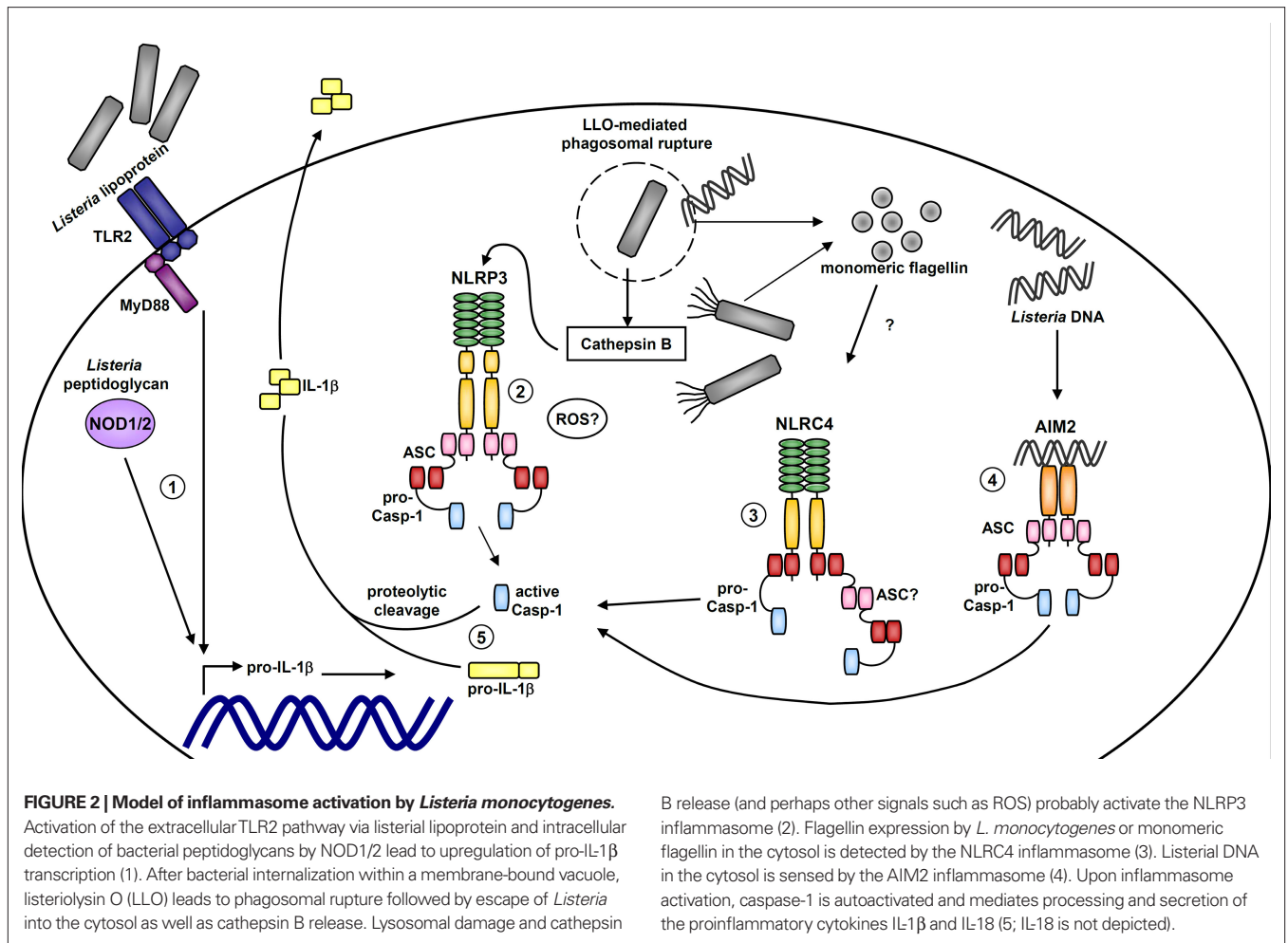


infected with *L. monocytogenes* deficient for the toxin listeriolysin O (LLO) did not secrete IL-1 β and IL-18 (Mariathasan et al., 2006; Ozoren et al., 2006; Hara et al., 2008). Similarly, NLRP3, ASC, and LLO were required for IL-1 β production in human peripheral blood mononuclear cells (PBMCs; Meixenberger et al., 2010). The LLO-mediated phagosomal rupture and release of cathepsin B into the cytosol might be involved in NLRP3 activation in human and murine cells (Meixenberger et al., 2010). However, several papers showed that *L. monocytogenes* activates caspase-1 through additional inflammasomes besides the NLRP3 complex.

NLRC4 has been shown to be required for caspase-1 activation in infections with different bacteria expressing flagellin (Franchi et al., 2006; Miao et al., 2006), and some studies showed a partial impairment of caspase-1 activation and IL-1 β production in NLRC4-deficient cells infected with *L. monocytogenes* (Warren et al., 2008; Wu et al., 2010). Other papers, however, found no evidence for a critical role of NLRC4 in *Listeria*-mediated caspase-1 activation (Kim et al., 2010; Meixenberger et al., 2010). It is known that the expression of flagellin is strictly inhibited at 37°C in some *L. monocytogenes* strains (Grundling et al., 2004; Way et al., 2004) and these differences in flagellin expression among bacterial strains are most likely responsible for the different results regarding NLRC4 involvement in *L. monocytogenes* infection.

In addition, several recent studies argue for a critical role of AIM2 in the recognition of *L. monocytogenes* in mouse macrophages via sensing *Listeria* DNA in the cytosol (Kim et al., 2010; Rathinam

et al., 2010; Tsuchiya et al., 2010; Warren et al., 2010; Wu et al., 2010). Knockdown of AIM2 in wild-type macrophages resulted in a reduced *L. monocytogenes*-stimulated caspase-1 activation, IL-1 β secretion, and cell death (Kim et al., 2010). Caspase-1 activation was completely inhibited in NLRP3-deficient macrophages treated with AIM2 siRNA. Accordingly, macrophages lacking AIM2 showed a lower but not abrogated caspase-1 activation and IL-1 β production after *L. monocytogenes* infection (Rathinam et al., 2010). It was indicated that lysis of some *Listeria* in the host-cell cytosol – maybe as a result of vacuolar acidification and lysosomal fusion before vacuolar escape or yet undefined antimicrobial mechanisms – leads to release of bacterial DNA and activation of the AIM2 inflammasome (Sauer et al., 2010; Warren et al., 2010). *Listeria* DNA colocalized with AIM2 and ASC specks in the host cytosol (Warren et al., 2010; Wu et al., 2010). The AIM2 inflammasome together with other inflammasomes trigger IL-1 β production as well as a weak pyroptotic cell death. *L. monocytogenes* mutants showing a higher amount of autolysis or *Listeria* treated with bactericidal antibiotics triggered a stronger AIM2-dependent cell death and IL-1 β production (Sauer et al., 2010). Together, these data show that AIM2 is activated by *Listeria* DNA which triggers caspase-1 activation, cell death, and secretion of IL-1 family cytokines. Considering that AIM2 is an IFN-stimulated gene, the findings of AIM2 involvement in *Listeria*-mediated inflammasome activation fit well to the previous observation that type I IFN signaling was required for strong *L. monocytogenes*-stimulated IL-1 β and IL-18 secretion (Henry et al., 2007).



Thus, multiple inflammasomes are involved in sensing *L. monocytogenes* infection. Warren et al. (2010) recently showed that NLRP3 most likely is temporally activated first, probably detecting vacuolar rupture. NLRC4 and AIM2 get activated at a later time point as flagellin monomers and bacterial DNA are released into the cytosol. Some studies, however, differ to some extent in conclusions regarding functional importance of the three different inflammasomes in *L. monocytogenes* infection (Franchi et al., 2007; Warren et al., 2008, 2010; Kim et al., 2010; Meixenberger et al., 2010; Rathinam et al., 2010; Sauer et al., 2010; Wu et al., 2010). This is likely attributed to the use of different *Listeria* strains that might vary in the expression and extent of release of the agonists of NLRP3, NLRC4, and AIM2. Moreover, some studies used LPS-primed macrophages in their experiments to induce strong pro-IL-1 β and NLRP3 expression, whereas others performed infections in unprimed cells.

Inflammasomes can be seen as major sentinels of the innate immune defense against *L. monocytogenes*. They contribute to pathogen sensing and control post-translational processing of the inflammatory cytokines IL-1 β and IL-18. IL-1 β and IL-18 activate via the IL-1 and IL-18 receptors a MyD88-dependent signaling and subsequent NF- κ B- and MAPK-regulated gene expression. Indeed,

signals stimulated by LLO leading to IL-1 β /IL-18 production, activation of the IL-1/IL-18 receptors and of MyD88-dependent signaling have been shown to compensate for lack of TLR2 in *Listeria* infection in mice (Gekara et al., 2009). IL-18 is crucial for IFN- γ induction, which is essential for the innate intracellular defense against *L. monocytogenes* as well as for the T cell-mediated acquired immunity (Tsuji et al., 2004). The significance of IL-1 in *Listeria* infections has been demonstrated by several groups. It has been shown that the blockade of IL-1 receptor exacerbates the disease and mice lacking caspase-1 or the IL-1 β receptor are significantly more susceptible to infections with *L. monocytogenes* (Havell et al., 1992; Labow et al., 1997; Tsuji et al., 2004). Moreover, IL-1 receptor antagonist (IL-1Ra)-deficient mice and IL-1Ra-overproducing mice are less or more susceptible, respectively, to listeriosis (Hirsch et al., 1996).

CONCLUSION

The interaction of *L. monocytogenes* with host cells is complex and involves entry of the bacterium to different cellular compartments as well as a multilayered host-cell surveillance system capable of detecting infection. NLR and AIM2 inflammasomes are key players of this innate immune surveillance system. They

are directly and indirectly activated by various PAMPs and virulence factors expressed by *L. monocytogenes* and mediate production of key inflammatory cytokines that alert the immune system, as well as host-cell death which helps to constrain bacterial infection.

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