frontiers
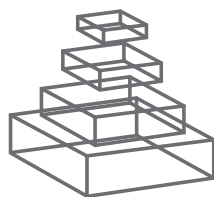## RESEARCH TOPICS

THE INFLAMMASOME AND ITS
ROLE IN INFECTIONS

Hosted by
Amal O. Amer

frontiers in
MICROBIOLOGY

frontiers

ABOUT FRONTIERS

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

FRONTIERS JOURNAL SERIES

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing.

All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

DEDICATION TO QUALITY

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.

Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view.

By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

WHAT ARE FRONTIERS RESEARCH TOPICS?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area!

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: researchtopics@frontiersin.org

THE INFLAMMASOME AND ITS ROLE IN INFECTIONS

Hosted By

Amal O. Amer, Division of Pulmonary, Allergy, Critical Care and Sleep Medicine, Center for Microbial Interface Biology, Department of Internal Medicine, Biological Research Tower, 460W 12th Avenue, Room 1014, Columbus, OH 43210, USA.
e-mail: amal.amer@osumc.edu
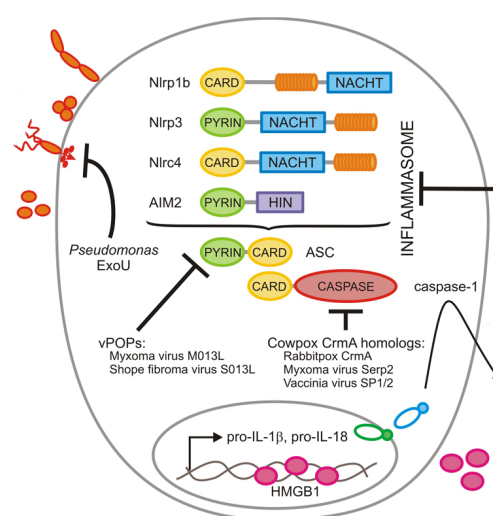
Host cells express a range of receptors that act as microbial sensors. These receptors sense microorganisms and transduce signals that activate immune responses. Host cells use several strategies to recognize specific pathogen-associated molecular patterns (PAMPs). Bacterial components, such as peptidoglycan, bacterial flagellin, and nucleic acid structures, are examples of PAMPs. One family of cell surface pattern recognition receptors (PRRs), named as Toll-like receptors TLRs, recognize PAMPs through an extracellular domain and initiate inflammatory signaling pathways through an intracellular domain. Sensing of the presence of microbes or their factors in the cytosol is mediated by NOD-like receptors (NLRs) and leads to signaling cascades that mediate the production of inflammatory cytokines, recruitment of phagocytic cells, and control of the acquired immune response. Most NLRs assemble in large multiprotein complex called the inflammasome leading to the activation of caspase-1. Caspase-1 is an inflammatory caspase that is required for the activation of cytokines such as IL-1β, IL-18 and IL-33. Caspase-1 can also induce a non-apoptotic form of cell death named pyroptosis. Dysfunction of the inflammasome can lead to exacerbated inflammation in several human conditions. On the other hand, the inflammasome response maybe absent in certain conditions, hence allowing some intracellular infections to pass unnoticed. In this issue, we will address several aspects of inflammasome activation and its role in health and disease conditions.

Table of Contents

The inflammasome

Amal O. Amer*

Internal Medicine, Ohio State University, Columbus, OH, USA
*Correspondence: amal.amer@osumc.edu

There is a clear need for interdisciplinary research and publications that bring together scientists who work on the inflammasome. This protein complex, termed the inflammasome and many of its components are implicated in disease disorders, autoimmune, and infectious diseases. The structure, activation, and regulation of the inflammasome complex have been and are still studied in increasing number of laboratories around the world. Our goal is to provide an issue summarizing every fascinating aspect of inflammasome activation and modulation of the innate immune response to microbial and to danger signals. This issue will bring the experts in inflammasome research up to speed with the most recent findings. However, several reviews are geared toward introducing the new scientists to the inflammasome complex and to the fundamental and essential information that will help them understand and even pursue their studies in this direction. By looking at the two sides of the coin, notably, some authors focused on the inflammasome as a major participant in innate immunity and tackled the infectious agents as modulators. Other authors considered the organism as the major player in the infected cell, while considering the inflammasome a contributor to immune response and to the fate of the pathogen.

The basics of the inflammasome and its essential functions in the cell are reviewed in details by Dr. Lamkanfi. As an expert in caspases and their activation, Dr. Lamkanfi presented the fundamental information regarding the composition and the activation of the inflammasome. He then supplied a nice overview on how bacterial and viral pathogens prevent the activation of the inflammasome, in most cases to their advantage. Dr. Saleh's review explored deeper aspects of the inflammasome during bacterial, viral, fungal, parasitic, and fungal infections. She provided a detailed description of how pathogens interact with the inflammasome, avoid, or suppress it. Then, Dr. Kanneganti polishes our knowledge about the inflammasome with her expert opinion on the major molecules involved in the modulation of the inflammasome and on human diseases impacted by the inflammasome.

Indeed, several biological pathways alter the inflammatory response to infectious agents. One of the recently recognized pathways is autophagy. Dr. Munz elegantly described how autophagy modulates inflammation and infection. He also provided information on how the inflammasome controls autophagy. On the other hand, microbe-focused reviews convince us that the pathogen ironically manipulates, exploits and evade recognition, and innate immune response by extraordinary tactics. Some organisms survive inside or outside enclosing vacuoles within eukaryotic cells such as *Listeria* reviewed by Dr. Opitz. He focused his review on the ability of this organism to differentially coordinate immune responses whether inside or outside an enclosing vacuole. Bacteria with increasing tendency to linger within enclosed vacuoles such as *Salmonella*, possess a plethora of virulence factors employed to modulate the innate immune response and inflammation is reviewed by Dr. Franchi. Surprisingly, vacuole-residing organisms such as *Mycobacteria* still modulate the inflammatory responses beyond its well protected vacuole. Dr. Torrelles describes in details how *Mycobacteria* perform this task. Dr. Gavrilin draws our attention to the important differences in innate immune responses between human and mice in response to *Francisella*. The review by Dr. Amer on why humans are susceptible to *Legionella* while mice are resistant further emphasizes this notion. This issue is embellished with two original research articles describing new findings in *Legionella* pathogenesis by Zamboni and Amer groups. Hope when the reader reaches the end of this issue, he or she will be fascinated with the interplay between the inflammasome and the pathogen.

Inflammasomes: caspase-1-activating platforms with critical roles in host defense

Lieselotte Vande Walle[1,2] and Mohamed Lamkanfi[1,2]*

[1] Department of Biochemistry, Ghent University, Ghent, Belgium
[2] Department of Medical Protein Research, Vlaams Instituut voor Biotechnologie, Ghent, Belgium

Activation of the inflammatory cysteine protease caspase-1 in inflammasome complexes plays a critical role in the host response to microbial infections. Inflammasome activation induces inflammation through secretion of the pro-inflammatory cytokines interleukin (IL)-1β and IL-18 and through extracellular release of the alarmin high mobility group box 1. Moreover, caspase-1 activation by inflammasomes counters bacterial replication and induces pyroptosis, a specialized cell death program that removes infected immune cells as part of the host defense system. It is thus not surprising that bacterial and viral pathogens evolved virulence factors targeting inflammasome activation and activity. Here, we provide an overview of the distinct inflammasome complexes that are activated in a pathogen-specific manner and discuss the diverse strategies employed by viruses and bacteria to modulate inflammasome function.

Keywords: caspase-1, inflammasome, NOD-like receptors, pathogen, interleukin, pyroptosis, infection

CASPASE-1: AN INFLAMMATORY CYSTEINE PROTEASE

Caspases are evolutionary conserved cysteine proteases that cleave their substrates behind aspartate residues (Lamkanfi et al., 2002). Caspase-mediated substrate proteolysis results in activation or inactivation of critical signaling cascades regulating programmed cell death, differentiation, and cell proliferation (Lamkanfi et al., 2006). Dysregulated activity of the founding father of mammalian caspases, interleukin (IL)-1β-converting enzyme and later renamed caspase-1, has been linked to inflammatory bowel diseases (Villani et al., 2009; Allen et al., 2010; Dupaul-Chicoine et al., 2010; Zaki et al., 2010a,b), gouty arthritis (Martinon et al., 2006), type II diabetes (Larsen et al., 2007), and less common autoinflammatory disorders that are collectively referred to as cryopyrinopathies (Lamkanfi and Kanneganti, 2010).

Caspase-1 modulates inflammatory and host defense responses against microbial pathogens by processing the precursor forms of the pro-inflammatory cytokines IL-1β and IL-18 into their biologically active forms (Kuida et al., 1995; Li et al., 1995; Ghayur et al., 1997; Gu et al., 1997). Caspase-1-mediated maturation of IL-1β and IL-18 is critical for their secretion from activated monocytes and macrophages. These related cytokines mediate critical aspects of the local and systemic immune response to infection including the induction of fever, transmigration of leukocytes into sites of injury or infection, and activation and polarization of T helper 1 (T_H1) and T_H2 responses (Dinarello, 2009). In addition to secreting IL-1β and IL-18, caspase-1 also mediates the release of the damage-associated molecular pattern (DAMP) high mobility group box 1 (HMGB1) from macrophages infected with the facultative intracellular pathogen *Salmonella typhimurium* (Lamkanfi et al., 2010). Moreover, caspase-1 was shown to activate the executioner caspase-7 (Lamkanfi et al., 2008; Akhter et al., 2009), to

inactivate glycolysis enzymes (Shao et al., 2007), and to induce a specialized form of cell death known as "pyroptosis" in macrophages infected with the bacterial pathogens *Shigella flexneri*, *S. typhimurium*, *Pseudomonas aeruginosa*, *Legionella pneumophila*, *Bacillus anthracis*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Francisella tularensis* (Lamkanfi and Dixit, 2010). In addition to preventing pathogen replication in infected immune cells, pyroptosis may enhance host defense responses by presenting intracellular microbial antigens to cells of the immune system (Lamkanfi and Dixit, 2010; Miao et al., 2010).

CASPASE-1 ACTIVATION BY INFLAMMASOMES

Caspase-1 is produced as an inactive zymogen that is recruited and activated by cytosolic multi-protein complexes known as inflammasomes (Lamkanfi and Dixit, 2009). These protein complexes are assembled in cells of myeloid and epithelial origin upon recognition of DAMPs and pathogen-associated molecular patterns in intracellular compartments, similar to the role of mammalian Toll-like receptors at the cell surface and within endosomes (Kawai and Akira, 2006). Inflammasomes contain members of the NOD-like receptor (NLR) or the HIN-200 receptor family, namely the NLRs Nlp1b, Nlrp3, and Nlrc4, or the HIN-200 protein absent in melanoma (AIM2; **Figure 1**). These receptors are recruited into the inflammasome in a pathogen-specific manner (Lamkanfi and Dixit, 2009). Nlrp3 is required for caspase-1 activation in response to microbial products with diverse molecular structures such as LPS, peptidoglycan, and lipoteichoic acid, upon exposure to microbial toxins and ionophores such as nigericin, endogenous alarmins such as ATP, and in response to infection with bacterial and fungal pathogens such as *S. aureus*, *Streptococcus pneumoniae*, and *Candida albicans*, respectively (Kanneganti et al., 2006a,b;
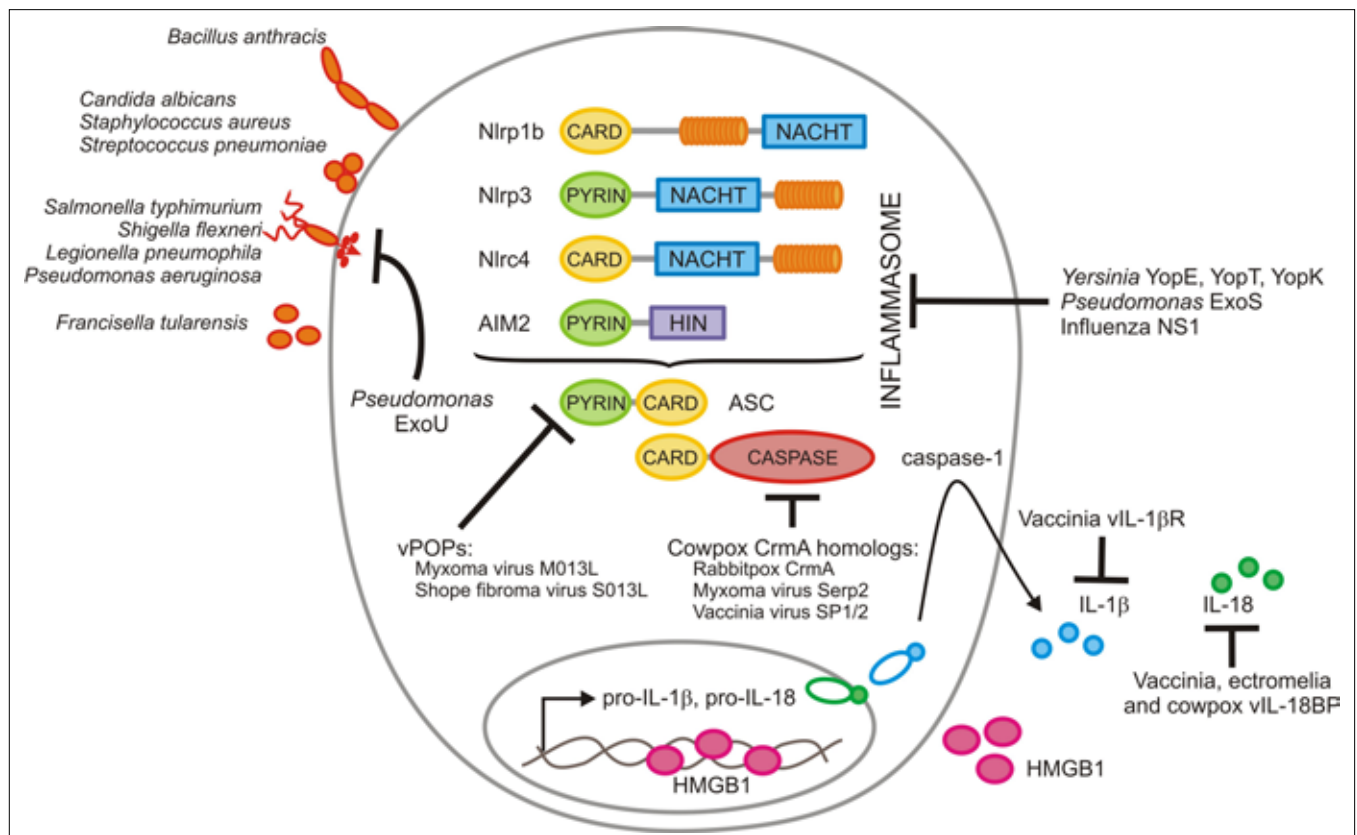
FIGURE 1 | Modulation of inflammasomes by microbial pathogens.
Bacillus anthracis activates the Nlrp1b inflammasome, whereas
Staphylococcus aureus, Streptococcus pneumonia, and *Candida albicans*
induce caspase-1 activation via Nlrp3. *Salmonella typhimurium, Shigella
flexneri, Legionella pneumophila,* and *Pseudomonas aeruginosa* all make use of
a bacterial type III or IV secretion system to inject effector proteins that are
recognized by the Nlrc4 inflammasome. Finally, *Francisella tularensis* induces
activation of the AIM2 inflammasome when genomic DNA of replicating
bacteria is detected in the cytosol of infected macrophages. Orthopoxviruses
interfere with inflammasome function at several steps. They produce pyrin-only
decoy proteins (vPOPs) that bind the inflammasome adaptor ASC to prevent
caspase-1 recruitment and inhibit caspase-1 activity directly with virally
encoded serpins such as the cowpox serpin CrmA and its homologs encoded
by myxoma and vaccinia virus. Finally, orthopoxviruses evolved scavenger
receptors for secreted IL-1β (vIL-1βR) and IL-18 (vIL-18BP) to prevent activation
of downstream signaling cascades. Influenza virus NS1 protein dampens
caspase-1 activation and secretion of IL-1β and IL-18 through a yet unknown
mechanism that requires its amino-terminal RNA-binding domain. The *Yersinia*
virulence factors YopE and YopT and the *Pseudomonas* effector ExoS inhibit
caspase-1 activation, possibly through an indirect mechanism involving
inhibition of Rho GTPase-mediated cytoskeletal changes. On the other hand,
the phospholipase A2 activity of *Pseudomonas* ExoU is required for inhibiting
caspase-1 activation, while *Yersinia* YopK prevents recognition of the bacterial
type III secretion system.

Mariathasan et al., 2006; Sutterwala et al., 2006; McNeela et al.,
2010). In addition, the DAMPs monosodium urate and calcium
pyrophosphate dehydrate crystals also activate the Nlrp3 inflam-
masome, suggesting a role for this inflammasome in the etiology of
gouty arthritis and pseudogout (Martinon et al., 2006). By contrast,
the Nlrc4 inflammasome is activated in macrophages infected with
intracellular pathogens such as *S. typhimurium, L. pneumophila,
P. aeruginosa,* and *S. flexneri* as illustrated by the observation that
caspase-1 activation is largely abolished in *Ipaf* deficient macro-
phages infected with these intracellular pathogens (Mariathasan
et al., 2004; Amer et al., 2006; Franchi et al., 2006, 2007; Miao et al.,
2006, 2008; Lamkanfi et al., 2007a; Sutterwala et al., 2007; Suzuki
et al., 2007). In contrast, *B. anthracis* Lethal Toxin (LT) triggers
activation of the Nlrp1b inflammasome in mouse macrophages and
mutations in the *Nlrp1b* gene were identified as the key susceptibil-
ity locus for Anthrax LT-induced macrophage death (Boyden and
Dietrich, 2006). Finally, additional inflammasome complexes such
as the recently identified AIM2 inflammasome, are responsible for

activation of caspase-1 in macrophages infected with *F. tularensis,
L. monocytogenes* and in response to DNA viruses such as cytome-
galovirus and vaccinia virus (Fernandes-Alnemri et al., 2010; Jones
et al., 2010; Rathinam et al., 2010; Sauer et al., 2010).

CASPASE-1 INHIBITION BY ORTHOPOXVIRUS-ENCODED SERPINS

Given the central role inflammasomes play in modulating repli-
cation and dissemination of microbial pathogens, certain viruses
and bacteria have evolved mechanisms to counter the induction
of caspase-1 activation and activity. One mechanism by which
microbial pathogens interfere with inflammasome functions is by
directly targeting the enzymatic activity of caspase-1. The cow-
pox virus protein Cytokine response modifier A (CrmA) and its
homologs in orthopoxviruses such as vaccinia, ectromelia, and
rabbitpox virus probably represent the best characterized exam-
ples of this class of inflammasome inhibitors (Dobbelstein and
Shenk, 1996; Macen et al., 1996; Turner et al., 2000; Best, 2008).

Although classified as serine protease inhibitors (serpins), these virus-encoded proteins function as pseudosubstrate inhibitors of caspase-1 (Ray et al., 1992; Komiyama et al., 1994; Best, 2008). They form covalent bonds with the active site cysteine after being processed by caspase-1, thus blocking its enzymatic activity. Indeed, CrmA inhibits caspase-1 activity with a K_I of 0.01 nM, rendering it one of the most effective inhibitors known for this protease. Notably, CrmA shares 54% amino acid identity with the human serpin PI-9 (Sprecher et al., 1995) and has two homologs in rodents (Sun et al., 1997). Although the reactive loop aspartate residue where caspase-mediated processing occurs in CrmA is mutated to glutamate in PI-9 (Annand et al., 1999), the latter serpin is capable of inhibiting caspases as illustrated by the observation that it prevents caspase-1-mediated processing of IL-1β and IL-18 in vascular smooth muscle cells (Young et al., 2000). Importantly, in addition to blocking caspase-1 activation, CrmA and its homologs inhibit the protease activity of the apoptotic initiators caspases-8 and -10 as well (Dobbelstein and Shenk, 1996; Macen et al., 1996; Turner et al., 2000; Best, 2008). Thus, poxviruses have devised a way to simultaneously interfere with inflammatory and apoptotic signaling cascades. Serpin-mediated caspase-1 inhibition prevents secretion of IL-1β and IL-18 and delays the induction of effective host defense responses. Moreover, by preventing programmed cell death of infected host cells, poxviruses can replicate in intracellular niches and spread to adjacent cells before being recognized by cells of the host's immune system.

The critical role of CrmA and other serpins in poxvirus virulence is demonstrated by the observation that CrmA deficiency reduced the lesion size and numbers on the chorio-allantoic membrane of developing chick embryos (Ray et al., 1992; Palumbo et al., 1994). Moreover, deletion of CrmA attenuated virulence in intranasally and intracranially infected Balb/c and C57BL/6 mice (Thompson et al., 1993; Palumbo et al., 1994; MacNeill et al., 2009). Similarly, deletion of the CrmA homolog Serp2 in myxoma virus causes a dramatic reduction in viral titers in infected rabbits (Messud-Petit et al., 1998). By contrast, vaccinia virus mutants lacking the CrmA homologs SPI-1 and SPI-2 did not display changes in virulence in intranasally infected Balb/c mice (Kettle et al., 1995). This may be explained by the observation that vaccinia virus encodes a scavenger receptor called virus-encoded IL-1β receptor (vIL-1βR) that neutralizes secreted IL-1β (Kettle et al., 1997). In addition, vaccinia, ectromelia, and cowpox viruses encode soluble IL-18-binding proteins (vIL-18BPs) that prevent activation of inflammatory pathways downstream of the IL-18 receptor (Smith et al., 2000). Thus, orthopoxviruses make use of serpins to inhibit caspase-1 activity and modulate signaling downstream of caspase-1 through scavenger receptors to increase virulence.

INHIBITION OF INFLAMMASOME ASSEMBLY BY ORTHOPOXVIRUS DECOY PROTEINS

In addition to modulating inflammasome signaling at the level of caspase-1 and its substrates, orthopoxviruses devised mechanisms to prevent activation of this inflammatory protease altogether. Indeed, Myxoma virus and Shope Fibroma virus encode pyrin-only decoy molecules that interfere with inflammasome assembly to inhibit caspase-1 activation (Johnston et al., 2005; Dorfleutner et al., 2007). In this regard, the myxoma virus M013L and Shope

Fibroma virus S013L pyrin-only proteins (POPs) resemble the POPs and caspase recruitment domain (CARD)-only proteins encoded in the human genome (Lamkanfi et al., 2007b; Stehlik and Dorfleutner, 2007). The human CARD-only proteins COP, INCA, ICEBERG, and CASP12$_S$ all bind the caspase-1 CARD to prevent activation of caspase-1 and the subsequent generation of IL-1β (Druilhe et al., 2001; Lee et al., 2001; Lamkanfi et al., 2004a,b; Saleh et al., 2006). In contrast, human cPOP1 and cPOP2, and viral POPs interact with the pyrin domain of the inflammasome adaptor protein ASC and pyrin motifs found in certain NLRs to prevent recruitment and activation of caspase-1 by inflammasomes (Stehlik and Dorfleutner, 2007). The relevance of vPOPs during infection is provided by the observation that deletion of the gene encoding myxoma virus M013L results in decreased viremia as a result of increased inflammatory responses and attenuated viral replication during myxomatosis (Johnston et al., 2005). Thus, the diverse mechanisms orthopoxviruses evolved to prevent and interfere with caspase-1 signaling suggest that inflammasomes play a critical role in the host response against these pathogens.

INHIBITION OF INFLAMMASOME SIGNALING BY INFLUENZA VIRUS

Viral modulation of inflammasome signaling is not limited to the orthopoxviruses described above. Interestingly, Influenza virus uses an unrelated mechanism to prevent caspase-1 activation and interfere with inflammasome signaling. Human influenza A/PR/8/34 (H1N1) virus was shown to inhibit production of IL-1β and IL-18 through inhibition of caspase-1 maturation in infected macrophages (Stasakova et al., 2005). Mutant viruses lacking the influenza NS1 gene were incapable of preventing caspase-1 activation and triggered secretion of significantly increased levels of IL-1β and IL-18 from infected host cells. These mutant viruses were attenuated *in vitro*, but it is unclear whether caspase-1 activation is solely responsible for this phenotype. Regardless, structure–function studies demonstrated that the amino-terminal RNA-binding/dimerization domain of NS1 is essential for inhibition of caspase-1 activation and the secretion of mature IL-1β and IL-18, whereas the carboxy-terminal effector domain was not required (Stasakova et al., 2005). Although the molecular mechanism by which influenza NS1 inhibits caspase-1 activation has not been uncovered, NS1 may interfere with a critical step involved in inflammasome assembly. Further study in this direction may unveil interesting new mechanisms by which viruses target inflammasomes.

NEGATIVE REGULATION OF INFLAMMASOME ACTIVATION BY BACTERIAL VIRULENCE FACTORS

In addition to the viruses described above, several Gram-positive and –Gram-negative bacterial pathogens have been demonstrated to interfere with inflammasome activation. Enteropathogenic *Yersinia enterocolitica* bacteria employ a set of intriguing mechanisms to prevent caspase-1 activation and secretion of IL-1β and IL-18 (Schotte et al., 2004). This Gram-negative pathogen makes use of a specialized type III secretion system to inject virulence factors called Yop proteins directly into the host cell cytosol. Among these effector proteins, YopE and YopT were shown to inhibit caspase-1 activation and the subsequent secretion of mature IL-1β (Schotte et al., 2004). These Yop proteins are known to target Rho GTPases,

which are critical for cytoskeletal reorganization and phagocytosis. YopE accelerates GTP hydrolysis in order to keep Rho GTPase family members in the inactive GDP-bound state. By contrast, YopT is as a cysteine protease that inactivates Rho GTPases by removing the C-terminal prenyl membrane anchor that attaches them to the plasma membrane. Experiments with dominant-negative proteins and chemical inhibitors of the Rho GTPase Rac1 suggested a critical role for this Rho GTPase in inflammasome assembly and caspase-1 activation (Schotte et al., 2004). In addition to YopE and YopT, the *Yersinia pseudotuberculosis* effector protein YopK prevents recognition of the bacterial type III secretion system by the Nlrp3 and Nlrc4 inflammasomes in order to promote bacterial survival in host macrophages (Brodsky et al., 2010). Together, these findings suggest that *Yersinia* spp. may prevent caspase-1 activation by inflammasomes by interfering with Rho GTPases and by masking the type III secretion system. However, the precise molecular chain of events by which Yop effector proteins affect inflammasome activation requires further analysis. *L. pneumophila*, the causative agent of Legionnaire's disease, uses a different strategy to inhibit caspase-1 activation in human phagocytes. This pathogen downregulates transcription of the inflammasome adaptor ASC to interfere with caspase-1-mediated restriction of bacterial replication in human monocytes (Abdelaziz et al., 2010). *P. aeruginosa* isolates expressing the virulence factor exoenzyme U (ExoU) represent another example of Gram-negative pathogens interfering with inflammasome activation. Notably, *Pseudomonas* ExoU phospholipase A2 activity was shown to be required for inhibiting the Nlrc4 inflammasome-driven secretion of IL-1β and IL-18 from infected macrophages (Sutterwala et al., 2007). ExoS is another *Pseudomonas* effector protein demonstrated to interfere with inflammasome-induced IL-1β

production. This virulence factor utilized its ADP-ribosyl transferase activity to inhibit caspase-1 activation (Galle et al., 2008). Also Gram-positive pathogens have evolved mechanisms to interfere with inflammasome function. For instance, *Mycobacterium tuberculosis* expresses a putative Zn^{2+} metalloprotease named Zmp1 that prevents inflammasome activation and IL-1β secretion (Master et al., 2008). Zmp1-mediated inhibition of caspase-1 activation was demonstrated to enhance bacterial survival in infected macrophages and to increase bacterial burdens in the lungs of aerosol-infected mice (Master et al., 2008).

CONCLUSIONS AND PERSPECTIVES

Caspase-1 activation by inflammasomes is a critical component of the host response to microbial pathogens. It induces secretion of HMGB1, IL-1β, and IL-18 and triggers pyroptosis of infected host cells in order to eliminate the infectious agent. It may thus be beneficial to microbial pathogens to prevent inflammasome assembly, to inhibit caspase-1 activation and activity and to interfere with downstream signaling cascades using decoy receptors. Studies showed that orthopoxviruses, influenza H1N1 virus and a variety of bacterial pathogens all evolved unique mechanisms to hijack the inflammasome machinery. Future studies will undoubtedly shed light on new and intriguing mechanisms by which bacterial and viral pathogens aim to silence the inflammasome.

ACKNOWLEDGMENTS

REFERENCES

Abdelaziz, D. H., Gavrilin, M. A., Akhter, A., Caution, K., Kotrange, S., Khweek, A. A., Abdulrahman, B. A., Grandhi, J., Hassan, Z. A., Marsh, M. D., and Amer, A. O. (2010). ASC controls *Legionella pneumophila* infection in human monocytes. *J. Biol. Chem.* doi: M110.197681 [pii] 10.1074/jbc.M110.197681. [Epub ahead of print].

Akhter, A., Gavrilin, M. A., Frantz, L., Washington, S., Ditty, C., Limoli, D., Day, C., Sarkar, A., Newland, C., Butchar, J., Marsh, C. B., Wewers, M. D., Tridandapani, S., Kanneganti, T. D., and Amer, A. O. (2009). Caspase-7 activation by the Nlrc4/Ipaf inflammasome restricts *Legionella pneumophila* infection. *PLoS Pathog.* 5, e1000361. doi: 10.1371/journal.ppat.1000361

Allen, I. C., TeKippe, E. M., Woodford, R. M., Uronis, J. M., Holl, E. K., Rogers, A. B., Herfarth, H. H., Jobin, C., and Ting, J. P. (2010). The NLRP3 inflammasome functions as a negative regulator of tumorigenesis during colitis-associated cancer. *J. Exp. Med.* 207, 1045–1056.

Amer, A., Franchi, L., Kanneganti, T. D., Body-Malapel, M., Ozoren, N., Brady, G., Meshinchi, S., Jagirdar, R., Gewirtz, A., Akira, S., and Nunez, G. (2006). Regulation of *Legionella* phagosome maturation and infection through flagellin and host Ipaf. *J. Biol. Chem.* 281, 35217–35223.

Annand, R. R., Dahlen, J. R., Sprecher, C. A., De, P., Dreu, Foster, D. C., Mankovich, J. A., Talanian, R. V., Kisiel, W., and Giegel, D. A. (1999). Caspase-1 (interleukin-1beta-converting enzyme) is inhibited by the human serpin analogue proteinase inhibitor 9. *Biochem. J.* 342(Pt 3), 655–665.

Best, S. M. (2008). Viral subversion of apoptotic enzymes: escape from death row. *Annu. Rev. Microbiol.* 62, 171–192.

Boyden, E. D., and Dietrich, W. F. (2006). Nalp1b controls mouse macrophage susceptibility to anthrax lethal toxin. *Nat. Genet.* 38, 240–244.

Brodsky, I. E., Palm, N. W., Sadanand, S., Ryndak, M. B., Sutterwala, F. S., Flavell, R. A., Bliska, J. B., and Medzhitov, R. (2010). A *Yersinia* effector protein promotes virulence by preventing inflammasome recognition of the type III secretion system. *Cell Host Microbe* 7, 376–387.

Dinarello, C. A. (2009). Immunological and inflammatory functions of the interleukin-1 family. *Annu. Rev. Immunol.* 27, 519–550.

Dobbelstein, M., and Shenk, T. (1996). Protection against apoptosis by the vaccinia virus SPI-2 (B13R) gene product. *J. Virol.* 70, 6479–6485.

Dorfleutner, A., Talbott, S. J., Bryan, N. B., Funya, K. N., Rellick, S. L., Reed, J. C., Shi, X., Rojanasakul, Y., Flynn, D. C., and Stehlik, C. (2007). A shope fibroma virus PYRIN-only protein modulates the host immune response. *Virus Genes* 35, 685–694.

Druilhe, A., Srinivasula, S. M., Razmara, M., Ahmad, M., and Alnemri, E. S. (2001). Regulation of IL-1beta generation by Pseudo-ICE and ICEBERG, two dominant negative caspase recruitment domain proteins. *Cell Death Differ.* 8, 649–657.

Dupaul-Chicoine, J., Yeretssian, G., Doiron, K., Bergstrom, K. S., McIntire, C. R., LeBlanc, P. M., Meunier, C., Turbide, C., Gros, P., Beauchemin, N., Vallance, B. A., and Saleh, M. (2010). Control of intestinal homeostasis, colitis, and colitis-associated colorectal cancer by the inflammatory caspases. *Immunity* 32, 367–378.

Fernandes-Alnemri, T., Yu, J. W., Juliana, C., Solorzano, L., Kang, S., Wu, J., Datta, P., McCormick, M., Huang, L., McDermott, E., Eisenlohr, L., Landel, C. P., and Alnemri, E. S. (2010). The AIM2 inflammasome is critical for innate immunity to *Francisella tularensis*. *Nat. Immunol.* 11, 385–393.

Franchi, L., Amer, A., Body-Malapel, M., Kanneganti, T. D., Ozoren, N., Jagirdar, R., Inohara, N., Vandenabeele, P., Bertin, J., Coyle, A., Grant, E. P., and Nunez, G. (2006). Cytosolic flagellin requires Ipaf for activation of caspase-1 and interleukin 1beta in *Salmonella*-infected macrophages. *Nat. Immunol.* 7, 576–582.

Franchi, L., Stoolman, J., Kanneganti, T. D., Verma, A., Ramphal, R., and Nunez, G. (2007). Critical role for Ipaf in *Pseudomonas aeruginosa*-induced caspase-1 activation. *Eur. J. Immunol.* 37, 3030–3039.

Galle, M., Schotte, P., Haegman, M., Wullaert, A., Yang, H. J., Jin, S., and Beyaert, R. (2008). The *Pseudomonas aeruginosa* Type III secretion system plays a dual role in the regulation of caspase-1 mediated IL-1beta maturation. *J. Cell. Mol. Med.* 12, 1767–1776.

Ghayur, T., Banerjee, S., Hugunin, M., Butler, D., Herzog, L., Carter, A.,

Quintal, L., Sekut, L., Talanian, R., Paskind, M., Wong, W., Kamen, R., Tracey, D., and Allen, H. (1997). Caspase-1 processes IFN-gamma-inducing factor and regulates LPS-induced IFN-gamma production. *Nature* 386, 619–623.

Gu, Y., Kuida, K., Tsutsui, H., Ku, G., Hsiao, K., Fleming, M. A., Hayashi, N., Higashino, K., Okamura, H., Nakanishi, K., Kurimoto, M., Tanimoto, T., Flavell, R. A., Sato, V., Harding, M. W., Livingston, D. J., and Su, M. S. (1997). Activation of interferon-gamma inducing factor mediated by interleukin-1beta converting enzyme. *Science* 275, 206–209.

Johnston, J. B., Barrett, J. W., Nazarian, S. H., Goodwin, M., Ricciuto, D., Wang, G., and McFadden, G. (2005). A poxvirus-encoded pyrin domain protein interacts with ASC-1 to inhibit host inflammatory and apoptotic responses to infection. *Immunity* 23, 587–598.

Jones, J. W., Kayagaki, N., Broz, P., Henry, T., Newton, K., O'Rourke, K., Chan, S., Dong, J., Qu, Y., Roose-Girma, M., Dixit, V. M., and Monack, D. M. (2010). Absent in melanoma 2 is required for innate immune recognition of *Francisella tularensis. Proc. Natl. Acad. Sci. U.S.A.* 107, 9771–9776.

Kanneganti, T. D., Ozoren, N., Body-Malapel, M., Amer, A., Park, J. H., Franchi, L., Whitfield, J., Barchet, W., Colonna, M., Vandenabeele, P., Bertin, J., Coyle, A., Grant, E. P., Akira, S., and Nunez, G. (2006a). Bacterial RNA and small antiviral compounds activate caspase-1 through cryopyrin/Nalp3. *Nature* 440, 233–236.

Kanneganti, T. D., Body-Malapel, M., Amer, A., Park, J. H., Whitfield, J., Franchi, L., Taraporewala, Z. F., Miller, D., Patton, J. T., Inohara, N., and Nunez, G. (2006b). Critical role for cryopyrin/Nalp3 in activation of caspase-1 in response to viral infection and double-stranded RNA. *J. Biol. Chem.* 281, 36560–36568.

Kawai, T., and Akira, S. (2006). TLR signaling. *Cell Death Differ.* 13, 816–825.

Kettle, S., Alcami, A., Khanna, A., Ehret, R., Jassoy, C., and Smith, G. L. (1997). Vaccinia virus serpin B13R (SPI-2) inhibits interleukin-1beta-converting enzyme and protects virus-infected cells from TNF- and Fas-mediated apoptosis, but does not prevent IL-1beta-induced fever. *J. Gen. Virol.* 78(Pt 3), 677–685.

Kettle, S., Blake, N. W., Law, K. M., and Smith, G. L. (1995). Vaccinia virus serpins B13R (SPI-2) and B22R (SPI-1) encode M(r) 38.5 and 40K, intracellular polypeptides that do not affect virus virulence in a murine intranasal model. *Virology* 206, 136–147.

Komiyama, T., Ray, C. A., Pickup, D. J., Howard, A. D., Thornberry, N. A., Peterson, E. P., and Salvesen, G. (1994).

Inhibition of interleukin-1 beta converting enzyme by the cowpox virus serpin CrmAn A., example of cross-class inhibition. *J. Biol. Chem.* 269, 19331–19337.

Kuida, K., Lippke, J. A., Ku, G., Harding, M. W., Livingston, D. J., Su, M. S., and Flavell, R. A. (1995). Altered cytokine export and apoptosis in mice deficient in interleukin-1 beta converting enzyme. *Science* 267, 2000–2003.

Lamkanfi, M., Amer, A., Kanneganti, T. D., Munoz-Planillo, R., Chen, G., Vandenabeele, P., Fortier, A., Gros, P., and Nunez, G. (2007a). The Nod-like receptor family member Naip5/Birc1e restricts *Legionella pneumophila* growth independently of caspase-1 activation. *J. Immunol.* 178, 8022–8027.

Lamkanfi, M., Festjens, N., Declercq, W., Vanden, T., Berghe, and Vandenabeele, P. (2007b). Caspases in cell survival, proliferation and differentiation. *Cell Death Differ.* 14, 44–55.

Lamkanfi, M., Declercq, W., Kalai, M., Saelens, X., and Vandenabeele, P. (2002). Alice in caspase land. A phylogenetic analysis of caspases from worm to man. *Cell Death Differ.* 9, 358–361.

Lamkanfi, M., Declercq, W., Vanden, T., Berghe, and Vandenabeele, P. (2006). Caspases leave the beaten track: caspase-mediated activation of NF-kappaB. *J. Cell Biol.* 173, 165–171.

Lamkanfi, M., Denecker, G., Kalai, M., D'Hondt, K., Meeus, A., Declercq, W., Saelens, X., and Vandenabeele, P. (2004a). INCA, a novel human caspase recruitment domain protein that inhibits interleukin-1beta generation. *J. Biol. Chem.* 279, 51729–51738.

Lamkanfi, M., Kalai, M., and Vandenabeele, P. (2004b). Caspase-12: an overview. *Cell Death Differ.* 11, 365–368.

Lamkanfi, M., and Dixit, V. M. (2009). The inflammasomes. *PLoS Pathog.* 5, e1000510. doi: 10.1371/journal. ppat.1000510

Lamkanfi, M., and Dixit, V. M. (2010). Manipulation of host cell death pathways during microbial infections. *Cell Host Microbe* 8, 44–54.

Lamkanfi, M., and Kanneganti, T. D. (2010). Nlrp3: an immune sensor of cellular stress and infection. *Int. J. Biochem. Cell Biol.* 42, 792–795.

Lamkanfi, M., Kanneganti, T. D., Van Damme, P., Vanden Berghe, T., Vanoverberghe, I., Vandekerckhove, J., Vandenabeele, P., Gevaert, K., and Nunez, G. (2008). Targeted peptidecentric proteomics reveals caspase-7 as a substrate of the caspase-1 inflammasomes. *Mol. Cell Proteomics* 7, 2350–2363.

Lamkanfi, M., Sarkar, A., Vande Walle, L., Vitari, A. C., Amer, A. O., Wewers, M. D., Tracey, K. J., Kanneganti, T. D., and

Dixit, V. M. (2010). Inflammasome-dependent release of the alarmin HMGB1 in endotoxemia. *J. Immunol.* 185, 4385–4392.

Larsen, C. M., Faulenbach, M., Vaag, A., Volund, A., Ehses, J. A., Seifert, B., Mandrup-Poulsen, T., and Donath, M. Y. (2007). Interleukin-1-receptor antagonist in type 2 diabetes mellitus. *N. Engl. J. Med.* 356, 1517–1526.

Lee, S. H., Stehlik, C., and Reed, J. C. (2001). Cop, a caspase recruitment domain-containing protein and inhibitor of caspase-1 activation processing. *J. Biol. Chem.* 276, 34495–34500.

Li, P., Allen, H., Banerjee, S., Franklin, S., Herzog, L., Johnston, C., McDowell, J., Paskind, M., Rodman, L., Salfeld, J., Towne, E., Tracey, D., Wardwell, S., Wei, F., Wong, W., Kamen, R., and Seshadri, T. (1995). Mice deficient in IL-1 beta-converting enzyme are defective in production of mature IL-1 beta and resistant to endotoxic shock. *Cell* 80, 401–411.

Macen, J. L., Garner, R. S., Musy, P. Y., Brooks, M. A., Turner, P. C., Moyer, R. W., McFadden, G., and Bleackley, R. C. (1996). Differential inhibition of the Fas- and granule-mediated cytolysis pathways by the orthopoxvirus cytokine response modifier A/SPI-2 and SPI-1 protein. *Proc. Natl. Acad. Sci. U.S.A.* 93, 9108–9113.

MacNeill, A. L., Moldawer, L. L., and Moyer, R. W. (2009). The role of the cowpox virus crmA gene during intratracheal and intradermal infection of C57BL/6 mice. *Virology* 384, 151–160.

Mariathasan, S., Newton, K., Monack, D. M., Vucic, D., French, D. M., Lee, W. P., Roose-Girma, M., Erickson, S., and Dixit, V. M. (2004). Differential activation of the inflammasome by caspase-1 adaptors ASC and Ipaf. *Nature* 430, 213–218.

Mariathasan, S., Weiss, D. S., Newton, K., McBride, J., O'Rourke, K., Roose-Girma, M., Lee, W. P., Weinrauch, Y., Monack, D. M., and Dixit, V. M. (2006). Cryopyrin activates the inflammasome in response to toxins and ATP. *Nature* 440, 228–232.

Martinon, F., Petrilli, V., Mayor, A., Tardivel, A., and Tschopp, J. (2006). Gout-associated uric acid crystals activate the NALP3 inflammasome. *Nature* 440, 237–241.

Master, S. S., Rampini, S. K., Davis, A. S., Keller, C., Ehlers, S., Springer, B., Timmins, G. S., Sander, P., and Deretic, V. (2008). *Mycobacterium tuberculosis* prevents inflammasome activation. *Cell Host Microbe* 3, 224–32.

McNeela, E. A., Burke, A., Neill, D. R., Baxter, C., Fernandes, V. E., Ferreira, D., Smeaton, S., El-Rachkidy, R., McLoughlin, R. M., Mori, A., Moran,

B., Fitzgerald, K. A., Tschopp, J., Petrilli, V., Andrew, P. W., Kadioglu, A., and Lavelle, E. C. (2010). Pneumolysin activates the NLRP3 inflammasome and promotes proinflammatory cytokines independently of TLR4. *PLoS Pathog.* 6, e1001191. doi: 10.1371/journal. ppat.1001191

Messud-Petit, F., Gelfi, J., Delverdier, M., Amardeilh, M. F., Py, R., Sutter, G., and Bertagnoli, S. (1998). Serp2, an inhibitor of the interleukin-1beta-converting enzyme, is critical in the pathobiology of myxoma virus. *J. Virol.* 72, 7830–7839.

Miao, E. A., Alpuche-Aranda, C. M., Dors, M., Clark, A. E., Bader, M. W., Miller, S. I., and Aderem, A. (2006). Cytoplasmic flagellin activates caspase-1 and secretion of interleukin 1beta via Ipaf. *Nat. Immunol.* 7, 569–575.

Miao, E. A., Ernst, R. K., Dors, M., Mao, D. P., and Aderem, A. (2008). *Pseudomonas aeruginosa* activates caspase 1 through Ipaf. *Proc. Natl. Acad. Sci. U.S.A.* 105, 2562–2567.

Miao, E. A., Leaf, I. A., Treuting, P. M., Mao, D. P., Dors, M., Sarkar, A., Warren, S. E., Wewers, M. D., and Aderem, A. (2010). Caspase-1-induced pyroptosis is an innate immune effector mechanism against intracellular bacteria. *Nat. Immunol.* 11, 1136–1142.

Palumbo, G. J., Buller, R. M., and Glasgow, W. C. (1994). Multigenic evasion of inflammation by poxviruses. *J. Virol.* 68, 1737–1749.

Rathinam, V. A., Jiang, Z., Waggoner, S. N., Sharma, S., Cole, L. E., Waggoner, L., Vanaja, S. K., Monks, B. G., Ganesan, S., Latz, E., Hornung, V., Vogel, S. N., Szomolanyi-Tsuda E., and Fitzgerald, K. A. (2010). The AIM2 inflammasome is essential for host defense against cytosolic bacteria and DNA viruses. *Nat. Immunol.* 11, 395–402.

Ray, C. A., Black, R. A., Kronheim, S. R., Greenstreet, T. A., Sleath, P. R., Salvesen, G. S., and Pickup, D. J. (1992). Viral inhibition of inflammation: cowpox virus encodes an inhibitor of the interleukin-1 beta converting enzyme. *Cell* 69, 597–604.

Saleh, M., Mathison, J. C., Wolinski, M. K., Bensinger, S. J., Fitzgerald, P., Droin, N., Ulevitch, R. J., Green, D. R., and Nicholson, D. W. (2006). Enhanced bacterial clearance and sepsis resistance in caspase-12-deficient mice. *Nature* 440, 1064–1068.

Sauer, J. D., Witte, C. E., Zemansky, J., Hanson, B., Lauer, P., and Portnoy, D. A. (2010). *Listeria monocytogenes* triggers AIM2-mediated pyroptosis upon infrequent bacteriolysis in the macrophage cytosol. *Cell Host Microbe* 7, 412–419.

Schotte, P., Denecker, G., Van Den Broeke, A., Vandenabeele, P., Cornelis, G. R.,

and Beyaert, R. (2004). Targeting Rac1 by the *Yersinia* effector protein YopE inhibits caspase-1-mediated maturation and release of interleukin-1beta. *J. Biol. Chem.* 279, 25134–25142.

Shao, W., Yeretssian, G., Doiron, K., Hussain, S. N., and Saleh, M. (2007). The caspase-1 digestome identifies the glycolysis pathway as a target during infection and septic shock. *J. Biol. Chem.* 282, 36321–36329.

Smith, V. P., Bryant, N. A., and Alcami, A. (2000). Ectromelia, vaccinia and cowpox viruses encode secreted interleukin-18-binding proteins. *J. Gen. Virol.* 81(Pt 5), 1223–1230.

Sprecher, C. A., Morgenstern, K. A., Mathewes, S., Dahlen, J. R., Schrader, S. K., Foster, D. C., and Kisiel, W. (1995). Molecular cloning, expression, and partial characterization of two novel members of the ovalbumin family of serine proteinase inhibitors. *J. Biol. Chem.* 270, 29854–29861.

Stasakova, J., Ferko, B., Kittel, C., Sereinig, S., Romanova, J., Katinger, H., and Egorov, A. (2005). Influenza A mutant viruses with altered NS1 protein function provoke caspase-1 activation in primary human macrophages, resulting in fast apoptosis and release of high levels of interleukins 1beta and 18. *J. Gen. Virol.* 86(Pt 1), 185–195.

Stehlik, C., and Dorfleutner, A. (2007). COPs and POPs: modulators of inflammasome activity. *J. Immunol.* 179, 7993–7998.

Sun, J., Ooms, L., Bird, C. H., Sutton, V. R., Trapani, J. A., and Bird, P. I. (1997). A new family of 10 murine ovalbumin serpins includes two homologs of proteinase inhibitor 8 and two homologs of the granzyme B inhibitor (proteinase inhibitor 9). *J. Biol. Chem.* 272, 15434–15441.

Sutterwala, F. S., Mijares, L. A., Li, L., Ogura, Y., Kazmierczak, B. I., and Flavell, R. A. (2007). Immune recognition of *Pseudomonas aeruginosa* mediated by the IPAF/NLRC4 inflammasome. *J. Exp. Med.* 204, 3235–3245.

Sutterwala, F. S., Ogura, Y., Szczepanik, M., Lara-Tejero, M., Lichtenberger, G. S., Grant, E. P., Bertin, J., Coyle, A. J., Galan, J. E., Askenase, P. W., and Flavell, R. A. (2006). Critical role for NALP3/CIAS1/cryopyrin in innate and adaptive immunity through its regulation of caspase-1. *Immunity* 24, 317–327.

Suzuki, T., Franchi, L., Toma, C., Ashida, H., Ogawa, M., Yoshikawa, Y., Mimuro, H., Inohara, N., Sasakawa, C., and Nunez, G. (2007). Differential regulation of caspase-1 activation, pyroptosis, and autophagy via Ipaf and ASC in Shigella-infected macrophages. *PLoS Pathog.* 3, e111. doi: 10.1371/journal.ppat.0030111

Thompson, J. P., Turner, P. C., Ali, A. N., Crenshaw, B. C., and Moyer, R. W. (1993). The effects of serpin gene mutations on the distinctive pathobiology of cowpox and rabbitpox virus following intranasal inoculation of Balb/c mice. *Virology* 197, 328–338.

Turner, S. J., Silke, J., Kenshole, B., and Ruby, J. (2000). Characterization of the ectromelia virus serpin, SPI-2. *J. Gen. Virol.* 81(Pt 10), 2425–2430.

Villani, A. C., Lemire, M., Fortin, G., Louis, E., Silverberg, M. S., Collette, C., Baba, N., Libioulle, C., Belaiche, J., Bitton, A., Gaudet, D., Cohen, A., Langelier, D., Fortin, P. R., Wither, J. E., Sarfati, M., Rutgeerts, P., Rioux, J. D., Vermeire, S., Hudson, T. J., and Franchimont, D. (2009). Common variants in the NLRP3 region contribute to Crohn's disease susceptibility. *Nat. Genet.* 41, 71–76.

Young, J. L., Sukhova, G. K., Foster, D., Kisiel, W., Libby, P., and Schonbeck, U. (2000). The serpin proteinase inhibitor 9 is an endogenous inhibitor of interleukin 1beta-converting enzyme (caspase-1) activity in human vascular smooth muscle cells. *J. Exp. Med.* 191, 1535–1544.

Zaki, M. H., Boyd, K. L., Vogel, P., Kastan, M. B., Lamkanfi, M., and Kanneganti, T. D. (2010a). The NLRP3 inflammasome protects against loss of epithelial integrity and mortality during experimental colitis. *Immunity* 32, 379–391.

Zaki, M. H., Vogel, P., Body-Malapel, M., Lamkanfi, M., and Kanneganti, T. D. (2010b). IL-18 production downstream of the Nlrp3 inflammasome confers protection against colorectal tumor formation. *J. Immunol.* 185, 4912–4920.

The inflammasomes: molecular effectors of host resistance against bacterial, viral, parasitic, and fungal infections

Alexander Skeldon[1] and Maya Saleh[1,2,3]*

[1] Department of Biochemistry, McGill University, Montreal, QC, Canada
[2] Department of Medicine, McGill University, Montreal, QC, Canada
[3] Department of Microbiology and Immunology, McGill University, Montreal, QC, Canada

The inflammasomes are large multi-protein complexes scaffolded by cytosolic pattern recognition receptors (PRRs) that form an important part of the innate immune system. They are activated following the recognition of microbial-associated molecular patterns or host-derived danger signals (danger-associated molecular patterns) by PRRs. This recognition results in the recruitment and activation of the pro-inflammatory protease caspase-1, which cleaves its preferred substrates pro-interleukin-1β (IL-1β) and pro-IL-18 into their mature biologically active cytokine forms. Through processing of a number of other cellular substrates, caspase-1 is also required for the release of "alarmins" and the induction and execution of an inflammatory form of cell death termed pyroptosis. A growing spectrum of inflammasomes have been identified in the host defense against a variety of pathogens. Reciprocally, pathogens have evolved effector strategies to antagonize the inflammasome pathway. In this review we discuss recent developments in the understanding of inflammasome-mediated recognition of bacterial, viral, parasitic, and fungal infections and the beneficial or detrimental effects of inflammasome signaling in host resistance.

Keywords: innate immunity, inflammation, Nod-like receptors, inflammasome, caspases, infection

INTRODUCTION

The innate immune system plays a primary role in the rapid recognition and elimination of invading microorganisms, through different processes such as phagocytosis and the induction of inflammation. It is also vital in priming and activating the adaptive immune system, which provides long-lasting immunity. Host cells, including among others cells of the myeloid and epithelial lineages, express a number of germline-encoded pattern recognition receptors (PRRs) that recognize conserved protein, lipid, polysaccharide, or nucleic acid motifs and activate different inflammatory and antimicrobial pathways. PRRs include, but are not limited to, trans-membrane Toll-like receptors (TLRs), C-type lectin receptors (CLRs), cytosolic Nod-like receptors (NLRs), retinoic acid inducible gene-I (RIG-I)-like helicases and HIN200 proteins (O'Neill and Bowie, 2010; Takeuchi and Akira, 2010). These receptors also sense and respond to endogenous, host-derived, danger signals that are released in response to stress, tissue injury or cell death (Bianchi, 2007; Medzhitov, 2008; Schroder and Tschopp, 2010).

To date, over 20 human NLRs have been identified. NLRs are related to disease resistance R proteins in plants (Belkhadir et al., 2004) and to apoptosis protease-activating factor 1 (Apaf1; Proell et al., 2008). They share with these proteins a tripartite structure consisting of an N-terminal protein–protein interaction module [which is either a pyrin domain (PYD), a caspase recruitment domain (CARD), or a baculovirus IAP repeat (BIR)], a central nucleotide binding and oligomerization domain (NOD or NAHCT) and a C-terminal agonist-sensing domain, which in NLRs is a series of leucine-rich repeats (LRRs), as found in TLRs.

The first identified NLRs, NOD1, and NOD2, were determined to recognize bacterial peptidoglycan moieties (Chamaillard et al., 2003; Girardin et al., 2003; Inohara et al., 2003) and trigger inflammation by activating NF-κB and MAP kinase (MAPK) pathways (Shaw et al., 2008). Further study of the NLR family revealed that some members are capable of forming multi-protein complexes, termed inflammasomes that recruit pro-caspase-1 and induce its "activation by proximity," either directly or through adaptor proteins such as apoptosis-associated speck-like protein containing a CARD (ASC; Martinon et al., 2002).

Multiple inflammasomes have been characterized, including those scaffolded by the NLR family members NLRP1, NLRP3, NLRC4, NAIP5 and the more recently described AIM2 and RIG-I inflammasomes that are stimulated by cytosolic DNA and viral RNA, respectively. The adaptor protein ASC is required for the recruitment of caspase-1 to the NLRP3, AIM2, and RIG-I inflammasomes while its role in the NLRP1, NLRC4, and NIAP5-associated complexes is less clear. Following inflammasome stimulation, the cellular pool of ASC coalesces into a large structure called the pyroptosome, which is the site of caspase-1 activation. Dependent on the associated PRR, inflammasomes are activated by a variety of signals and may have redundant functions during infection. Dysregulation and/or genetic mutations or variations in genes encoding inflammasome components have been linked to genetic disorders in humans, including a continuum of autoinflammatory syndromes ranging from familial cold urticaria and Muckle–Wells syndrome to neonatal onset multisystem inflammatory disease, Crohn's disease, sepsis as well as susceptibility to infection with certain pathogens (Rodrigue-Gervais and Saleh, 2010).

Caspase-1 processes a number of cellular substrates, which is a prerequisite to the induction of an inflammatory response. Most notably, caspase-1 converts pro-IL-1β and pro-IL-18 into their mature biologically active cytokine forms (Thornberry et al., 1992; Ghayur et al., 1997; Gu et al., 1997). Moreover, caspase-1 is required for the release of a number of pro-inflammatory molecules, which are not necessarily caspase-1 substrates, including IL-1α (Keller et al., 2008). In addition to its pro-inflammatory effects, excessive activation of caspase-1 leads to a form of cell death called pyroptosis, with characteristics of both apoptosis and necrosis (Fink and Cookson, 2005; Labbe and Saleh, 2008). Among the different caspase-1 substrates, we have shown that it cleaves a number of glycolysis enzymes (Shao et al., 2007), which is possibly a primary mechanism to execute cell death. Moreover, caspase-1 has been shown to cleave and activate the pro-apoptotic executioner caspase, caspase-7 (Lamkanfi et al., 2008), suggesting that this enzyme might provide a secondary amplification loop during pyroptosis.

INFLAMMASOME REGULATION

Because of the paramount effects of caspase-1 during infection and inflammation, regulation of the inflammasome pathway is tightly controlled through a number of checkpoints (**Figure 1**). Firstly, inflammasome activation depends on a two-step process, whereby signal 1 "primes" the inflammasome via transcriptional induction of inflammasome components and cytokine proforms (usually mediated by NF-κB downstream of PRRs or cytokine receptors) and signal 2, which "activates" the inflammasome.

In addition to this bi-modal mechanism of activation, the inflammasome is kept in check via a number of positive and negative regulators (**Figure 1**). For instance, murine caspase-11 and its closest human homolog caspase-5 (Lin et al., 2000) are required for caspase-1 activation in some contexts (Wang et al., 1998), presumably through direct interaction with the inflammasome (Martinon et al., 2002). Conversely, caspase-12 is a negative regulator of caspase-1 (Saleh et al., 2006). Two groups of proteins termed COPs (CARD-only proteins) and POPs (PYD-only proteins) also inhibit caspase-1 activation by preventing inflammasome assembly (Stehlik and Dorfleutner, 2007). In addition to these dominant negative proteins, the anti-apoptotic factors Bcl-2 and Bcl-X_L can bind and suppress NLRP1 oligomerization and caspase-1 activation by inhibiting ATP binding (Bruey et al., 2007; Faustin et al., 2009). The IL-1 pathway is also highly regulated. Secreted IL-1β binds to the IL-1 receptor I (IL-1RI) and recruits the IL-1R accessory protein (IL-1RAcP), which is required for signaling (Gabay et al., 2010). The dominant negative receptor IL-1RII and soluble IL-1 receptor antagonist (IL-RA) are able to inhibit this process (Gabay et al., 2010). Together, these factors add an additional layer of regulation on the inflammasome pathway.

Given the widespread activation of inflammasomes in response to various microbes, it is not surprising that pathogens have devised multiple strategies to alter inflammasome function and downstream signaling. Bacteria utilize effector molecules, injected through secretion systems into the host cytosol, for this purpose. For instance, *Y. enterocolitica* YopE and YopT (Schotte et al., 2004), *Y. pseudotuberculosis* YopK (Brodsky et al., 2010), and *P. aeruginosa* ExoU (Sutterwala et al., 2007) have been reported to blunt inflammasome activation. Viruses also encode proteins that tar-

get this pathway including influenza NS1 (Stasakova et al., 2005), Myxoma virus M13L-PYD and Shope fibroma virus gp013L (Johnston et al., 2005; Dorfleutner et al., 2007) that act as POPs. Vaccinia virus encodes a soluble IL-1β receptor, B15R, that blunts IL-1 signaling (Alcami and Smith, 1992), whereas Molluscum contagiosum poxvirus produces two IL-18 inhibitors, MC53L and MC54L (Xiang and Moss, 1999). The active inhibition of the inflammasome by various pathogens supports the notion that its pro-inflammatory effects together with the induction of pyroptosis are deleterious for the pathogen.

INFLAMMASOME ACTIVATION

A spectrum of agonists activate the inflammasomes, with some being more specific than others depending on the associated NLR. NLRP3 forms a multi-protein complex with ASC and caspase-1, and is currently the most well characterized inflammasome. It can be activated by various structurally unrelated stimuli including microbial-associated molecular patterns (MAMPs), and danger-associated molecular patterns (DAMPs). For instance, elevated concentrations of ATP (Mariathasan et al., 2006), pore-forming toxins (Mariathasan et al., 2006), UVB irradiation and particulate matter such as crystalline forms of monosodium urate (MSU; Martinon et al., 2006), asbestos and silica (Cassel et al., 2008; Dostert et al., 2008; Hornung et al., 2008), and amyloid β aggregates (Halle et al., 2008) have all been reported to trigger NLRP3 activation. Due to the high disparity of these agonists, it is suggested that a downstream signal is instead sensed by NLRP3. In the case of particulate agonists, disruption of the lysosomal membrane along with cathepsins appear to be upstream of inflammasome activation. For instance, chemical inhibition of cathepsin B, cathepsin B-deficiency, or treatment of cells with inhibitors of the vacuolar H^+ ATPase result in reduced caspase-1 activation (Halle et al., 2008; Hornung et al., 2008). On the other hand, inflammasome activation triggered by ATP is not affected by these inhibitors. ATP activates the P2X7 receptor cation channel, which induces potassium efflux and causes the recruitment of the pannexin-1 channel that amplifies this response (Pelegrin and Surprenant, 2006). Treatment of macrophages with nigericin, a *Streptomyces*-derived potassium ionophore (Mariathasan et al., 2006), or with α-hemolysin, a *Staphylococcus aureus* pore-forming toxin, similarly triggers NLRP3 inflammasome activation (Craven et al., 2009). It has been further suggested that reactive oxygen species (ROS) may be involved in this process. Depletion of the p22^{phox} subunit of the ROS-generating NADPH complex in the human monocytic cell line THP-1 results in reduced IL-1β processing in response to asbestos, but not MSU crystals (Dostert et al., 2008). The inhibition of cellular autophagy results in the accumulation of damaged, ROS producing mitochondria that also triggers NLRP3 activation (Zhou et al., 2011). Therefore, different ligands appear to require an assortment of mechanisms to activate NLRP3. The precise signal sensed by NLRP3 remains unclear but may be a combination of those mentioned above. Unlike NLRP3, the other known inflammasomes, namely NLRP1, NLRP4, AIM2, and RIG-I, have more defined activators and primarily play a role in the detection of pathogens. Recently, we have gained significant insights into the understanding of how the inflammasomes detect infectious microorganisms and the contribution of inflammasome signaling
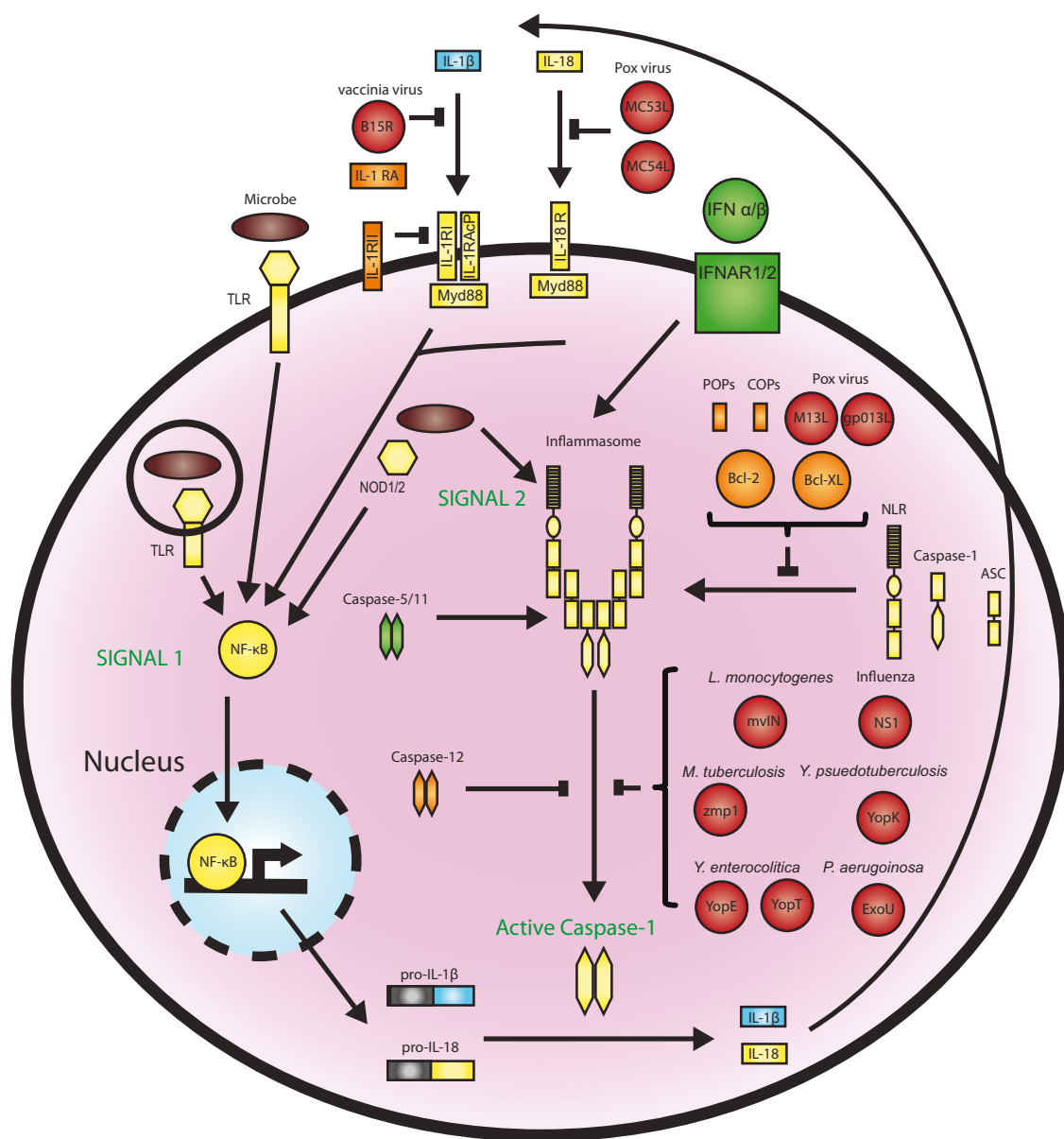
FIGURE 1 | Regulation of the inflammasomes by host factors and pathogen effectors. Inflammasomes are activated in a two-step process beginning with PRR-mediated induction of inflammasome components and pro-IL-1β production through NF-κB, followed by a second signal that activates the inflammasome and caspase-1 catalysis. This process can be regulated at multiple steps by host proteins that function as positive regulators (green) or inhibitors (orange) or targeted by pathogen effectors (red). Host COPs and POPs, the poxvirus proteins M13L and gp013L, and the anti-apoptotic factors Bcl-2 and Bcl-X$_L$ inhibit inflammasome assembly. Caspase-1 activation is inhibited by caspase-12, and multiple pathogen effectors, while murine caspase-11 and human caspase-5 are required for caspase-1 activation in response to certain stimuli. Type I IFN is required for AIM2 inflammasome activation in response to cytosolic DNA. The IL-1β and IL-18 pathways are also highly regulated. Endogenous IL-1 receptor antagonist (RA) prevents IL-1 signaling by binding to the IL-1 receptor, while the vaccinia virus proteins B15R and Molluscum contagiosum poxvirus MC53L and MC54L can bind and inhibit IL-1 and IL-18, respectively.

to the immune response. In this review, we focus our discussion on the role of the inflammasomes in bacterial, viral, parasitic, and fungal infections.

BACTERIA

The innate immune system plays an important role in eliminating bacterial pathogens and preventing their replication and damage to the host. The human body is also home to numerous commensal microorganisms and it is therefore imperative that a distinction can be made in the response to these species versus pathogens to prevent the development of inflammatory conditions. During bacterial infection, inflammation is generally beneficial, but if uncontrolled could lead to deleterious effects such as septic shock.

Some pathogenic bacteria have an intracellular lifestyle within the host and are either engulfed by phagocytes or capable of invading non-phagocytic cells. This aids in the avoidance of immune

detection and reduces exposure to antimicrobial components of the immune system, such as antimicrobial peptides, complements, and immunoglobulins. Inside the cell, bacteria are capable of manipulating the endocytic pathways that would otherwise result in their degradation within lysosomes. Interfering with these pathways allows the pathogen to survive and in many cases replicate. This is often achieved through bacterial secretion systems, which mediate the translocation of virulence factors or effector molecules into the host cell cytosol, where they become active. Following replication within the cell, the increase in bacterial load triggers cell death, allowing dissemination to new host cells.

Salmonella enterica is the best characterized intracellular bacterium with respect to inflammasome activation (**Table 1**). The study of *Salmonella* has greatly expanded our understanding of how the inflammasome recognizes pathogenic intracellular bacteria and how this contributes to the immune response. *S. enterica* serovar Typhimurium induces gastroenteritis and systemic typhoid-like disease in mice (Valdez et al., 2009). The bacterium employs a *Salmonella* Pathogenicity Island 1 (SPI-1) Type III Secretion System (T3SS) to invade epithelial cells and cross the intestinal barrier. *S. typhimurium* is then exposed to cells of the immune system and is capable of surviving within macrophages. The SPI-2 T3SS creates an intracellular niche called the *Salmonella*-containing vacuole (SCV) by inhibiting intracellular trafficking that would result in bacterial degradation (Haraga et al., 2008).

Early studies have determined that *Salmonella* induces caspase-1-dependent cell death in infected macrophages (Hersh et al., 1999). Subsequently, the inflammasome components Asc and Nlrc4 and a *Salmonella* SPI-1 T3SS component SipB were shown to be required for this response (Hersh et al., 1999; Mariathasan et al., 2004). The Nlrp3 inflammasome was thought to be irrelevant in this context, as Nlrp3-deficient macrophages showed no defect in caspase-1 activation or IL-1β secretion in *in vitro* studies (Mariathasan et al., 2006). A beneficial role for caspase-1 during *S. typhimurium* infection was identified *in vivo* using caspase-1-deficient mice. These mice displayed reduced survival, increased bacterial counts in the spleen, Peyer's patch, and mesenteric lymph node (MLN), and increased intestinal inflammation after oral infection compared to wild-type animals (Lara-Tejero et al., 2006; Raupach et al., 2006). In contrast, mice singly deficient in one of the inflammasome components Nlrp3, Nlrc4, or Asc were phenotypically equivalent to wild-type mice, suggesting that *Salmonella* induces redundant pathways to activate caspase-1 *in vivo* (Lara-Tejero et al., 2006). Consistently, it has been recently demonstrated that both Nlrp3 and Nlrc4 are activated during *Salmonella* infection (Broz et al., 2010). Earlier *in vitro* work has demonstrated that *Asc*$^{-/-}$ macrophages have reduced IL-1β secretion and caspase-1 processing in response to *Salmonella* compared to wild-type cells (Mariathasan et al., 2004, 2006; Miao et al., 2006). In addition, *Asc*$^{-/-}$ macrophages have a more blunted response compared to Nlrp3-deficient cells, suggesting that Asc plays a role independent of the Nlrp3 inflammasome (Broz et al., 2010). Notably, deficiency in both Asc and Nlrc4 reduces IL-1β levels to that of caspase-1 null macrophages, suggesting that Asc may work to enhance Nlrc4 activity during *Salmonella* infection. However, a recent study has demonstrated that flagellin-dependent Nlrc4 inflammasome activation resulted

in macrophage pyroptosis, independently of Asc, leading to bacterial release and subsequent killing by neutrophils (Miao et al., 2010a). *Nlrc4*$^{-/-}$ mice were impaired in clearing the infection, pointing to a critical role of Nlrc4 in bacterial elimination that is independent of IL-1r, IL-18, Nlrp3, and Asc.

Multiple *Salmonella* motifs trigger inflammasome activation (**Figure 2**). The SPI-1 (Miao et al., 2006) and SPI-2 (Broz et al., 2010) T3SS and flagellin (Franchi et al., 2006; Miao et al., 2006) stimulate NLRC4, while NLRP3 is activated by a currently unknown signal (Broz et al., 2010). The T3SS effector SopE (Muller et al., 2009; Hoffmann et al., 2010) is also capable of caspase-1 activation through an undetermined inflammasome. *Salmonella* strains lacking flagellin have reduced ability to activate caspase-1 or induce IL-1β secretion in macrophages, and transfected flagellin can activate the Nlrc4 inflammasome (Franchi et al., 2006; Miao et al., 2006; Broz et al., 2010). In addition to their role in flagellin translocation, T3SSs independently stimulate the Nlrc4 inflammasome. Indeed, a conserved component of the SPI-1 T3SS, the periplasmic rod-forming protein PrgJ, is recognized by Nlrc4 (Miao et al., 2010b). In contrast, The SPI-2 rod protein SsaI is not detected, suggesting that repression of SPI-1 in phagocytes is an adaptation mechanism to evade recognition by NLRC4. Homologous rod proteins from *Escherichia coli, Shigella flexneri,* and *Pseudomonas aeruginosa* are similarly sensed by Nlrc4, indicating that this is a conserved mechanism of caspase-1 activation found in a number of bacterial species. This may provide a possible explanation as to why *Shigella* and *Pseudomonas* strains that lack flagella are still capable of activating the Nlrc4 inflammasome (Sutterwala et al., 2007; Suzuki et al., 2007). A recent study has suggested that potassium efflux is required, but not sufficient, for NLRC4 activation induced by *Salmonella* or *P. aeruginosa* infection (Arlehamn et al., 2010). This is at odds with earlier reports that used lower extracellular potassium concentrations and different infection time points (Franchi et al., 2007; Petrilli et al., 2007; Fink et al., 2008).

In addition to flagellin, bacterial effector proteins translocated through T3SSs are also capable of inflammasome activation. For instance, SopE, a guanine nucleotide exchange factor (GEF) of Rho GTPases that induces *Salmonella* invasion into epithelial cells (Hardt et al., 1998), leads to caspase-1 activation *in vitro*, in a manner dependent on its GEF activity and the Rho GTPases Rac1 and Cdc42 (Muller et al., 2009). This effect appears to be independent of flagellin (Hoffmann et al., 2010). *In vivo*, SopE triggers the caspase-1-IL-18 axis of inflammation, predominantly in non-hematopoietic cells (Muller et al., 2009). *Y. enterocolitica*'s effector YopE, a GTPase activating protein (GAP) and therefore an inhibitor of Rho GTPases, conversely inhibits caspase-1 activation (Schotte et al., 2004). It is currently unknown how Rho GTPases impact caspase-1 activation, but this raises the possibility that the large number of pathogen effector molecules targeting Rho GTPase functions might also play an important role in inflammasome signaling. Taking into account the different signals involved in *Salmonella*-induced caspase-1 activation, it is clear that the T3SS is a conserved inflammasome activator.

Legionella pneumophila, the causative agent of Legionnaires' disease in humans, is another bacterial pathogen that can survive and replicate within macrophages. This bacterium employs the Dot/Icm Type IV Secretion System (T4SS) to recruit endoplasmic reticulum components to a *Legionella*-containing vacuole (LCV), inhibiting

Table 1 | Inflammasome-mediated pathogen recognition and response.

| Organism	PRR	PAMP(s), Activator(s)	Other coactivators	Inflammasome *in vitro* function	Inflammasome *in vivo* function	Inflammasome Inhibitors	References
BACTERIA							
S. enterica	NLRC4	Flagellin, PrgJ, SopE	K + efflux?	IL-1β secretion, caspase-1 processing, pyroptosis	Host survival, bacterial clearance		(Mariathasan et al., (2004, 2006), Franchi et al., (2006), Lara-Tejero et al., (2006), Mariathasan *et al.*, 2006, Miao et al., (2006, 2010a,b), Raupach et al., (2006), Sun et al., (2007), Muller et al., (2009), Arlehamn et al. (2010), Broz et al., (2010), Miao et al., 2010a, Miao *et al.*, 2010b, Arlehamn *et al.*, 2010)
	NLRP3	?		IL-1β secretion, caspase-1 processing	Host survival, bacterial clearance		
L. pneumophila	NLRC4	Flagellin	Naip5	IL-1β secretion, caspase-1 processing, pyroptosis, bacterial clearance, reduced ER recruitment to LCV	Bacterial clearance		(Diez et al., (2003), Wright et al., (2003), Amer et al., (2006), Molofsky et al., (2006), Ren et al., (2006), Zamboni et al., (2006), Coers et al., (2007), Fortier et al., (2007), Lamkanfi et al., (2007), Lightfield et al., (2008), Vinzing et al., (2008), Case et al., (2009), Fortier et al., (2009), Silveira and Zamboni *et al.*, (2010))
M. tuberculosis	NLRP3	ESAT-6, Ag85	K + efflux	IL-1β secretion, caspase-1 processing, prevent phagosome maturation arrest, bacterial clearance	ASC-dependent granuloma formation?	zmp1	(Koo et al., (2008), Master et al., (2008), Kurenuma et al., (2009), Carlsson et al., (2010), Mayer-Barber et al., (2010), McElvania Tekippe et al., (2010), Mishra et al., (2010), Walter et al., (2010))
L. monocytogenes	NLRC4	Flagellin	LLO	IL-1β secretion, caspase-1 processing, pyroptosis, bacterial clearance	Host survival		(Tsuji et al., (2004), Hara et al., (2008), Warren et al., (2008), Kim et al., (2010), Meixenberger et al., (2010), Rathinam et al., (2010), Sauer et al., (2010), Tsuchiya et al., (2010), Wu et al., (2010))
	NLRP3	LLO	cathepsin B? Type I IFN				
	AIM2	DNA					
F. tularensis	AIM2	DNA	Lysosomal activation, Type I IFN	IL-1β secretion, caspase-1 processing, pyroptosis,	Host survival, bacterial clearance,	mvlN	(Gavriin et al., (2006), Henry et al., (2007), Fernandes-Alnemri et al., (2010), Jones et al., (2010), Rathinam et al., (2010), Ulland et al., (2010))
B. anthracis	NLRP1	Lethal Toxin, muramyl-dipeptide	NOD2? Endosome acidification, proteasome activity, K + efflux	IL-1β secretion, caspase-1 processing, pyroptosis	Host survival		(Boyden and Dietrich *et al.*, (2006), Squires et al., (2007), Fink et al., (2008), Hsu et al., (2008), Wickliffe et al., (2008), Newman et al., (2010), Terra et al., (2010))
FUNGI							
C. albicans	NLRP3		SYK, K + efflux, ROS, cathepsin B?	IL-1β secretion, caspase-1 processing, B cell activation	Host survival, fungal clearance,		(Gross et al., (2009), Hise et al., (2009), Joly et al., (2009), Kumar et al., (2009), van de Veerdonk et al., (2009))

(Continued)

Table 1 | Continued

Organism	PRR	PAMP(s), Activator(s)	Other coactivators	Inflammasome *in vitro* function	Inflammasome *in vivo* function	Inflammasome Inhibitors	References
Plasmodium	NLRP3	Hemozoin?	SYK/LYN, cathepsin B?	IL-1β secretion, caspase-1 processing	Reduced host survival?		(Dostert et al., (2009), Griffith et al., (2009), Shio et al., (2009), Labbe et al., (2010), Reimer et al., (2010))
VIRUSES							
Influenza	NLRP3	M2 ion channel, ssRNA	K + efflux, cathepsin B, ROS	IL-1β secretion, caspase-1 processing	IL-1β secretion, Host survival, pulmonary healing?	NS1	(Schmitz et al., (2005), Stasakova et al., (2005), Allen et al., (2009), Ichinohe et al., (2009, 2010), Thomas et al., (2009), Ichinohe et al., 2010)
Vesicular stomatitis virus	RIG-I	5' triphosphate on RNA	K + efflux	IL-1β secretion, caspase-1 processing			(Poeck et al., (2010))
mCMV	AIM2	DNA		IL-1β secretion, caspase-1 processing	Reduced splenic viral titre, Increased IFN-γ NK cells		(Rathinam et al., (2010))
Vaccinia virus	AIM2	DNA		IL-1β secretion, caspase-1 processing			(Hornung et al., (2009), Rathinam et al., (2010), Fernandes-Alnemri et al., (2009))

its acidification and fusion with the lysosome (Isberg et al., 2009). Caspase-1 and Nlrc4, but not IL-1β are required to restrict *Legionella* growth in macrophages *in vitro* and in the lungs of mice *in vivo* (Zamboni et al., 2006). Nlrc4-dependent activation of caspase-1 in this case leads to macrophage pyroptosis through pore formation (Silveira and Zamboni, 2010). The absence of caspase-1 also leads to enhanced recruitment of endoplasmic reticulum to LCV, which inhibits LCV fusion with the lysosome (Amer et al., 2006).

A second NLR, Naip5, encoded by the *Birc1e* gene, is also required for flagellin recognition and host defense against *L. pneumophila* (Molofsky et al., 2006; Ren et al., 2006). Unlike human cells, murine macrophages are generally non-permissive to *Legionella* replication, with the exception of the A/J mouse line that is susceptible to infection due to polymorphisms in *Birc1e* (Diez et al., 2003; Wright et al., 2003). It has now been determined that Naip5 is involved in the recognition of a 35 amino acid motif in flagellin, and along with Nlrc4 leads to caspase-1 activation, pyroptosis, and phagosomal maturation of the LCV (Lightfield et al., 2008; **Figure 2**). Flagellin regions outside this motif can activate Nlrc4, independently of Naip5. In addition, Naip5 is only partially required for recognition of *Pseudomonas* or *Salmonella* flagellin (Lightfield et al., 2008; Miao et al., 2008). C57BL/6 mice, harboring the restrictive *Birc1e* allele, display increased bacterial burden in their lungs when infected with a flagellin-deficient strain of *Legionella*, which correlates with macrophage resistance to cell death and increased bacterial replication (Amer et al., 2006; Molofsky et al., 2006; Ren et al., 2006). The role of Naip5 in LCV acidification seems to depend on the activation of caspase-7 by caspase-1; caspase-7 promotes lysosomal fusion with the LCV and reduces bacterial replication in macrophages in an Nlrc4, Naip5, and caspase-1 dependent manner. Consistently, caspase-7-deficient mice have increased bacterial counts in their lungs after infection (Akhter et al., 2009).

The adaptor Asc is required for IL-1β secretion by *Legionella*-infected macrophages, although it has no role in the inhibition of *Legionella* replication in macrophages *in vitro* and *in vivo* (Zamboni et al., 2006; Case et al., 2009). Interestingly, Asc-mediated inflammasome activation does not lead to pyroptosis and is independent of the flagellin-Nlrc4 pathway or Nlrp3 (Case et al., 2009). Instead, it can be inhibited by high concentrations of extracellular potassium, suggesting the involvement of a third unknown inflammasome sensor in response to *Legionella*.

Interestingly, Naip5's effects in host defense to *Legionella* may be mediated by both caspase-1 dependent and independent pathways. Notably, the A/J hypomorphic Naip5 allele that is associated with permissiveness to *Legionella* replication, does not fully impair the caspase-1 pathway (Lamkanfi et al., 2007), whereas macrophages from *Naip5⁻/⁻* mice that are similarly permissive to bacterial replication, exhibit significantly blunted caspase-1 activation (Lightfield et al., 2008). Together, these studies suggest that Naip5's function in inhibiting bacterial replication is distinct from its role in caspase-1 activation. Notably, Naip5 and Nlrc4 are also able to activate Interferon regulatory factor 1 (Irf1) and Irf8, which inhibit *Legionella* replication in macrophages (Fortier et al., 2009). The human homologs of NLRC4 and NAIP5 were also demonstrated to inhibit *Legionella* replication in human macrophages and epithelial cells (Vinzing et al., 2008).
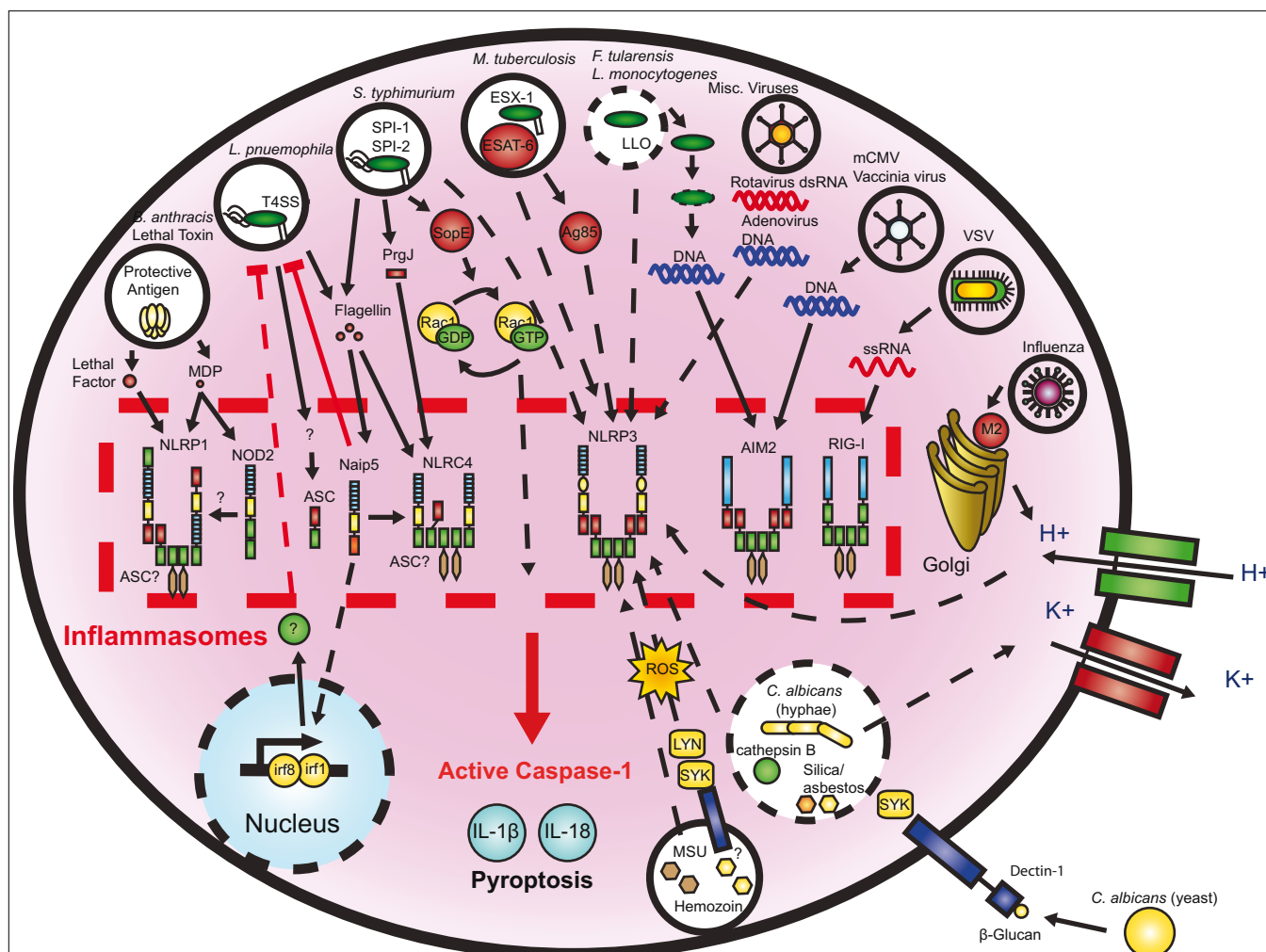
FIGURE 2 | Microbial activation of the inflammasomes. Pathogenic microorganisms activate the inflammasomes through multiple agonists and pathways. *S. typhimurium*, *L. pneumophila*, and *M. tuberculosis* reside within the host cell phagosome and are capable of activating inflammasomes through secreted flagellin, effectors, or undefined NLRP3 agonists. *F. tularensis* and *L. monocytogenes*, which escape the phagosome activate AIM2 that senses cytosolic DNA. *B. anthracis* lethal toxin activates the NLRP1 inflammasome. *C. albicans* and hemozoin activate NLRP3 through SYK signaling. Viral-mediated inflammasome activation is heavily dependent on the detection of nucleic acids by NLRP3, AIM2, and RIG-I. Dotted lines indicate signaling through an unknown mechanism.

Mycobacterium tuberculosis, the causative agent of tuberculosis in humans, survives and replicates within alveolar macrophages (Flynn and Chan, 2001). *M. tuberculosis* is capable of inflammasome activation and the Il-1r pathway is critical for the control of infection (Juffermans et al., 2000; Fremond et al., 2007). Caspase-1 and IL-1β prevent the arrest of *Mycobacteria* phagosome maturation and reduce bacterial survival in macrophages (Master et al., 2008). The ESX-1 or Type 7 Secretion System is required for IL-1β and IL-18 secretion by *M. tuberculosis*- or *M. marinum*-infected macrophages (Koo et al., 2008). Nlrp3 and Asc, but not Nlrc4, are implicated in ESX-1-induced caspase-1 activation, and the ESX-1 effector molecule ESAT-6 acts upstream of NLRP3 activation (Mishra et al., 2010; **Figure 2**). ESAT-6 functions by disrupting cell membranes and allowing for the *M. tuberculosis* protein Ag85 to enter into the cytosol and stimulate the inflammasome (Mishra et al., 2010). Interestingly, a putative Zn^{2+} metalloprotease, zmp1,

which is required for *M. tuberculosis* virulence in mice, was found to inhibit inflammasome activation and IL-1β secretion in macrophages (Master et al., 2008). *M. bovis* BCG zmp1 mutants do not block phagolysosome maturation and are eliminated by a caspase-1, Asc, and Nlrc4 dependent mechanism. Wild-type *M. bovis* BCG induced low levels of IL-1β in macrophages, which may be explained by the lack of the ESX-1 secretion system. However, the precise molecular mechanism by which zmp1 impacts caspase-1 or whether this inhibition occurs *in vivo* remain to be determined.

Recent *in vivo* mouse studies have begun to determine the role of the inflammasome pathway in *Mycobacteria* infection. In one study, Nlrp3-deficient mice infected with *M. tuberculosis* did not exhibit enhanced histopathology or increased bacterial burden in the lungs, despite reduced IL-1β and IL-18 levels (Walter et al., 2010). Consistently, a second report demonstrated that ablation of either Nlrp3 or caspase-1 did not affect mouse mortality following

M. tuberculosis infection. Surprisingly, however, a third study demonstrated that Asc, but not caspase-1 or Nlrp3, promoted mouse survival (McElvania Tekippe et al., 2010). Asc-mediated protection was independent of IL-1β levels in the lung or bacterial clearance. Instead, Asc was required to contain *M. tuberculosis* within granulomas, suggesting that Asc has important caspase-1-independent roles during infection. In striking contrast, a study using *M. marinum* found that Asc promoted lung pathology, as Asc-deficient mice had reduced inflammation and lung histophathology (Carlsson et al., 2010). The presence of the ESX-1 secretion system triggered this inflammatory response through an Asc-dependent mechanism. The different *Mycobacteria* species combined with a different infectious dose and method (tail injection versus aerosol in all other studies) may explain the differences observed here with regard to Asc. Given the conflicting data in these studies, further work is required to clarify the role of inflammasome signaling during *M. tuberculosis* infection *in vivo*. Interestingly, *casp1*$^{-/-}$ mice retain the ability to produce mature biologically active IL-1β during *M. tuberculosis* infection, suggesting that other redundant mechanisms are capable of generating IL-1β (Mayer-Barber et al., 2010). Caspase-1-independent IL-1β processing in response to *M. tuberculosis* was found *in vivo*, but not *in vitro*, underlining the importance of animal models when examining responses to infection. Overall, these studies indicate that the inflammasomes and ASC in particular have additional roles during infection with *Mycobacteria* species, possibly independent of IL-1 and IL-18 secretion. Moreover, enzymes other than caspase-1, presumably neutrophil elastase or other proteases, are also deployed *in vivo* for the generation of the key IL-1 family cytokines.

Francisella tularensis is an intracellular bacterium responsible for tularaemia in humans. Following, phagocytosis by host macrophages, the bacteria escape from the phagosome into the cytosol and replicate in this compartment (Santic et al., 2006; **Figure 2**). Early studies have demonstrated that caspase-1 and Asc, but not Nlrp3 and Nlrc4, were essential for macrophage cell death, bacterial clearance *in vitro* and *in vivo* and mouse survival (Mariathasan et al., 2005). This suggested that caspase-1 activation was mediated by a different inflammasome that required Asc. It was recently discovered that absent in melanoma 2 (AIM2), a member of the hematopoietic interferon-inducible nuclear protein (HIN-200) family that senses cytosolic DNA was the primary inflammasome triggered during *F. tularensis* infection (Fernandes-Alnemri et al., 2010; Jones et al., 2010; Rathinam et al., 2010). Lysosomal activation was shown to be required to stimulate this inflammasome during infection, possibly due to lysis of bacteria, which allows the release of DNA into the cytosol (Fernandes-Alnemri et al., 2010; Jones et al., 2010).

In contrast to previously discussed bacterial pathogens, caspase-1 activation in response to *F. novicida* requires IRF3-dependent Type I IFN signaling (Henry et al., 2007). *Salmonella*, which resides in vacuoles within the macrophage, does not require Type I IFN, but the cytosolic bacteria *L. monocytogenes* does (Henry et al., 2007). Type I IFN was demonstrated to increase AIM2 expression and hence AIM2-dependent caspase-1 activation, which was reduced in *Irf3*$^{-/-}$ macrophages (Fernandes-Alnemri et al., 2010; Jones et al., 2010). Interestingly, while phagosome lysis was required for AIM2 sensing, NLRP3 thought to be activated by vacuole lysis, was not

activated in *Francisella* infection (Fernandes-Alnemri et al., 2010). Notably, the *F. tularensis* protein mvIN, which promotes virulence, exerts its effects by inhibiting AIM2-mediated caspase-1 activation (Ulland et al., 2010).

Similarly to *Francisella*, *L. monocytogenes* escapes from the phagosome into the cytosol, through the membrane-disrupting enzyme listeriolysin O (LLO). *In vivo*, caspase-1-deficient mice display reduced survival following infection and could be partially rescued through exogenous IL-18 administration (Tsuji et al., 2004), suggesting that inflammasome signaling is protective during infection. Macrophage infection results in caspase-1 and Asc-dependent cell death, with involvement of AIM2, Nlrp3, and Nlrc4 (Kim et al., 2010; Sauer et al., 2010). LLO is required for cell death and LLO-deficient strains, which remain in the phagosome, are unable to activate caspase-1 or induce IL-1β secretion (Mariathasan et al., 2006; Ozoren et al., 2006; Cervantes et al., 2008). Purified LLO can directly induce Nlrp3-dependent IL-1β secretion (Meixenberger et al., 2010; Sauer et al., 2010). Interestingly, however, entry into the cytosol alone is not sufficient to trigger caspase-1 activation. Indeed, LLO substitution with the *L. ivanovii* homolog ivanolysin O is not permissive for inflammasome activation (Hara et al., 2008), suggesting that LLO may have additional roles in this process.

Different reports have implicated the AIM2, NLRC4, and NLRP3 inflammasomes to varying degrees in the response to *Listeria* infection, however there have been some conflicting evidence. The earliest study identified Nlrp3 and Asc as essential for caspase-1 activation and IL-1β secretion in macrophages (Mariathasan et al., 2006). The role of these inflammasomes was confirmed along with Nlrc4, which was implicated in the detection of *Listeria* flagellin, and also dependent on LLO (Warren et al., 2008). Asc-deficient macrophages infected with flagellin deleted strains resulted in reduced IL-1β secretion than similarly infected Nlrp3 null macrophages, suggesting that an unknown inflammasome, signaling through Asc, was required. Similarly to recognition of *Francisella*, AIM2 was found to activate caspase-1 and induce IL-1β secretion (Kim et al., 2010; Rathinam et al., 2010; Sauer et al., 2010; Wu et al., 2010). Lysis of the bacteria is important for inflammasome recognition as treatment of intracellular bacteria with β-lactam antibiotics or the use of mutant bacteria that undergo rapid lysis in the cytosol resulted in increased inflammasome activation and pyroptosis (Sauer et al., 2010). *L. monocytogenes* DNA was also found to colocalize with ASC pyroptosomes (Warren et al., 2010). Another study using siRNA depletion in human PBMCs determined that NLRP3 was required for IL-1β secretion, but not AIM2 or NLRC4 (Meixenberger et al., 2010). The *Listeria* strain used however, expresses lower levels of flagellin, which may have resulted in the lack of requirement for NLRC4. Similarly other studies have implicated Nlrc4 along with Aim2, Nlrp3, and Asc (Tsuchiya et al., 2010; Wu et al., 2010), while others did not find that it played a role (Kim et al., 2010). Inflammasomes appear to have a protective effect during *L. monocytogenes* infection, through pyroptosis. Interestingly, *Listeria* attempts to evade inflammasome recognition by downregulating activators such as flagellin at 37°C (Shen and Higgins, 2006).

While some pathogenic organisms evade the immune system through an intracellular lifestyle, other pathogenic bacteria survive outside of the cell. Extracellular species often secrete toxins that

can cause tissue damage and inflammation. Upon attachment and adhesion to surfaces, such as epithelial cells, extracellular bacteria often produce a biofilm, a polysaccharide rich matrix that provides protection from leucocytes and components of the immune system. *B. anthracis*, is a well known extracellular bacterial pathogen that is the cause of anthrax. The bacteria secrete lethal toxin (LeTx), which is composed of the protective antigen (PA) and the zinc-dependent metalloprotease lethal factor (LF) subunits (Collier and Young, 2003). PA mediates toxin entry into the cytosol while LF cleaves MAPK to disrupt cell signaling (Duesbery et al., 1998). Polymorphisms in the gene encoding Nlrp1 confer susceptibility or resistance to LeTx in mouse macrophages. LeTx is responsible for caspase-1-dependent pyroptosis in murine macrophages through activation of the Nlrp1 inflammasome (Boyden and Dietrich, 2006), but the adaptor Asc is not required in this process (Reig et al., 2008). The exact mechanism by which LeTx triggers Nlrp1 activation is currently unknown. Endosome acidification, proteasome activity, and potassium efflux are required for cell death (Squires et al., 2007; Fink et al., 2008; Wickliffe et al., 2008). Nlrp1-mediated cell lysis appears to have a protective effect in rats as it reduces mortality to infection with *B. anthracis* (Newman et al., 2010). *B. anthracis* spores are also capable of inducing caspase-1 activation through Nlrp1 *in vivo*, which confers protection in mice (Terra et al., 2010). Human NLRP1 has also been shown to recognize the bacterial cell wall product muramyl-dipeptide (Faustin et al., 2007).

In summary, multiple inflammasomes have been implicated in response to intracellular and extracellular bacteria. While this can often lead to pyroptotic cell death, the overall effect in mouse models suggests that caspase-1 activation and IL-1β and IL-18 secretion promote bacterial clearance and host survival. Caspase-1-mediated pyroptosis of cells infected with intracellular bacteria may play a beneficial role in eliminating their replicative niche. The discovery of bacterial proteins that inhibit the inflammasomes, such as *P. aeruginosa* ExoU (Sutterwala et al., 2007) and *M. tuberculosis* zmp1 (Master et al., 2008) further indicates that the effects of caspase-1 are likely beneficial for the host rather than the pathogen. Activation can be initiated by flagellin, secretion system components, and bacterial effector molecules injected in the host cell cytosol through these secretion systems. These signals are relatively conserved and it is likely that the NLRs have evolved to detect these distinct molecules. The most recent evidence suggests that there is significant redundancy in inflammasome activation during bacterial infections. In addition to promoting host defense against pathogenic bacteria, the inflammasome has been recently implicated in sensing commensal microorganisms in the gut and maintaining intestinal homeostasis and immune tolerance to the microflora. In addition, upon tissue damage, Nlrp3 inflammasome-dependent production of IL-18 was shown to be required for tissue repair and protection from colitis and colitis-associated colorectal cancer (Allen et al., 2010; Dupaul-Chicoine et al., 2010; Saleh and Trinchieri, 2011; Zaki et al., 2010).

VIRUSES

Viruses infect and replicate within host cells, utilizing the host cellular machinery to manufacture viral particles. In mammals, both the innate and adaptive branches of the immune system play an important role in fighting viral infections. Different PRRs are capable of recognizing viral motifs, primarily those with nucleic acids, and initiate type I interferon production which inhibits viral replication (Saito and Gale Jr., 2007). Multiple inflammasomes including NLRP3, AIM2, and RIG-I have been linked to viral infections (**Table 1**).

Prior to the discovery of the inflammasome, it has been reported that influenza infection induced IL-1 production by infected macrophages and in mice (Hennet et al., 1992; Pirhonen et al., 1999), which mediated mouse survival by enhancing adaptive immunity to the virus (Schmitz et al., 2005). The Nlrp3 inflammasome was subsequently identified to play a role in influenza and Sendai virus infection in macrophages (Kanneganti et al., 2006). *In vitro*, the NLRP3 inflammasome is triggered by various viruses and viral PAMPs, including viral RNA analogs poly(I:C) and ssRNA40 (Kanneganti et al., 2006; Allen et al., 2009), transfected adenovirus DNA (Muruve et al., 2008), and rotavirus dsRNA (Kanneganti et al., 2006). However, *in vivo*, there have been some conflicting findings as to the role of the inflammasome, and the involvement of its different components, in influenza virus infection. Notably, Asc, caspase-1, and Nlrp3, but not Nlrc4 were shown to be essential for mouse survival (Allen et al., 2009; Thomas et al., 2009), but this result was only reproduced for Asc, caspase-1 and IL-1R, but not Nlrp3 (Ichinohe et al., 2009). These discrepancies may be largely due to different infectious doses. Higher infectious doses (>1000 pfu) trigger marked inflammatory cell infiltration to the airways, pulmonary necrosis and fibrosis, that are reduced in Nlrp3 and caspase-1 deficient mice (Allen et al., 2009; Thomas et al., 2009), suggesting a role of the inflammasome in lung tissue healing. Lower infectious doses (10 pfu), on the other hand, are less injurious, which allows for the examination of inflammasome function in immune responses. Under these conditions, *casp1*$^{-/-}$ and *Asc*$^{-/-}$ mice exhibit reduced CD4 and CD8 T cell responses and decreased immunoglobulin titers (Ichinohe et al., 2009). A recently discovered influenza M2 ion channel was demonstrated to localize to the host cell Golgi and activate Nlrp3 by inducing cytosolic acidification (Ichinohe et al., 2010).

Vesicular stomatitis virus (VSV), another RNA virus, is recognized by the intracellular receptor with RNA helicase activity RIG-I. RIG-I detects 5′ triphosphate RNA as a viral signature and induces type I IFN production and NF-κB activation through recruitment of the adaptor protein MAVS (Nakhaei et al., 2009). Interestingly, infection with VSV also triggers IL-1β secretion in a RIG-I-dependent manner (Poeck et al., 2010). ASC interacts with RIG-I and is required for VSV-induced caspase-1 activation, which requires potassium efflux, but not Type I IFN. These results indicate that RIG-I serves multiple roles during the innate immune response to viruses, as it triggers Type I IFN expression and activates the NF-κB and caspase-1 pro-inflammatory pathways.

DNA viruses are also capable of activating caspase-1 through the AIM2 inflammasome. As previously discussed, AIM2 detects cytosolic DNA, and is engaged in response to vaccinia virus and mouse cytomegalovirus (mCMV), but interestingly not herpes simplex virus type 1 (HSV-1) (Rathinam et al., 2010). Similarly to its response to bacterial DNA, AIM2 requires ASC, but not NLRP3 (Hornung et al., 2009; Rathinam et al., 2010). mCMV-infected AIM2 deficient mice have reduced IL-18 serum levels that correlate with a reduced population of IFNγ+ NK cells. IL-18 is a known

activator of IFNγ production in NK cells (Hyodo et al., 1999; Chaix et al., 2008), which play an important role in eliminating tumor or virus-infected cells, and is likely one of the primary contributors to the higher viral titers found in the spleen of AIM2-deficient mice (Rathinam et al., 2010).

Caspase-1-induced inflammation and pyroptosis are deleterious for virus replication and dissemination. Consequently, viruses have evolved proteins to target caspase-1 function and its downstream signaling, including a poxvirus PYD containing protein (Johnston et al., 2005) and influenza NS1 (Stasakova et al., 2005).

PARASITES

The protozoan *Plasmodium* genus is the causative agent of malaria, which is endemic to the tropics and sub-Saharan Africa. The parasite is transmitted by mosquito bite into the host, where after an incubation period, replicates within erythrocytes (Sachs and Malaney, 2002). The breakdown of hemoglobin by *Plasmodium* results in the release of heme, which is then converted into the crystalline product hemozoin (Hanscheid et al., 2007). Recent work has suggested that the NLRP3 inflammasome might play a role in the recognition of hemozoin. Hemozoin was reported to activate caspase-1 and induce IL-1β secretion *in vitro*, in an ASC and NLRP3, but not NLRC4, dependent manner (Dostert et al., 2009; Shio et al., 2009), but this was not reproduced in one study (Griffith et al., 2009). Mechanistically, hemozoin is not likely to be directly recognized by NLRP3, but instead activates the inflammasome through other signals, such as uric acid crystal release (Griffith et al., 2009) and/or activation of cathepsin B downstream of the SYK and LYN kinases (Shio et al., 2009). The role of cathepsin B is controversial however, as cathepsin B-deficient macrophages did not exhibit a defect in caspase-1 activation in response to hemozoin (Dostert et al., 2009). Interestingly, deficiency in Nlrp3 but not Asc or caspase-1 increased mouse survival after injection with red blood cells infected with the mouse virulent strain *P. chabaudi adami* (Shio et al., 2009). Another mouse model of cerebral malaria using *P. berghei* similarly showed that *Nlrp3⁻/⁻* mice, but not *Asc⁻/⁻*, *casp1⁻/⁻*, or *Il1b⁻/⁻* mice, had a greater time to death than wild-type animals (Reimer et al., 2010). This suggests that NLRP3 may have a deleterious role in cerebral malaria, independently of caspase-1 and IL-1β. Consistently, we have shown that *casp1⁻/⁻* mice were equivalent to wild-type mice in their response to the *P. chabaudi* and *P. berghei* models of malaria (Labbe et al., 2010). In contrast, mice deficient in caspase-12, an inhibitor of caspase-1 and NF-κB, showed enhanced inflammatory and immune response to the *Plasmodium* parasites and were susceptible to cerebral malaria. This phenotype was, however, independent of caspase-1, but resulted from excessive NF-κB activation and IFNγ production (Labbe et al., 2010). *Plasmodium* is currently the only parasite characterized with respect to inflammasome activation, and the study of other protozoa and helminths may lead to a better understanding of the immune response to these organisms and to insights into unknown functions of the inflammasome and NLRs.

FUNGI

Fungi are eukaryotic organisms that often cause opportunistic infections in immuno-compromised hosts. The most common cause of fungal infections is *Candida albicans*, a commensal that resides in the majority of the human population. It exists as a unicellular yeast or a filamentous hyphae, the latter associated with invasion (Lo et al., 1997). *C. albicans* and other opportunistic fungi are cleared in healthy individuals and various PRRs, such as TLRs and CLRs have been implicated as important players in the immune response to fungi (Netea and Marodi, 2010). IL-1β is essential in host defense in candidiasis (Vonk et al., 2006) and recently the inflammasome has been linked to this response.

Caspase-1, IL-1R, ASC, and NLRP3, have been implicated in controlling fungal dissemination and host survival in mice (Gross et al., 2009; Hise et al., 2009; Joly et al., 2009). Caspase-1, Asc, or Il-1r deficient mice have reduced survival and increased organ fungal burden upon *C. albicans* infection (Hise et al., 2009). *Nlrp3⁻/⁻* mice have increased mortality following infection (Gross et al., 2009) and higher tissue colony counts (Gross et al., 2009; Hise et al., 2009; Joly et al., 2009). The fungal cell wall component β-glucan is recognized by the CLR, dectin-1, which recruits SYK via phosphorylated ITAMs in its cytoplasmic tail leading to synthesis of pro-IL-1β and NLRP3 inflammasome components (Rogers et al., 2005; Gross et al., 2009; Hise et al., 2009; Kumar et al., 2009; van de Veerdonk et al., 2009). TLR2 has also been implicated in this recognition (Hise et al., 2009; van de Veerdonk et al., 2009). While these receptors provide signal 1 toward NLRP3 activation, signal 2 is mediated by potassium efflux and ROS production (Gross et al., 2009). Consistently, *Syk⁻/⁻* dendritic cells display reduced caspase-1 activation and production of IL-1β in response to *C. albicans*, but not *Salmonella*, ATP or nigericin (Gross et al., 2009). The role of cathepsin B in *C. albicans* inflammasome activation is unclear as IL-1β secretion from cathepsin B-deficient dendritic cells was unaffected in one study (Gross et al., 2009), while cell treatment with the cathepsin B cell permeable inhibitor CA-074-Me caused a significant decrease in secreted IL-1β in another (Joly et al., 2009).

Interestingly, the transition from the yeast to the filamentous form of *C. albicans* is required for Nlrp3 inflammasome activation, as mutant fungi unable to transform to the hyphae stage trigger blunted IL-1β production (Joly et al., 2009). The hyphae form is able to disrupt the phagosome (Lo et al., 1997), and it is hypothesized that this event is sensed as signal 2. The observation that cathepsin B inhibitors reduced IL-1β secretion from macrophages (Joly et al., 2009) supports this hypothesis as vacuole rupture and cathepsin B activity have been linked to NLRP3 activation (Hornung et al., 2008). Notably, UV-inactivated *C. albicans* yeast cells are unable to activate the inflammasome (Hise et al., 2009; Joly et al., 2009). However, in human PBMCs heat-killed yeast cells could induce IL-1β secretion (van de Veerdonk et al., 2009), presumably due to the ability of these cells to produce excessive levels of ATP which activates the inflammasome in the presence of a signal 1, provided in this case by the heat-killed cells.

Interestingly, treatment of B cells with β-glucan resulted in Nlrp3 activation and antibody production. This occurred independently of IL-1R signaling, as determined by the use of *Myd88⁻/⁻* cells. This is suggestive of a link between NLRP3 and the adaptive immune system in response to fungal infections (Kumar et al., 2009). While the large majority of studies on the role of inflammasomes in fungal infections has been discovered using *C. albicans*, another opportunistic fungus, *Aspergillus fumigates*, was also found to activate the

NLRP3 inflammasome via similar mechanisms (Said-Sadier et al., 2010). Altogether, these studies indicate that the NLRP3 inflammasome plays a protective role against fungal infections.

CONCLUSION

Innate immunity and inflammation are important for the host defense against invading microbes. The rapidly expanding number of studies on the inflammasomes has begun to unravel their roles in response to bacterial, parasitic, fungal, and viral infections. Current evidence supports that inflammasome activation during infection confers protection to the host through various mechanisms including pyroptosis, pro-inflammatory cytokine secretion, and stimulation of the adaptive immune system. While pyroptosis of infected macrophages may intuitively seem deleterious for the host, it can permit exposure of intracellular bacteria to other bactericidal host factors. Consequently many pathogens have evolved different mechanisms to inhibit inflammasome activation and function. There have been many recent advances in understanding the different motifs that are recognized by the inflammasomes and how they work. However, much remains to be done for a full understanding of the molecular events linking cellular perturbations and DAMP sensing to inflammasome activation. While the majority of studies researching the inflammasomes have focused on the NLRP3 and NLRC4 complexes, the recent discoveries of AIM2 and RIG-I in caspase-1 activation open a new window of investigation. Moreover, it is likely that additional inflammasomes have not yet been discovered. Importantly, it is becoming clear that while NLRs and ASC assemble inflammasomes, their functions are not always necessarily mediated by the caspase-1 pathway. Similarly, caspase-1 dependent effector cytokines could be produced by other proteases during infection. Additionally, NLR signaling pathways are significantly redundant and overlapping with those of other PRRs. Therefore, a stronger understanding of inflammasome-mediated innate immune responses is warranted toward the development of therapeutic strategies for infectious disease.

REFERENCES

Akhter, A., Gavrilin, M. A., Frantz, L., Washington, S., Ditty, C., Limoli, D., Day, C., Sarkar, A., Newland, C., Butchar, J., Marsh, C. B., Wewers, M. D., Tridandapani, S., Kanneganti, T. D., and Amer, A. O. (2009). Caspase-7 activation by the Nlrc4/Ipaf inflammasome restricts *Legionella pneumophila* infection. *PLoS Pathog.* 5, e1000361. doi: 10.1371/journal.ppat.1000361

Alcami, A., and Smith, G. L. (1992). A soluble receptor for interleukin-1 beta encoded by vaccinia virus: a novel mechanism of virus modulation of the host response to infection. *Cell* 71, 153–167.

Allen, I. C., Scull, M. A., Moore, C. B., Holl, E. K., Mcelvania-Tekippe, E., Taxman, D. J., Guthrie, E. H., Pickles, R. J., and Ting, J. P. (2009). The NLRP3 inflammasome mediates in vivo innate immunity to influenza A virus through recognition of viral RNA. *Immunity* 30, 556–565.

Allen, I. C., Tekippe, E. M., Woodford, R. M. T., Uronis, J. M., Holl, E. K., Rogers, A. B., Herfarth, H. H., Jobin, C., and Ting, J. P. Y. (2010). The NLRP3 inflammasome functions as a negative regulator of tumorigenesis during colitis-associated cancer. *J. Exp. Med.* 207, 1045–1056.

Amer, A., Franchi, L., Kanneganti, T. D., Body-Malapel, M., Ozoren, N., Brady, G., Meshinchi, S., Jagirdar, R., Gewirtz, A., Akira, S., and Nunez, G. (2006). Regulation of *Legionella* phagosome maturation and infection through flagellin and host Ipaf. *J. Biol. Chem.* 281, 35217–35223.

Arlehamn, C. S., Petrilli, V., Gross, O., Tschopp, J., and Evans, T. J. (2010). The role of potassium in inflammasome activation by bacteria. *J. Biol. Chem.* 285, 10508–10518.

Belkhadir, Y., Subramaniam, R., and Dangl, J. L. (2004). Plant disease resistance protein signaling: NBS-LRR proteins and their partners. *Curr. Opin. Plant Biol.* 7, 391–399.

Bianchi, M. E. (2007). DAMPs, PAMPs and alarmins: all we need to know about danger. *J. Leukoc. Biol.* 81, 1–5.

Boyden, E. D., and Dietrich, W. F. (2006). Nalp1b controls mouse macrophage susceptibility to anthrax lethal toxin. *Nat. Genet.* 38, 240–244.

Brodsky, I. E., Palm, N. W., Sadanand, S., Ryndak, M. B., Sutterwala, F. S., Flavell, R. A., Bliska, J. B., and Medzhitov, R. (2010). A *Yersinia* effector protein promotes virulence by preventing inflammasome recognition of the type III secretion system. *Cell Host Microbe* 7, 376–387.

Broz, P., Newton, K., Lamkanfi, M., Mariathasan, S., Dixit, V. M., and Monack, D. M. (2010). Redundant roles for inflammasome receptors NLRP3 and NLRC4 in host defense against *Salmonella*. *J. Exp. Med.* 207, 1745–1755.

Bruey, J. M., Bruey-Sedano, N., Luciano, F., Zhai, D., Balpai, R., Xu, C., et al. (2007). Bcl-2 and Bcl-XL regulate proinflammatory caspase-1 activation by interaction with NALP1. *Cell* 129, 45–56.

Carlsson, F., Kim, J., Dumitru, C., Barck, K. H., Carano, R. A., Sun, M., Diehl, L., and Brown, E. J. (2010). Host-detrimental role of Esx-1-mediated inflammasome activation in mycobacterial infection. *PLoS Pathog.* 6, e1000895. doi: 10.1371/journal.ppat.1000895

Case, C. L., Shin, S., and Roy, C. R. (2009). Asc and Ipaf Inflammasomes direct distinct pathways for caspase-1 activation in response to *Legionella pneumophila*. *Infect. Immun.* 77, 1981–1991.

Cassel, S. L., Eisenbarth, S. C., Iyer, S. S., Sadler, J. J., Colegio, O. R., Tephly, L. A., Carter, A. B., Rothman, P. B., Flavell, R. A., and Sutterwala, F. S. (2008). The Nalp3 inflammasome is essential for the development of silicosis. *Proc. Natl. Acad. Sci. U.S.A.* 105, 9035–9040.

Cervantes, J., Nagata, T., Uchijima, M., Shibata, K., and Koide, Y. (2008). Intracytosolic *Listeria monocytogenes* induces cell death through caspase-1 activation in murine macrophages. *Cell. Microbiol.* 10, 41–52.

Chaix, J., Tessmer, M. S., Hoebe, K., Fuseri, N., Ryffel, B., Dalod, M., Alexopoulou, L., Beutler, B., Brossay, L., Vivier, E., and Walzer, T. (2008). Cutting edge: priming of NK cells by IL-18. *J. Immunol.* 181, 1627–1631.

Chamaillard, M., Hashimoto, M., Horie, Y., Masumoto, J., Qiu, S., Saab, L., Ogura, Y., Kawasaki, A., Fukase, K., Kusumoto, S., Valvano, M. A., Foster, S. J., Mak, T. W., Nunez, G., and Inohara, N. (2003). An essential role for NOD1 in host recognition of bacterial peptidoglycan containing diaminopimelic acid. *Nat. Immunol.* 4, 702–707.

Coers, J., Vance, R. E., Fontana, M. F., and Dietrich, W. F. (2007). Restriction of *Legionella pneumophila* growth in macrophages requires the concerted action of cytokine and Naip5/Ipaf signalling pathways. *Cell. Microbiol.* 9, 2344–2357.

Collier, R. J., and Young, J. A. (2003). Anthrax toxin. *Annu. Rev. Cell Dev. Biol.* 19, 45–70.

Craven, R. R., Gao, X., Allen, I. C., Gris, D., Bubeck Wardenburg, J., Mcelvania-Tekippe, E., Ting, J. P., and Duncan, J. A. (2009). *Staphylococcus aureus* α-hemolysin activates the NLRP3-inflammasome in human and mouse monocytic cells. *PLoS ONE* 4, e7446. doi: 10.1371/journal.pone.0007446

Diez, E., Lee, S. H., Gauthier, S., Yaraghi, Z., Tremblay, M., Vidal, S., and Gros, P. (2003). Birc1e is the gene within the Lgn1 locus associated with resistance to *Legionella pneumophila*. *Nat. Genet.* 33, 55–60.

Dorfleutner, A., Bryan, N. B., Talbott, S. J., Funya, K. N., Rellick, S. L., Reed, J. C., Shi, X., Rojanasakul, Y., Flynn, D. C., and Stehlik, C. (2007). Cellular pyrin domain-only protein 2 is a candidate regulator of inflammasome activation. *Infect. Immun.* 75, 1484–1492.

Dostert, C., Guarda, G., Romero, J. F., Menu, P., Gross, O., Tardivel, A., Suva, M. L., Stehle, J. C., Kopf, M., Stamenkovic, I., Corradin, G., and Tschopp, J. (2009). Malarial hemozoin is a Nalp3 inflammasome activating danger signal. *PLoS ONE* 4, e6510. doi: 10.1371/journal.pone.0006510

Dostert, C., Petrilli, V., Van Bruggen, R., Steele, C., Mossman, B. T., and Tschopp, J. (2008). Innate immune activation through Nalp3 inflammasome sensing of asbestos and silica. *Science* 320, 674–677.

Duesbery, N. S., Webb, C. P., Leppla, S. H., Gordon, V. M., Klimpel, K. R., Copeland, T. D., Ahn, N. G., Oskarsson, M. K., Fukasawa, K., Paull, K. D., and Vande Woude, G. F. (1998). Proteolytic inactivation of MAP-kinase-kinase by anthrax lethal factor. *Science* 280, 734–737.

Dupaul-Chicoine, J., Yeretssian, G., Doiron, K., Bergstrom, K. S., Mcintire, C. R., Leblanc, P. M., Meunier, C., Turbide, C., Gros, P., Beauchemin, N., Vallance, B. A., and Saleh, M. (2010). Control of intestinal homeostasis, colitis, and colitis-associated colorectal cancer by the inflammatory caspases. *Immunity* 32, 367–378.

Faustin, B., Chen, Y., Zhai, D. Y., Le Negrate, G., Lartigue, L., Satterthwait,

A., and Reed, J. C. (2009). Mechanism of Bcl-2 and Bcl-X-L inhibition of NLRP1 inflammasome: loop domain-dependent suppression of ATP binding and oligomerization. *Proc. Natl. Acad. Sci. U.S.A.* 106, 3935–3940.

Faustin, B., Lartigue, L., Bruey, J. M., Luciano, F., Sergienko, E., Bailly-Maitre, B., Volkmann, N., Hanein, D., Rouiller, I., and Reed, J. C. (2007). Reconstituted NALP1 inflammasome reveals two-step mechanism of caspase-1 activation. *Mol. Cell* 25, 713–724.

Fernandes-Alnemri, T., Yu, J. W., Datta, P., Wu, J., and Alnemri, E. S. (2009). AIM2 activates the inflammasome and cell death in response to cytoplasmic DNA. *Nature* 458, 509–513.

Fernandes-Alnemri, T., Yu, J. W., Juliana, C., Solorzano, L., Kang, S., Wu, J., Datta, P., Mccormick, M., Huang, L., Mcdermott, E., Eisenlohr, L., Landel, C. P., and Alnemri, E. S. (2010). The AIM2 inflammasome is critical for innate immunity to *Francisella tularensis*. *Nat. Immunol.* 11, 385–393.

Fink, S. L., Bergsbaken, T., and Cookson, B. T. (2008). Anthrax lethal toxin and *Salmonella* elicit the common cell death pathway of caspase-1-dependent pyroptosis via distinct mechanisms. *Proc. Natl. Acad. Sci. U.S.A.* 105, 4312–4317.

Fink, S. L., and Cookson, B. T. (2005). Apoptosis, pyroptosis, and necrosis: mechanistic description of dead and dying eukaryotic cells. *Infect. Immun.* 73, 1907–1916.

Flynn, J. L., and Chan, J. (2001). Immunology of tuberculosis. *Annu. Rev. Immunol.* 19, 93–129.

Fortier, A., de Chastellier, C., Balor, S., and Gros, P. (2007). Birc1e/Naip5 rapidly antagonizes modulation of phagosome maturation by *Legionella pneumophila*. *Cell. Microbiol.* 9, 910–923.

Fortier, A., Doiron, K., Saleh, M., Grinstein, S., and Gros, P. (2009). Restriction of *Legionella pneumophila* replication in macrophages requires concerted action of the transcriptional regulators Irf1 and Irf8 and nod-like receptors Naip5 and Nlrc4. *Infect. Immun.* 77, 4794–4805.

Franchi, L., Amer, A., Body-Malapel, M., Kanneganti, T. D., Ozoren, N., Jagirdar, R., Inohara, N., Vandenabeele, P., Bertin, J., Coyle, A., Grant, E. P., and Nunez, G. (2006). Cytosolic flagellin requires Ipaf for activation of caspase-1 and interleukin 1beta in *Salmonella*-infected macrophages. *Nat. Immunol.* 7, 576–582.

Franchi, L., Kanneganti, T. D., Dubyak, G. R., and Nunez, G. (2007). Differential requirement of P2X7 receptor and intracellular K+ for caspase-1 activation induced by intracellular and extracellular bacteria. *J. Biol. Chem.* 282, 18810–18818.

Fremond, C. M., Togbe, D., Doz, E., Rose, S., Vasseur, V., Maillet, I., Jacobs, M., Ryffel, B., and Quesniaux, V. F. (2007). IL-1 receptor-mediated signal is an essential component of MyD88-dependent innate response to *Mycobacterium tuberculosis* infection. *J. Immunol.* 179, 1178–1189.

Gabay, C., Lamacchia, C., and Palmer, G. (2010). IL-1 pathways in inflammation and human diseases. *Nat. Rev. Rheumatol.* 6, 232–241.

Gavrilin, M. A., Bouakl, I. J., Knatz, N. L., Duncan, M. D., Hall, M. W., Gunn, J. S., and Wewers, M. D. (2006). Internalization and phagosome escape required for *Francisella* to induce human monocyte IL-1beta processing and release. *Proc. Natl. Acad. Sci. U.S.A.* 103, 141–146.

Ghayur, T., Banerjee, S., Hugunin, M., Butler, D., Herzog, L., Carter, A., Quintal, L., Sekut, L., Talanian, R., Paskind, M., Wong, W., Kamen, R., Tracey, D., and Allen, H. (1997). Caspase-1 processes IFN-gamma-inducing factor and regulates LPS-induced IFN-gamma production. *Nature* 386, 619–623.

Girardin, S. E., Boneca, I. G., Carneiro, L.a.M., Antignac, A., Jehanno, M., Viala, J., Tedin, K., Taha, M. K., Labigne, A., Zahringer, U., Coyle, A. J., Bertin, J., Sansonetti, P. J., and Philpott, D. J. (2003). Nod1 detects a unique muropeptide from Gram-negative bacterial peptidoglycan. *Science* 300, 1584–1587.

Griffith, J. W., Sun, T., McIntosh, M. T., and Bucala, R. (2009). Pure hemozoin is inflammatory in vivo and activates the NALP3 inflammasome via release of uric acid. *J. Immunol.* 183, 5208–5220.

Gross, O., Poeck, H., Bscheider, M., Dostert, C., Hannesschlager, N., Endres, S., Hartmann, G., Tardivel, A., Schweighoffer, E., Tybulewicz, V., Mocsai, A., Tschopp, J., and Ruland, J. (2009). Syk kinase signalling couples to the Nlrp3 inflammasome for anti-fungal host defence. *Nature* 459, 433–436.

Gu, Y., Kuida, K., Tsutsui, H., Ku, G., Hsiao, K., Fleming, M. A., Hayashi, N., Higashino, K., Okamura, H., Nakanishi, K., Kurimoto, M., Tanimoto, T., Flavell, R. A., Sato, V., Harding, M. W., Livingston, D. J., and Su, M. S. S. (1997). Activation of interferon-gamma inducing factor mediated by interleukin-1 beta converting enzyme. *Science* 275, 206–209.

Halle, A., Hornung, V., Petzold, G. C., Stewart, C. R., Monks, B. G., Reinheckel, T., Fitzgerald, K. A., Latz, E., Moore, K. J., and Golenbock, D. T. (2008). The NALP3 inflammasome is involved in the innate immune response to amyloid-beta. *Nat. Immunol.* 9, 857–865.

Hanscheid, T., Egan, T. J., and Grobusch, M. P. (2007). Haemozoin: from melatonin pigment to drug target, diagnostic tool, and immune modulator. *Lancet Infect. Dis.* 7, 675–685.

Hara, H., Tsuchiya, K., Nomura, T., Kawamura, I., Shoma, S., and Mitsuyama, M. (2008). Dependency of caspase-1 activation induced in macrophages by *Listeria monocytogenes* on cytolysin, listeriolysin O, after evasion from phagosome into the cytoplasm. *J. Immunol.* 180, 7859–7868.

Haraga, A., Ohlson, M. B., and Miller, S. I. (2008). Salmonellae interplay with host cells. *Nat. Rev. Microbiol.* 6, 53–66.

Hardt, W. D., Chen, L. M., Schuebel, K. E., Bustelo, X. R., and Galan, J. E. (1998). *S. typhimurium* encodes an activator of Rho GTPases that induces membrane ruffling and nuclear responses in host cells. *Cell* 93, 815–826.

Hennet, T., Ziltener, H. J., Frei, K., and Peterhans, E. (1992). A kinetic study of immune mediators in the lungs of mice infected with influenza A virus. *J. Immunol.* 149, 932–939.

Henry, T., Brotcke, A., Weiss, D. S., Thompson, L. J., and Monack, D. M. (2007). Type I interferon signaling is required for activation of the inflammasome during *Francisella* infection. *J. Exp. Med.* 204, 987–994.

Hersh, D., Monack, D. M., Smith, M. R., Ghori, N., Falkow, S., and Zychlinsky, A. (1999). The *Salmonella* invasin SipB induces macrophage apoptosis by binding to caspase-1. *Proc. Natl. Acad. Sci. U.S.A.* 96, 2396–2401.

Hise, A. G., Tomalka, J., Ganesan, S., Patel, K., Hall, B. A., Brown, G. D., and Fitzgerald, K. A. (2009). An essential role for the NLRP3 inflammasome in host defense against the human fungal pathogen *Candida albicans*. *Cell Host Microbe* 5, 487–497.

Hoffmann, C., Galle, M., Dilling, S., Kappeli, R., Muller, A. J., Songhet, P., Beyaert, R., and Hardt, W. D. (2010). In macrophages, caspase-1 activation by SopE and the type III secretion system-1 of *S. typhimurium* can proceed in the absence of flagellin. *PLoS ONE* 5, e12477. doi: 10.1371/journal.pone.0012477

Hornung, V., Ablasser, A., Charrel-Dennis, M., Bauernfeind, F., Horvath, G., Caffrey, D. R., Latz, E., and Fitzgerald, K. A. (2009). AIM2 recognizes cytosolic dsDNA and forms a caspase-1-activating inflammasome with ASC. *Nature* 458, 514–518.

Hornung, V., Bauernfeind, F., Halle, A., Samstad, E. O., Kono, H., Rock, K. L., Fitzgerald, K. A., and Latz, E. (2008). Silica crystals and aluminum salts activate the NALP3 inflammasome through phagosomal destabilization. *Nat. Immunol.* 9, 847–856.

Hsu, L. C., Ali, S. R., Mcgillivray, S., Tseng, P. H., Mariathasan, S., Humke, E. W., Eckmann, L., Powell, J. J., Nizet, V., Dixit, V. M., and Karin, M. (2008). A NOD2-NALP1 complex mediates caspase-1-dependent IL-1beta secretion in response to *Bacillus anthracis* infection and muramyl dipeptide. *Proc. Natl. Acad. Sci. U.S.A.* 105, 7803–7808.

Hyodo, Y., Matsui, K., Hayashi, N., Tsutsui, H., Kashiwamura, S., Yamauchi, H., Hiroishi, K., Takeda, K., Tagawa, Y., Iwakura, Y., Kayagaki, N., Kurimoto, M., Okamura, H., Hada, T., Yagita, H., Akira, S., Nakanishi, K., and Higashino, K. (1999). IL-18 up-regulates perforin-mediated NK activity without increasing perforin messenger RNA expression by binding to constitutively expressed IL-18 receptor. *J. Immunol.* 162, 1662–1668.

Ichinohe, T., Lee, H. K., Ogura, Y., Flavell, R., and Iwasaki, A. (2009). Inflammasome recognition of influenza virus is essential for adaptive immune responses. *J. Exp. Med.* 206, 79–87.

Ichinohe, T., Pang, I. K., and Iwasaki, A. (2010). Influenza virus activates inflammasomes via its intracellular M2 ion channel. *Nat. Immunol.* 11, 404–410.

Inohara, N., Ogura, Y., Fontalba, A., Gutierrez, O., Pons, F., Crespo, J., Fukase, K., Inamura, S., Kusumoto, S., Hashimoto, M., Foster, S. J., Moran, A. P., Fernandez-Luna, J. L., and Nunez, G. (2003). Host recognition of bacterial muramyl dipeptide mediated through NOD2. *J. Biol. Chem.* 278, 5509–5512.

Isberg, R. R., O'Connor, T. J., and Heidtman, M. (2009). The *Legionella pneumophila* replication vacuole: making a cosy niche inside host cells. *Nat. Rev. Microbiol.* 7, 13–24.

Johnston, J. B., Barrett, J. W., Nazarian, S. H., Goodwin, M., Ricciuto, D., Wang, G., and McFadden, G. (2005). A poxvirus-encoded pyrin domain protein interacts with ASC-1 to inhibit host inflammatory and apoptotic responses to infection. *Immunity* 23, 587–598.

Joly, S., Ma, N., Sadler, J. J., Soll, D. R., Cassel, S. L., and Sutterwala, F. S. (2009). Cutting edge: *Candida albicans* hyphae formation triggers activation of the Nlrp3 inflammasome. *J. Immunol.* 183, 3578–3581.

Jones, J. W., Kayagaki, N., Broz, P., Henry, T., Newton, K., O'Rourke, K., Chan,

S., Dong, J., Qu, Y., Roose-Girma, M., Dixit, V. M., and Monack, D. M. (2010). Absent in melanoma 2 is required for innate immune recognition of *Francisella tularensis*. *Proc. Natl. Acad. Sci. U.S.A.* 107, 9771–9776.

Juffermans, N. P., Florquin, S., Camoglio, L., Verbon, A., Kolk, A. H., Speelman, P., Van Deventer, S. J., and Van Der Poll, T. (2000). Interleukin-1 signaling is essential for host defense during murine pulmonary tuberculosis. *J. Infect. Dis.* 182, 902–908.

Kanneganti, T. D., Body-Malapel, M., Amer, A., Park, J. H., Whitfield, J., Franchi, L., Taraporewala, Z. F., Miller, D., Patton, J. T., Inohara, N., and Nunez, G. (2006). Critical role for cryopyrin/Nalp3 in activation of caspase-1 in response to viral infection and double-stranded RNA. *J. Biol. Chem.* 281, 36560–36568.

Keller, M., Ruegg, A., Werner, S., and Beer, H. D. (2008). Active caspase-1 is a regulator of unconventional protein secretion. *Cell* 132, 818–831.

Kim, S., Bauernfeind, F., Ablasser, A., Hartmann, G., Fitzgerald, K. A., Latz, E., and Hornung, V. (2010). *Listeria monocytogenes* is sensed by the NLRP3 and AIM2 inflammasome. *Eur. J. Immunol.* 40, 1545–1551.

Koo, I. C., Wang, C., Raghavan, S., Morisaki, J. H., Cox, J. S., and Brown, E. J. (2008). ESX-1-dependent cytolysis in lysosome secretion and inflammasome activation during mycobacterial infection. *Cell. Microbiol.* 10, 1866–1878.

Kumar, H., Kumagai, Y., Tsuchida, T., Koenig, P. A., Satoh, T., Guo, Z., Jang, M. H., Saitoh, T., Akira, S., and Kawai, T. (2009). Involvement of the NLRP3 inflammasome in innate and humoral adaptive immune responses to fungal beta-glucan. *J. Immunol.* 183, 8061–8067.

Kurenuma, T., Kawamura, I., Hara, H., Uchiyama, R., Daim, S., Dewamitta, S. R., Sakai, S., Tsuchiya, K., Nomura, T., and Mitsuyama, M. (2009). The RD1 locus in the *Mycobacterium tuberculosis* genome contributes to activation of caspase-1 via induction of potassium ion efflux in infected macrophages. *Infect. Immun.* 77, 3992–4001.

Labbe, K., Miu, J., Yeretssian, G., Serghides, L., Tam, M., Finney, C. A., Erdman, L. K., Goulet, M. L., Kain, K. C., Stevenson, M. M., and Saleh, M. (2010). Caspase-12 dampens the immune response to malaria independently of the inflammasome by targeting NF-kappaB signaling. *J. Immunol.* 185, 5495–5502.

Labbe, K., and Saleh, M. (2008). Cell death in the host response to infection. *Cell Death Differ.* 15, 1339–1349.

Lamkanfi, M., Amer, A., Kanneganti, T. D., Munoz-Planillo, R., Chen, G., Vandenabeele, P., Fortier, A., Gros, P., and Nunez, G. (2007). The Nod-like receptor family member Naip5/Birc1e restricts *Legionella pneumophila* growth independently of caspase-1 activation. *J. Immunol.* 178, 8022–8027.

Lamkanfi, M., Kanneganti, T. D., Van Damme, P., Vanden Berghe, T., Vanoverberghe, I., Vandekerckhove, J., Vandenabeele, P., Gevaert, K., and Nunez, G. (2008). Targeted peptide-centric proteomics reveals caspase-7 as a substrate of the caspase-1 inflammasomes. *Mol. Cell Proteomics* 7, 2350–2363.

Lara-Tejero, M., Sutterwala, F. S., Ogura, Y., Grant, E. P., Bertin, J., Coyle, A. J., Flavell, R. A., and Galan, J. E. (2006). Role of the caspase-1 inflammasome in *Salmonella typhimurium* pathogenesis. *J. Exp. Med.* 203, 1407–1412.

Lightfield, K. L., Persson, J., Brubaker, S. W., Witte, C. E., Von Moltke, J., Dunipace, E. A., Henry, T., Sun, Y. H., Cado, D., Dietrich, W. F., Monack, D. M., Tsolis, R. M., and Vance, R. E. (2008). Critical function for Naip5 in inflammasome activation by a conserved carboxy-terminal domain of flagellin. *Nat. Immunol.* 9, 1171–1178.

Lin, X. Y., Choi, M. S. K., and Porter, A. G. (2000). Expression analysis of the human caspase-1 subfamily reveals specific regulation of the CASP5 gene by lipopolysaccharide and interferon-gamma. *J. Biol. Chem.* 275, 39920–39926.

Lo, H. J., Kohler, J. R., DiDomenico, B., Loebenberg, D., Cacciapuoti, A., and Fink, G. R. (1997). Nonfilamentous *C. albicans* mutants are avirulent. *Cell* 90, 939–949.

Mariathasan, S., Newton, K., Monack, D. M., Vucic, D., French, D. M., Lee, W. P., Roose-Girma, M., Erickson, S., and Dixit, V. M. (2004). Differential activation of the inflammasome by caspase-1 adaptors ASC and Ipaf. *Nature* 430, 213–218.

Mariathasan, S., Weiss, D. S., Dixit, V. M., and Monack, D. M. (2005). Innate immunity against *Francisella tularensis* is dependent on the ASC/caspase-1 axis. *J. Exp. Med.* 202, 1043–1049.

Mariathasan, S., Weiss, D. S., Newton, K., Mcbride, J., O'rourke, K., Roose-Girma, M., Lee, W. P., Weinrauch, Y., Monack, D. M., and Dixit, V. M. (2006). Cryopyrin activates the inflammasome in response to toxins and ATP. *Nature* 440, 228–232.

Martinon, F., Burns, K., and Tschopp, J. (2002). The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. *Mol. Cell* 10, 417–426.

Martinon, F., Petrilli, V., Mayor, A., Tardivel, A., and Tschopp, J. (2006). Gout-associated uric acid crystals activate the NALP3 inflammasome. *Nature* 440, 237–241.

Master, S. S., Rampini, S. K., Davis, A. S., Keller, C., Ehlers, S., Springer, B., Timmins, G. S., Sander, P., and Deretic, V. (2008). *Mycobacterium tuberculosis* prevents inflammasome activation. *Cell Host Microbe* 3, 224–232.

Mayer-Barber, K. D., Barber, D. L., Shenderov, K., White, S. D., Wilson, M. S., Cheever, A., Kugler, D., Hieny, S., Caspar, P., Nunez, G., Schlueter, D., Flavell, R. A., Sutterwala, F. S., and Sher, A. (2010). Caspase-1 independent IL-1beta production is critical for host resistance to *Mycobacterium tuberculosis* and does not require TLR signaling in vivo. *J. Immunol.* 184, 3326–3330.

McElvania Tekippe, E., Allen, I. C., Hulseberg, P. D., Sullivan, J. T., McCann, J. R., Sandor, M., Braunstein, M., and Ting, J. P. (2010). Granuloma formation and host defense in chronic *Mycobacterium tuberculosis* infection requires PYCARD/ASC but not NLRP3 or caspase-1. *PLoS ONE* 5, e12320. doi: 10.1371/journal.pone.0012320

Medzhitov, R. (2008). Origin and physiological roles of inflammation. *Nature* 454, 428–435.

Meixenberger, K., Pache, F., Eitel, J., Schmeck, B., Hippenstiel, S., Slevogt, H., N'guessan, P., Witzenrath, M., Netea, M. G., Chakraborty, T., Suttorp, N., and Opitz, B. (2010). *Listeria monocytogenes*-infected human peripheral blood mononuclear cells produce IL-1beta, depending on listeriolysin O and NLRP3. *J. Immunol.* 184, 922–930.

Miao, E. A., Alpuche-Aranda, C. M., Dors, M., Clark, A. E., Bader, M. W., Miller, S. I., and Aderem, A. (2006). Cytoplasmic flagellin activates caspase-1 and secretion of interleukin 1beta via Ipaf. *Nat. Immunol.* 7, 569–575.

Miao, E. A., Ernst, R. K., Dors, M., Mao, D. P., and Aderem, A. (2008). *Pseudomonas aeruginosa* activates caspase 1 through Ipaf. *Proc. Natl. Acad. Sci. U.S.A.* 105, 2562–2567.

Miao, E. A., Leaf, I. A., Treuting, P. M., Mao, D. P., Dors, M., Sarkar, A., Warren, S. E., Wewers, M. D., and Aderem, A. (2010a). Caspase-1-induced pyroptosis is an innate immune effector mechanism against intracellular bacteria. *Nat. Immunol.* 11, 1136–1194.

Miao, E. A., Mao, D. P., Yudkovsky, N., Bonneau, R., Lorang, C. G., Warren, S. E., Leaf, I. A., and Aderem, A. (2010b).

Innate immune detection of the type III secretion apparatus through the NLRC4 inflammasome. *Proc. Natl. Acad. Sci. U.S.A.* 107, 3076–3080.

Mishra, B. B., Moura-Alves, P., Sonawane, A., Hacohen, N., Griffiths, G., Moita, L. F., and Anes, E. (2010). *Mycobacterium tuberculosis* protein ESAT-6 is a potent activator of the NLRP3/ASC inflammasome. *Cell. Microbiol.* 12, 1046–1063.

Molofsky, A. B., Byrne, B. G., Whitfield, N. N., Madigan, C. A., Fuse, E. T., Tateda, K., and Swanson, M. S. (2006). Cytosolic recognition of flagellin by mouse macrophages restricts *Legionella pneumophila* infection. *J. Exp. Med.* 203, 1093–1104.

Muller, A. J., Hoffmann, C., Galle, M., Van Den Broeke, A., Heikenwalder, M., Falter, L., Misselwitz, B., Kremer, M., Beyaert, R., and Hardt, W. D. (2009). The *S. typhimurium* effector SopE induces caspase-1 activation in stromal cells to initiate gut inflammation. *Cell Host Microbe* 6, 125–136.

Muruve, D. A., Petrilli, V., Zaiss, A. K., White, L. R., Clark, S. A., Ross, P. J., Parks, R. J., and Tschopp, J. (2008). The inflammasome recognizes cytosolic microbial and host DNA and triggers an innate immune response. *Nature* 452, 103–107.

Nakhaei, P., Genin, P., Civas, A., and Hiscott, J. (2009). RIG-I-like receptors: sensing and responding to RNA virus infection. *Semin. Immunol.* 21, 215–222.

Netea, M. G., and Marodi, L. (2010). Innate immune mechanisms for recognition and uptake of *Candida* species. *Trends Immunol.* 31, 346–353.

Newman, Z. L., Printz, M. P., Liu, S., Crown, D., Breen, L., Miller-Randolph, S., Flodman, P., Leppla, S. H., and Moayeri, M. (2010). Susceptibility to anthrax lethal toxin-induced rat death is controlled by a single chromosome 10 locus that includes rNlrp1. *PLoS Pathog.* 6, e1000906. doi: 10.1371/journal.ppat.1000906

O'Neill, L. A., and Bowie, A. G. (2010). Sensing and signaling in antiviral innate immunity. *Curr. Biol.* 20, R328–R333.

Ozoren, N., Masumoto, J., Franchi, L., Kanneganti, T. D., Body-Malapel, M., Erturk, I., Jagirdar, R., Zhu, L., Inohara, N., Bertin, J., Coyle, A., Grant, E. P., and Nunez, G. (2006). Distinct roles of TLR2 and the adaptor ASC in IL-1beta/IL-18 secretion in response to *Listeria monocytogenes*. *J. Immunol.* 176, 4337–4342.

Pelegrin, P., and Surprenant, A. (2006). Pannexin-1 mediates large pore formation and interleukin-1beta release

by the ATP-gated P2X7 receptor. *EMBO J.* 25, 5071–5082.

Petrilli, V., Papin, S., Dostert, C., Mayor, A., Martinon, F., and Tschopp, J. (2007). Activation of the NALP3 inflammasome is triggered by low intracellular potassium concentration. *Cell Death Differ.* 14, 1583–1589.

Pirhonen, J., Sareneva, T., Kurimoto, M., Julkunen, I., and Matikainen, S. (1999). Virus infection activates IL-1 beta and IL-18 production in human macrophages by a caspase-1-dependent pathway. *J. Immunol.* 162, 7322–7329.

Poeck, H., Bscheider, M., Gross, O., Finger, K., Roth, S., Rebsamen, M., Hannesschlager, N., Schlee, M., Rothenfusser, S., Barchet, W., Kato, H., Akira, S., Inoue, S., Endres, S., Peschel, C., Hartmann, G., Hornung, V., and Ruland, J. (2010). Recognition of RNA virus by RIG-I results in activation of CARD9 and inflammasome signaling for interleukin 1 beta production. *Nat. Immunol.* 11, 63–69.

Proell, M., Riedl, S. J., Fritz, J. H., Rojas, A. M., and Schwarzenbacher, R. (2008). The Nod-like receptor (NLR) family: a tale of similarities and differences. *PLoS ONE* 3, e2119. doi: 10.1371/journal.pone.0002119

Rathinam, V. A., Jiang, Z., Waggoner, S. N., Sharma, S., Cole, L. E., Waggoner, L., Vanaja, S. K., Monks, B. G., Ganesan, S., Latz, E., Hornung, V., Vogel, S. N., Szomolanyi-Tsuda, E., and Fitzgerald, K. A. (2010). The AIM2 inflammasome is essential for host defense against cytosolic bacteria and DNA viruses. *Nat. Immunol.* 11, 395–402.

Raupach, B., Peuschel, S. K., Monack, D. M., and Zychlinsky, A. (2006). Caspase-1-mediated activation of interleukin-1beta (IL-1beta) and IL-18 contributes to innate immune defenses against *Salmonella enterica* serovar Typhimurium infection. *Infect. Immun.* 74, 4922–4926.

Reig, N., Jiang, A., Couture, R., Sutterwala, F. S., Ogura, Y., Flavell, R. A., Mellman, I., and Van Der Goot, F. G. (2008). Maturation modulates caspase-1-independent responses of dendritic cells to anthrax lethal toxin. *Cell. Microbiol.* 10, 1190–1207.

Reimer, T., Shaw, M. H., Franchi, L., Coban, C., Ishii, K. J., Akira, S., Horii, T., Rodriguez, A., and Nunez, G. (2010). Experimental cerebral malaria progresses independently of the Nlrp3 inflammasome. *Eur. J. Immunol.* 40, 764–769.

Ren, T., Zamboni, D. S., Roy, C. R., Dietrich, W. F., and Vance, R. E. (2006). Flagellin-deficient *Legionella* mutants evade caspase-1- and Naip5-mediated macrophage immunity.

PLoS Pathog. 2, e18. doi: 10.1371/journal.ppat.0020018

Rodrigue-Gervais, I. G., and Saleh, M. (2010). Genetics of inflammasome-associated disorders: a lesson in the guiding principals of inflammasome function. *Eur. J. Immunol.* 40, 643–648.

Rogers, N. C., Slack, E. C., Edwards, A. D., Nolte, M. A., Schulz, O., Schweighoffer, E., Williams, D. L., Gordon, S., Tybulewicz, V. L., Brown, G. D., and Reis E. Sousa, C. (2005). Syk-dependent cytokine induction by Dectin-1 reveals a novel pattern recognition pathway for C type lectins. *Immunity* 22, 507–517.

Sachs, J., and Malaney, P. (2002). The economic and social burden of malaria. *Nature* 415, 680–685.

Said-Sadier, N., Padilla, E., Langsley, G., and Ojcius, D. M. (2010). *Aspergillus fumigatus* stimulates the NLRP3 inflammasome through a pathway requiring ROS production and the Syk tyrosine kinase. *PLoS ONE* 5, e10008. doi: 10.1371/journal.pone.0010008

Saito, T. and Gale, M. Jr. (2007). Principles of intracellular viral recognition. *Curr. Opin. Immunol.* 19, 17–23.

Saleh, M., Mathison, J. C., Wolinski, M. K., Bensinger, S. J., Fitzgerald, P., Droin, N., Ulevitch, R. J., Green, D. R., and Nicholson, D. W. (2006). Enhanced bacterial clearance and sepsis resistance in caspase-12-deficient mice. *Nature* 440, 1064–1068.

Saleh, M., and Trinchieri, G. (2011). Innate immune mechanisms of colitis and colitis-associated colorectal cancer. *Nat. Rev. Immunol.* 11, 9–20.

Santic, M., Molmeret, M., Klose, K. E., and Abu Kwaik, Y. (2006). *Francisella tularensis* travels a novel, twisted road within macrophages. *Trends Microbiol.* 14, 37–44.

Sauer, J. D., Witte, C. E., Zemansky, J., Hanson, B., Lauer, P., and Portnoy, D. A. (2010). *Listeria monocytogenes* triggers AIM2-mediated pyroptosis upon infrequent bacteriolysis in the macrophage cytosol. *Cell Host Microbe* 7, 412–419.

Schmitz, N., Kurrer, M., Bachmann, M. F., and Kopf, M. (2005). Interleukin-1 is responsible for acute lung immunopathology but increases survival of respiratory influenza virus infection. *J. Virol.* 79, 6441–6448.

Schotte, P., Denecker, G., Van Den Broeke, A., Vandenabeele, P., Cornelis, G. R., and Beyaert, R. (2004). Targeting Rac1 by the *Yersinia* effector protein YopE inhibits caspase-1-mediated maturation and release of interleukin-1beta. *J. Biol. Chem.* 279, 25134–25142.

Schroder, K., and Tschopp, J. (2010). The inflammasomes. *Cell* 140, 821–832.

Shao, W., Yeretssian, G., Doiron, K., Hussain, S. N., and Saleh, M. (2007). The caspase-1 digestome identifies the glycolysis pathway as a target during infection and septic shock. *J. Biol. Chem.* 282, 36321–36329.

Shaw, M. H., Reimer, T., Kim, Y. G., and Nunez, G. (2008). NOD-like receptors (NLRs): bona fide intracellular microbial sensors. *Curr. Opin. Immunol.* 20, 377–382.

Shen, A., and Higgins, D. E. (2006). The MogR transcriptional repressor regulates nonhierarchal expression of flagellar motility genes and virulence in *Listeria monocytogenes*. *PLoS Pathog.* 2, e30. doi: 10.1371/journal.ppat.0020030

Shio, M. T., Eisenbarth, S. C., Savaria, M., Vinet, A. F., Bellemare, M. J., Harder, K. W., Sutterwala, F. S., Bohle, D. S., Descoteaux, A., Flavell, R. A., and Olivier, M. (2009). Malarial hemozoin activates the NLRP3 inflammasome through Lyn and Syk kinases. *PLoS Pathog.* 5, e1000559. doi: 10.1371/journal.ppat.1000559

Silveira, T. N., and Zamboni, D. S. (2010). Pore formation triggered by *Legionella* spp. is an Nlrc4 inflammasome-dependent host cell response that precedes pyroptosis. *Infect. Immun.* 78, 1403–1413.

Squires, R. C., Muehlbauer, S. M., and Brojatsch, J. (2007). Proteasomes control caspase-1 activation in anthrax lethal toxin-mediated cell killing. *J. Biol. Chem.* 282, 34260–34267.

Stasakova, J., Ferko, B., Kittel, C., Sereinig, S., Romanova, J., Katinger, H., and Egorov, A. (2005). Influenza A mutant viruses with altered NS1 protein function provoke caspase-1 activation in primary human macrophages, resulting in fast apoptosis and release of high levels of interleukins 1beta and 18. *J. Gen. Virol.* 86, 185–195.

Stehlik, C., and Dorfleutner, A. (2007). COPs and POPs: modulators of inflammasome activity. *J. Immunol.* 179, 7993–7998.

Sun, Y. H., Rolan, H. G., and Tsolis, R. M. (2007). Injection of flagellin into the host cell cytosol by *Salmonella enterica* serotype Typhimurium. *J. Biol. Chem.* 282, 33897–33901.

Sutterwala, F. S., Mijares, L. A., Li, L., Ogura, Y., Kazmierczak, B. I., and Flavell, R. A. (2007). Immune recognition of Pseudomonas aeruginosa mediated by the IPAF/NLRC4 inflammasome. *J. Exp. Med.* 204, 3235–3245.

Suzuki, T., Franchi, L., Toma, C., Ashida, H., Ogawa, M., Yoshikawa, Y., Mimuro, H., Inohara, N., Sasakawa, C., and

Nunez, G. (2007). Differential regulation of caspase-1 activation, pyroptosis, and autophagy via Ipaf and ASC in *Shigella*-infected macrophages. *PLoS Pathog.* 3, e111. doi: 10.1371/journal.ppat.0030111

Takeuchi, O., and Akira, S. (2010). Pattern recognition receptors and inflammation. *Cell* 140, 805–820.

Terra, J. K., Cote, C. K., France, B., Jenkins, A. L., Bozue, J. A., Welkos, S. L., Levine, S. M., and Bradley, K. A. (2010). Cutting edge: resistance to *Bacillus anthracis* infection mediated by a lethal toxin sensitive allele of Nalp1b/Nlrp1b. *J. Immunol.* 184, 17–20.

Thomas, P. G., Dash, P., Aldridge, J. R. Jr., Ellebedy, A. H., Reynolds, C., Funk, A. J., Martin, W. J., Lamkanfi, M., Webby, R. J., Boyd, K. L., Doherty, P. C., and Kanneganti, T. D. (2009). The intracellular sensor NLRP3 mediates key innate and healing responses to influenza A virus via the regulation of caspase-1. *Immunity* 30, 566–575.

Thornberry, N. A., Bull, H. G., Calaycay, J. R., Chapman, K. T., Howard, A. D., Kostura, M. J., Miller, D. K., Molineaux, S. M., Weidner, J. R., Aunins, J., Elliston, K. O., Ayala, J. M., Casano, F. J., Chin, J., Ding, G. J. F., Egger, L. A., Gaffney, E. P., Limjuco, G., Palyha, O. C., Raju, S. M., Rolando, A. M., Salley, J. P., Yamin, T. T., Lee, T. D., Shively, J. E., Maccross, M., Mumford, R. A., Schmidt, J. A., and Tocci, M. J. (1992). A novel heterodimeric cysteine protease is required for interleukin-1-beta processing in monocytes. *Nature* 356, 768–774.

Tsuchiya, K., Hara, H., Kawamura, I., Nomura, T., Yamamoto, T., Daim, S., Dewamitta, S. R., Shen, Y., Fang, R., and Mitsuyama, M. (2010). Involvement of absent in melanoma 2 in inflammasome activation in macrophages infected with *Listeria monocytogenes*. *J. Immunol.* 185, 1186–1195.

Tsuji, N. M., Tsutsui, H., Seki, E., Kuida, K., Okamura, H., Nakanishi, K., and Flavell, R. A. (2004). Roles of caspase-1 in Listeria infection in mice. *Int. Immunol.* 16, 335–343.

Ulland, T. K., Buchan, B. W., Ketterer, M. R., Fernandes-Alnemri, T., Meyerholz, D. K., Apicella, M. A., Alnemri, E. S., Jones, B. D., Nauseef, W. M., and Sutterwala, F. S. (2010). Cutting edge: mutation of *Francisella tularensis* mviN leads to increased macrophage absent in melanoma 2 inflammasome activation and a loss of virulence. *J. Immunol.* 185, 2670–2674.

Valdez, Y., Ferreira, R. B. R., and Finlay, B. B. (2009). Molecular mechanisms of *Salmonella* virulence and host resistance. *Curr. Top. Microbiol.* 337, 93–127.

Van De Veerdonk, F. L., Joosten, L. A., Devesa, I., Mora-Montes, H. M., Kanneganti, T. D., Dinarello, C. A., Van Der Meer, J. W., Gow, N. A., Kullberg, B. J., and Netea, M. G. (2009). Bypassing pathogen-induced inflammasome activation for the regulation of interleukin-1beta production by the fungal pathogen *Candida albicans*. *J. Infect. Dis.* 199, 1087–1096.

Vinzing, M., Eitel, J., Lippmann, J., Hocke, A. C., Zahlten, J., Slevogt, H., N'guessan, P., D., Gunther, S., Schmeck, B., Hippenstiel, S., Flieger, A., Suttorp, N., and Opitz, B. (2008). NAIP and Ipaf control *Legionella pneumophila* replication in human cells. *J. Immunol.* 180, 6808–6815.

Vonk, A. G., Netea, M. G., van Krieken, J. H., Iwakura, Y., van der Meer, J. W., and Kullberg, B. J. (2006). Endogenous interleukin (IL)-1 alpha and IL-1 beta are crucial for host defense against disseminated candidiasis. *J. Infect. Dis.* 193, 1419–1426.

Walter, K., Holscher, C., Tschopp, J., and Ehlers, S. (2010). NALP3 is not necessary for early protection against experimental tuberculosis. *Immunobiology* 215, 804–811.

Wang, S. Y., Miura, M., Jung, Y. K., Zhu, H., Li, E., and Yuan, J. Y. (1998). Murine caspase-11, an ICE-interacting protease, is essential for the activation of ICE. *Cell* 92, 501–509.

Warren, S. E., Armstrong, A., Hamilton, M. K., Mao, D. P., Leaf, I. A., Miao, E. A., and Aderem, A. (2010). Cutting edge: cytosolic bacterial DNA activates the inflammasome via Aim2. *J. Immunol.* 185, 818–821.

Warren, S. E., Mao, D. P., Rodriguez, A. E., Miao, E. A., and Aderem, A. (2008). Multiple Nod-like receptors activate caspase 1 during *Listeria monocytogenes* infection. *J. Immunol.* 180, 7558–7564.

Wickliffe, K. E., Leppla, S. H., and Moayeri, M. (2008). Anthrax lethal toxin-induced inflammasome formation and caspase-1 activation are late events dependent on ion fluxes and the proteasome. *Cell. Microbiol.* 10, 332–343.

Wright, E. K., Goodart, S. A., Growney, J. D., Hadinoto, V., Endrizzi, M. G., Long, E. M., Sadigh, K., Abney, A. L., Bernstein-Hanley, I., and Dietrich, W. F. (2003). Naip5 affects host susceptibility to the intracellular pathogen *Legionella pneumophila*. *Curr. Biol.* 13, 27–36.

Wu, J., Fernandes-Alnemri, T., and Alnemri, E. S. (2010). Involvement of the AIM2, NLRC4, and NLRP3 inflammasomes in caspase-1 activation by *Listeria monocytogenes*. *J. Clin. Immunol.* 30, 693–702.

Xiang, Y., and Moss, B. (1999). IL-18 binding and inhibition of interferon gamma induction by human poxvirus-encoded proteins. *Proc. Natl. Acad. Sci. U.S.A.* 96, 11537–11542.

Zaki, M. H., Boyd, K. L., Vogel, P., Kastan, M. B., Lamkanfi, M., and Kanneganti, T. D. (2010). The NLRP3 inflammasome protects against loss of epithelial integrity and mortality during experimental colitis. *Immunity* 32, 379–391.

Zamboni, D. S., Kobayashi, K. S., Kohlsdorf, T., Ogura, Y., Long, E. M., Vance, R. E., Kuida, K., Mariathasan, S., Dixit, V. M., Flavell, R. A., Dietrich, W. F., and Roy, C. R. (2006). The Birc1e cytosolic pattern-recognition receptor contributes to the detection and control of *Legionella pneumophila* infection. *Nat. Immunol.* 7, 318–325.

Zhou, R., Yazdi, A. S., Menu, P., and Tschopp, J. (2011). A role for mitochondria in NLRP3 inflammasome activation. *Nature* 469, 221–225.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Role of the Nlrp3 inflammasome in microbial infection

Paras K. Anand, R. K. Subbarao Malireddi and Thirumala-Devi Kanneganti*

Department of Immunology, St Jude Children's Research Hospital, Memphis, TN, USA

Edited by:
Amal Amer, The Ohio State University, USA

Reviewed by:
Amal Amer, The Ohio State University, USA
Kevin Coombs, University of Manitoba, Canada

*Correspondence:
Thirumala-Devi Kanneganti, Department of Immunology, St Jude Children's Research Hospital, Memphis, TN 38105, USA.
e-mail: thirumala-devi.kanneganti@ stjude.org

The intracellular Nod-like receptor Nlrp3 has emerged as the most versatile innate immune receptor because of its broad specificity in mediating immune response to a wide range of microbial or danger signals. Nlrp3 mediates assembly of the inflammasome complex in the presence of microbial components leading to the activation of caspase-1 and the processing and release of the pro-inflammatory cytokines IL-1β and IL-18. In this review, we give an update on the recent literature examining the role of Nlrp3 inflammasome in response to fungal, bacterial, and viral infections.

Keywords: NLR, Nlrp3, inflammasome, caspase-1, IL-1β, IL-18

INTRODUCTION

The innate immune system depends on germline encoded pattern recognition receptors (PRRs) for the detection of various microbial components. PRRs belong to different classes of receptors such as toll-like receptors (TLRs) that are localized at the cell surface or in endosomes and the cytosolic RIG-I-like receptors (RLRs), Nod-like receptors (NLRs), and the recently identified HIN-200 family members (Palsson-McDermott and O'Neill, 2007; Hornung and Latz, 2010; Unterholzner et al., 2010). Upon perceiving a microbial or danger stimuli, these receptors activate downstream signaling events leading to generation of the appropriate immune response (Creagh and O'Neill, 2006). Inflammasomes are molecular platforms that assemble by hetero-oligomerization of a nucleotide-binding oligomerization domain, LRR containing receptor (NLR), an adaptor protein ASC and pro-caspase-1, and triggers caspase-1 activation and downstream maturation and secretion of the pro-inflammatory cytokines IL-1β and IL-18 (Kanneganti et al., 2006a, 2007; Lamkanfi and Kanneganti, 2010).

The requirement for a particular NLR within the inflammasome complex depends upon the upstream trigger. The Nlrp3 inflammasome, for example, acts as a global sensor that responds to a wide array of stimuli whereas Nlrc4 and Nlrp1 inflammasomes are more specific; they are activated only by bacterial flagellin and anthrax toxin, respectively (Boyden and Dietrich, 2006; Franchi et al., 2006; Miao et al., 2006). Many studies have now uncovered the crucial role of the Nlrp3 inflammasome in different microbial infections. The purpose of this review is to give an update on the recent literature highlighting the role of Nlrp3 inflammasome during host responses to various pathogens.

FUNGAL INFECTION

Most of the fungi are non-pathogenic in healthy individuals; however, they are long known to cause severe systemic and superficial infections in patients with AIDS, cancer or other immunocompromised conditions (Romani, 2004). Although the antifungal effects of IL-1β and IL-18 were known previously (Mencacci et al., 2000;

Vonk et al., 2006), the inflammasome dependent processing of these cytokines was not addressed in those studies. One report suggested that caspase-1 is constitutively active in human monocytes and does not require inflammasome assembly for its activation (van de Veerdonk et al., 2009). In contrast, other reports in mouse models established the inflammasome dependent caspase-1 activation and IL-1β production in response to pathogen-associated molecular patterns from *Candida albicans*, *Aspergillus fumigatus*, and *Saccharomyces cerevisiae* (Gross et al., 2009; Hise et al., 2009; Joly et al., 2009; Kumar et al., 2009; Lamkanfi et al., 2009; Kankkunen et al., 2010; Poeck and Ruland, 2010; Said-Sadier et al., 2010). Intriguingly, mice deficient in Nlrp3 are hyper-susceptible to *C. albicans* in several infection models (Gross et al., 2009; Hise et al., 2009; Joly et al., 2009). However, the *in vivo* role of Asc and caspase-1 in *C. albicans* infection is not known. Interestingly, hyphael stages of these heteromorphic fungi are more virulent and are suggested to be more aggressive inducers of inflammation (Lo et al., 1997). Indeed, yeast forms of *A. fumigatus* and *C. albicans* either did not induce or showed poor Nlrp3 inflammasome activation, respectively providing evidence for the differential regulation of immune responses based on the morphological forms of fungi (Hise et al., 2009; Joly et al., 2009; Said-Sadier et al., 2010). Accordingly, appearance of hyphael forms of fungi is a positive prognosis factor for the rapidly spreading fungal infections in affected tissues and organs.

The Dectin-CARD9 signaling pathway through syk kinase regulates transcriptional up-regulation of cytokines downstream of fungal recognition (Gross et al., 2009; Poeck and Ruland, 2010). Interestingly, inhibition of syk kinase, either pharmacologically or through shRNA-based knock down, resulted not only in the inhibition of transcription but also reduced the Nlrp3 inflammasome activation (Gross et al., 2006; Said-Sadier et al., 2010). These observations thus suggest that the syk kinase signaling may contribute to the Nlrp3 inflammasome activation by providing the necessary signals required either for its up-regulation at the transcriptional level and/or for its assembly by a yet unidentified mechanism.

BACTERIAL INFECTION

Nlrp3 inflammasome has been shown to be particularly important in response to several bacterial pathogens. *Staphylococcus aureus* induced IL-1β secretion, for example, requires Nlrp3 inflammasome activation (Mariathasan et al., 2006; Craven et al., 2009). By using purified α-hemolysin Craven et al. (2009) discovered a crucial role for *Staphylococcus aureus* hemolysins in Nlrp3 inflammasome activation in THP-1 monocytes. However, Mariathasan et al. (2006) reported no role for *Staphylococcus aureus* hemolysins (α-, β-, or γ-hemolysins) in the induction of Nlrp3 inflammasome in bone marrow-derived macrophages by using *Staphylococcus aureus* hemolysin mutants. The differences observed between these two studies might be due to differences in the cell types used or to the fact that other redundant factors released by *Staphylococcus aureus* hemolysin mutants activate Nlrp3 as efficiently.

Salmonella typhimurium is a flagellated bacterium that has been shown to activate the Nlrc4 inflammasome (Franchi et al., 2006; Miao et al., 2006). However, Broz et al. (2010) recently reported activation of both the Nlrc4 and Nlrp3 inflammasomes via SPI-1 and SPI-2 dependent mechanisms. Unlike previous studies, which had focused on the SPI-1-dependent mechanism of caspase-1 activation that occurs rapidly and activates Nlrc4, this study focused on *Salmonella* SPI-2 dependent mechanisms that activate the Nlrp3 inflammasome. During *Salmonella* infection, Nlrp3 inflammasome dependent IL-1β production was observed between 17 and 20 h after infection. Interestingly, both Nlrp3 and Nlrc4 were recruited into a single ASC focus in response to *Salmonella* that correlated well with the amount of IL-1β and IL-18 released (Broz et al., 2010). Accordingly, mice lacking both of these NLRs were found more susceptible to infection than mice deficient in either Nlrc4 or Nlrp3 alone (Broz et al., 2010). However, the role of Nlrp3 in *Salmonella* infection needs further verification. Nonetheless, these observations indicate redundant roles for inflammasomes during infection.

The redundant nature of the inflammasomes is also evident during *Listeria* infection. *Listeria monocytogenes* activates inflammasome in an Nlrp3-dependent manner (Mariathasan et al., 2006). However, recent studies also show the activation of Nlrc4 and Aim2 inflammasomes upon *Listeria* infection (Warren et al., 2008; Wu et al., 2010). In particular, Nlrp3 inflammasome is activated in response to phagosomal membrane damage caused by expression of listeriolysin O (LLO) by *Listeria* (Wu et al., 2010). Indeed, membrane damage resulting in cathepsin B release has been shown previously to result in Nlrp3 activation (Hornung et al., 2008). Critical role for the Nlrp3 inflammasome has also been reported during *Mycobacterium* infection (Carlsson et al., 2010; McElvania Tekippe et al., 2010). Asc-deficient mice were found to be more susceptible to *M. tuberculosis* infection because of defective granuloma formation in these mice (McElvania Tekippe et al., 2010). In contrast, another study reported similar *M. marinum* burden in WT and Asc-deficient mice (Carlsson et al., 2010). Notably, the two studies differ in the *Mycobacterium* spp. examined and the route of the infection.

The role of Nlrp3 inflammasome in other bacterial infections has also been studied. *Streptococcus pyogenes* activates the Nlrp3 inflammasome in a streptolysin O (SLO) dependent manner (Harder et al., 2009). Nlrp3 was essential for IL-1β production but the mutant mice were equally susceptible to *Streptococcus pyogenes* infection as

wild-type mice (Harder et al., 2009). *Vibrio* spp. also induced Nlrp3 inflammasome activation mediated by hemolysins and toxins (Toma et al., 2010). *Staphylococcus aureus* activated Nlrp3 inflammasome dependent on hemolysins and bacterial lipoproteins secreted in culture supernatants (Munoz-Planillo et al., 2009). Similarly, *Neisseria gonorrhoeae* induced IL-1β production via Nlrp3 inflammasome that was dependent upon the secreted virulence factor lipo-oligosaccharide (Duncan et al., 2009).

VIRAL INFECTION

Within mammalian hosts, viruses are recognized by TLR3 and TLR7 in the endosomes and by RIG-I in the cytoplasm mounting robust immune responses through the regulation of type-1 interferons (Ichinohe et al., 2009; Kanneganti, 2010). Initial evidence implicating Nlrp3 inflammasome in viral infection came from reports of caspase-1 activation and production of IL-1β and IL-18 during Sendai virus and influenza virus infections (Kanneganti et al., 2006b). Infection with the modified vaccinia virus Ankara also activates the Nlrp3 inflammasome (Delaloye et al., 2009). Several lines of evidence indicate that the Nlrp3 inflammasome might detect the presence of viral RNA and DNA in intracellular compartments. For example, Nlrp3 has been implicated in the detection of viral DNA from adenovirus in cell culture (Muruve et al., 2008). Additionally, transfection of human or mouse cell lines with ssRNA or dsRNA analogs, such as polyinosinic–polycytidylic acid (poly(I:C)), is sufficient to activate Nlrp3 (Allen et al., 2009). *In vivo* administration of poly(I:C) or the purified ssRNA of influenza A virus to mice also led to IL-1β secretion and inflammation due to Nlrp3 activation (Kanneganti et al., 2006b; Allen et al., 2009; Thomas et al., 2009).

Several recent studies reported activation of the Nlrp3 inflammasome in response to influenza A virus in mouse bone marrow-derived macrophages, dendritic cells, monocytic THP-1 cells and *in vivo* (Allen et al., 2009; Ichinohe et al., 2009; Thomas et al., 2009). Perhaps, the Nlrp3 inflammasome activation in response to viruses has been best characterized by using influenza A virus. Influenza A virus infection led to reduced production of cytokines and chemokines in mice lacking components of the Nlrp3 inflammasome leading to decreased recruitment of neutrophils and monocytes (Allen et al., 2009; Thomas et al., 2009). This was accompanied by epithelial necrosis and collagen deposition, an effect that was more severe in the bronchia of the Nlrp3 mutant mice. Despite these facts, Nlrp3 inflammasome had no role in either virus control or generation of adaptive immunity (Thomas et al., 2009). In contrast, another study reported importance of the Nlrp3 in viral clearance (Allen et al., 2009). The apparent discrepancy might be due to different doses of infection or evaluation of viral plaque-forming units at different days after infection. Still another study by Ichinohe et al. (2009), however, reported a role for Nlrp3 only in certain cell types, but observed no role for it in the generation of adaptive immune responses similar to the study by Thomas et al. (2009). Interestingly, Ichinohe et al. (2010) proposed a role for the viral M2 ion channel in transporting H+ out of the trans-Golgi network. The authors postulated that this perturbation somehow activates other plasma membrane channels responsible for K+ efflux thus activating the Nlrp3 inflammasome (Ichinohe et al., 2010; Kanneganti, 2010).

MECHANISMS OF INFLAMMASOME ACTIVATION

The Nlrp3 inflammasome is generally believed to require a two-signal mechanism. Stimulation with LPS leads to TLR activation resulting in synthesis of precursor forms of the cytokines IL-1β and IL-18. Further stimulation of these cells with ATP activates P2X7R, allowing K^+ efflux through membrane pores that results in Nlrp3 inflammasome activation. Recent reports have proposed that besides transcriptional up-regulation of IL-1β and IL-18, LPS also leads to up-regulation of Nlrp3 expression in an NF-κB dependent manner (Bauernfeind et al., 2009; Franchi et al., 2009). However, a recent study reported that infection with *V. cholerae* did not up-regulate Nlrp3 expression suggesting that it is not indispensable for caspase-1 activation, at least in *Vibrio* infection (Toma et al., 2010).

Many pathogens bypass the necessary second signal (i.e., P2X7R activation) required for inflammasome activation through the formation of membrane pores. *Streptococcus pyogenes*, for example, activates Nlrp3 inflammasome in a P2X7R-independent manner (Harder et al., 2009). *Streptococcus pyogenes* synthesizes the pore-forming toxin SLO which may therefore provide the necessary functions of ATP and, as has been proposed before allows the delivery of microbial molecules (Nakagawa et al., 2004) to the cytosol thereby triggering Nlrp3 activation. Similarly, *Staphylococcus aureus* hemolysins (α and β) trigger caspase-1 activation in conjunction with released lipoproteins independently of P2X7R (Munoz-Planillo et al., 2009) again suggesting a role for bacterial toxins and hemolysins in fulfilling the second signal necessary for inflammasome activation.

Although studied extensively, the mechanism of Nlrp3 inflammasome activation has not been established so far. Efflux of K^+ has long been considered to be the mechanism for activation of this inflammasome (Petrilli et al., 2007). Activation of P2X7R results in rapid efflux of K^+. However, P2X7R activation also influences the levels of other ions such as Na^+ and Ca^{2+} (Dietl and Volkl, 1994; Schilling et al., 1999; North, 2002). Another mechanism proposed suggests activation of Nlrp3 by cathepsin B released from ruptured lysosomes following phagocytosis of monosodium urate and alum (Dostert et al., 2008; Hornung et al., 2008). This was demonstrated by using cathepsin B inhibitors in cell culture. However, cathepsin B-deficient macrophages showed IL-1β levels comparable to wild-type macrophages in response to monosodium urate and alum (Dostert et al., 2009). Recently, reactive oxygen species (ROS) have also been proposed to be an upstream inducer of the Nlrp3 inflammasome complex (Zhou et al., 2010). However, the role of ROS is again controversial given the fact that cells from patients with chronic granulomatous disease or macrophages from gp91phox – deficient mice (that are defective in ROS generation) produced similar levels of inflammasome activation as their normal counterparts (Meissner et al., 2010; van de Veerdonk et al., 2010).

CONCLUDING REMARKS

Nlrp3 inflammasome is activated by a variety of microbial stimuli (**Table 1**). This variety obscures efforts to determine the upstream mechanism of Nlrp3 inflammasome activation. Although multiple mechanisms have been proposed for Nlrp3

Table 1 | Microbes and microbial components that induce Nlrp3 inflammasome activation.

Pathogens	Microbial/host components involved
FUNGAL	
Candida albicans	Hyphael forms are better inducers of Nlrp3. (Gross et al., 2009; Hise et al., 2009; Joly et al., 2009)
Aspergillus fumigatus	Nlrp3 activation is strictly dependent on hyphael forms. (Said-Sadier et al., 2010)
Saccharomyces cerevisiae	Cell wall components (zymosan, mannan, and β - glucans) activate Nlrp3. (Kumar et al., 2009; Lamkanfi et al., 2009)
BACTERIAL	
Staphylococcus aureus	Nlrp3 activation is dependent on bacterial hemolysins. (Mariathasan et al., 2006; Craven et al., 2009; Munoz-Planillo et al., 2009)
Salmonella typhimurium	Nlrp3 and Nlrc4 are activated. Mice deficient in both Nlrp3 and Nlrc4 are more susceptible. (Broz et al., 2010; Franchi et al., 2006; Miao et al., 2006)
Listeria monocytogenes	Nlrp3, Nlrc4 and Aim2 are activated. (Warren et al., 2008; Wu et al., 2010)
Mycobacteriuma marinum	Nlrp3 activation is dependent on ESX-1 secretion system. (Carlsson et al., 2010)
Mycobacteriuma tuberculosis	$Asc^{-/-}$ mice are more susceptible. (McElvania Tekippe et al., 2010)
Streptococcus pyogenes	Nlrp3 activation is dependent on streptolysin O release. (Harder et al., 2009)
Vibrio cholerae	Nlrp3 activation is dependent on bacterial hemolysins and toxins. (Toma et al., 2010)
Chlamydia pneumoniae	Nlrp3 dependent IL-1β release is crucial for host defense against bacterial pneumonia. (He et al., 2010)
Neisseria gonorrhoeae	Nlrp3 activation is dependent on lipo-oligosaccharide release. (Duncan et al., 2009)
VIRAL	
Sendai virus	Nlrp3 inflammasome is activated. (Kanneganti et al., 2006b)
Modified vaccinia virus Ankara (MVA)	Innate immune sensing is mediated by Nlrp3 inflammasome. (Delaloye et al., 2009)
Adenovirus	$Nlrp3^{-/-}$ and $Asc^{-/-}$ mice show poor inflammatory responses. (Muruve et al., 2008)
Influenza A virus	Mice deficient in components of Nlrp3 inflammasome show reduced cytokine and chemokine production. (Kanneganti et al., 2006b; Allen et al., 2009; Ichinohe et al., 2009; Thomas et al., 2009)
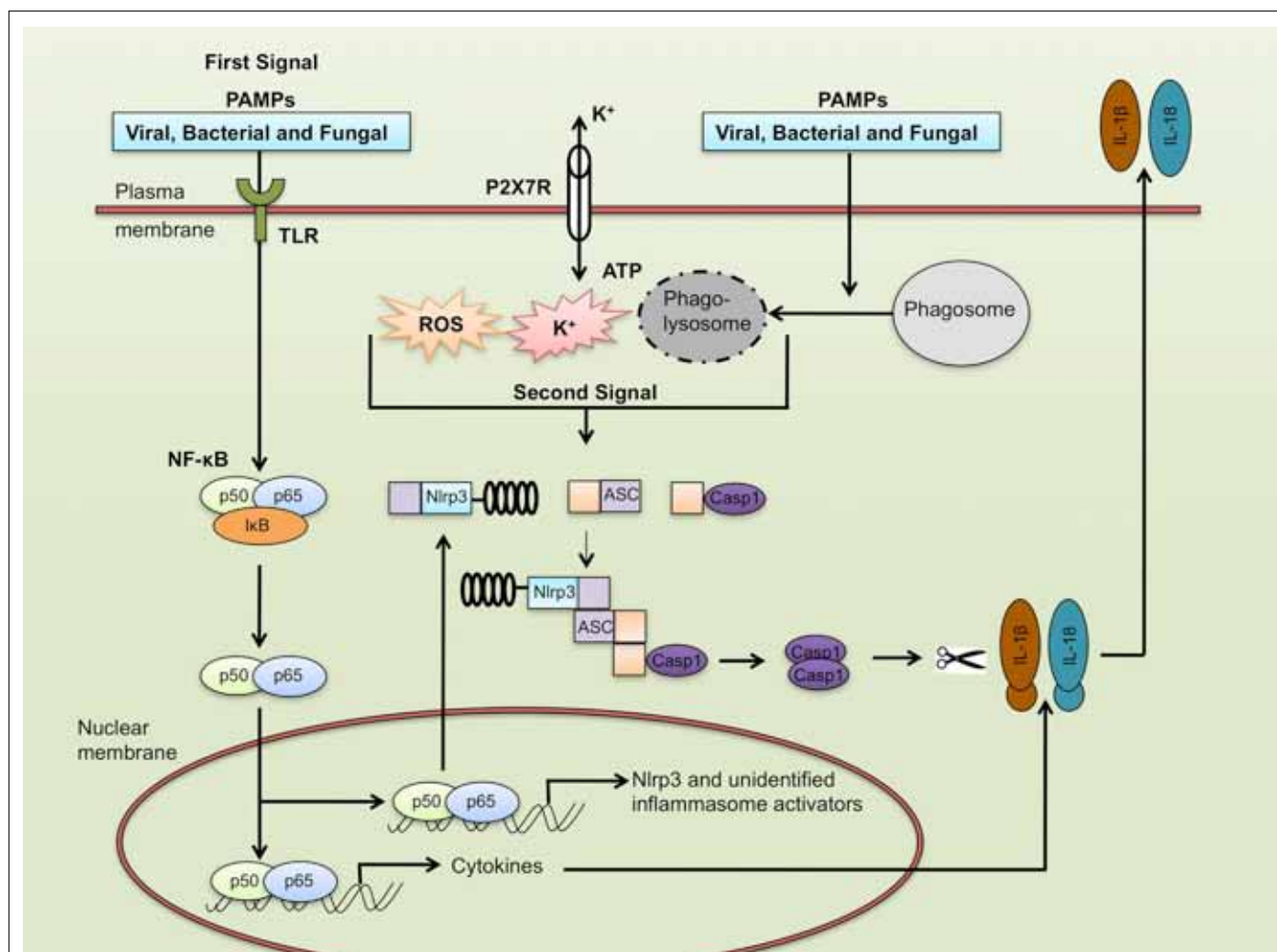
FIGURE 1 | Signaling mechanisms proposed for Nlrp3 inflammasome activation. Extracellular and intracellular pathogen-associated molecular patterns (PAMPs) are sensed by toll-like receptors (TLRs; first signal) leading to NF-κB activation and transcription of cytokines. Additionally, NF-κB is also proposed to up-regulate Nlrp3 expression, which might be a limiting factor for inflammasome assembly. A wide range of pathogens trigger Nlrp3 inflammasome activation. The physiological insults resulting from exposure to these PAMPs can be narrowed down to a few mechanisms that drive Nlrp3 activation. These mechanisms include K+ efflux, lysosomal damage and reactive oxygen species (ROS) production (second signal). Activation of P2X7R by ATP results in membrane pores that allow K+ efflux and entry of extracellular factors into the cytoplasm resulting in Nlrp3 activation. Phagocytosis of certain pathogenic microbes leads to rupture of lysosomes thereby releasing cathepsin B into the cytoplasm and causing Nlrp3 activation. Generation of ROS downstream of microbial infection has also been proposed to trigger Nlrp3. Nlrp3 inflammasome assembly consisting of the adaptor molecule Asc and pro-caspase-1 leads to caspase-1 activation, which results in processing and secretion of cytokines IL-1β and IL-18.

inflammasome activation (**Figure 1**), still no clearly defined consensus has emerged yet. It is highly probable that the different proposed mechanisms of Nlrp3 activation are not mutually exclusive and some common intersecting points exist between these various pathways. Future studies are likely to shed more light on this aspect besides deciphering the novel roles for Nlrp3 inflammasome.

REFERENCES

Allen, I. C., Scull, M. A., Moore, C. B., Holl, E. K., McElvania-TeKippe, E., Taxman, D. J., Guthrie, E. H., Pickles, R. J., and Ting, J. P. (2009). The NLRP3 inflammasome mediates in vivo innate immunity to influenza A virus through recognition of viral RNA. *Immunity* 30, 556–565.

Bauernfeind, F. G., Horvath, G., Stutz, A., Alnemri, E. S., MacDonald, K., Speert, D., Fernandes-Alnemri, T., Wu, J., Monks, B. G., Fitzgerald, K. A., Hornung, V., and Latz, E. (2009). Cutting edge: NF-kappaB activating pattern recognition and cytokine receptors license NLRP3 inflammasome activation by regulating NLRP3 expression. *J. Immunol.* 183, 787–791.

Boyden, E. D., and Dietrich, W. F. (2006). Nalp1b controls mouse macrophage susceptibility to anthrax lethal toxin. *Nat. Genet.* 38, 240–244.

Broz, P., Newton, K., Lamkanfi, M., Mariathasan, S., Dixit, V. M., and Monack, D. M. (2010). Redundant roles for inflammasome receptors NLRP3 and NLRC4 in host defense against *Salmonella. J. Exp. Med.* 207, 1745–1755.

Carlsson, F., Kim, J., Dumitru, C., Barck, K. H., Carano, R. A., Sun, M., Diehl, L., and Brown, E. J. (2010). Host-

detrimental role of Esx-1-mediated inflammasome activation in mycobacterial infection. *PLoS Pathog.* 6, e1000895. doi: 10.1371/journal.ppat.1000895

Craven, R. R., Gao, X., Allen, I. C., Gris, D., Wardenburg, J. B., McElvania-Tekippe, E., Ting, J. P., and Duncan, J. A. (2009). *Staphylococcus aureus* α-hemolysin activates the NLRP3-inflammasome in human and mouse monocytic cells. *PLoS ONE* 4, e7446. doi: 10.1371/journal.pone.0007446

Creagh, E. M., and O'Neill, L. A. (2006). TLRs, NLRs and RLRs: a trinity of pathogen sensors that co-operate in innate immunity. *Trends Immunol.* 27, 352–357.

Delaloye, J., Roger, T., Steiner-Tardivel, Q. G., Le Roy, D., Knaup Reymond, M., Akira, S., Petrilli, V., Gomez, C. E., Perdiguero, B., Tschopp, J., Pantaleo, G., Esteban, M., and Calandra, T. (2009). Innate immune sensing of modified vaccinia virus Ankara (MVA) is mediated by TLR2–TLR6, MDA-5 and the NALP3 inflammasome. *PLoS Pathog.* 5, e1000480. doi: 10.1371/journal.ppat.1000480

Dietl, P., and Volkl, H. (1994). Maitotoxin activates a nonselective cation channel and stimulates Ca2+ entry in MDCK renal epithelial cells. *Mol. Pharmacol.* 45, 300–305.

Dostert, C., Guarda, G., Romero, J. F., Menu, P., Gross, O., Tardivel, A., Suva, M. L., Stehle, J. C., Kopf, M., Stamenkovic, I., Corradin, G., and Tschopp, J. (2009). Malarial hemozoin is a Nalp3 inflammasome activating danger signal. *PLoS ONE* 4, e6510. doi: 10.1371/journal.pone.0006510

Dostert, C., Petrilli, V., Van Bruggen, R., Steele, C., Mossman, B. T., and Tschopp, J. (2008). Innate immune activation through Nalp3 inflammasome sensing of asbestos and silica. *Science* 320, 674–677.

Duncan, J. A., Gao, X., Huang, M. T., O'Connor, B. P., Thomas, C. E., Willingham, S. B., Bergstralh, D. T., Jarvis, G. A., Sparling, P. F., and Ting, J. P. (2009). *Neisseria gonorrhoeae* activates the proteinase cathepsin B to mediate the signaling activities of the NLRP3 and ASC-containing inflammasome. *J. Immunol.* 182, 6460–6469.

Franchi, L., Amer, A., Body-Malapel, M., Kanneganti, T. D., Ozoren, N., Jagirdar, R., Inohara, N., Vandenabeele, P., Bertin, J., Coyle, A., Grant, E. P., and Nunez, G. (2006). Cytosolic flagellin requires Ipaf for activation of caspase-1 and interleukin 1beta in *Salmonella*-infected macrophages. *Nat. Immunol.* 7, 576–582.

Franchi, L., Eigenbrod, T., and Nunez, G. (2009). Cutting edge: TNF-alpha mediates sensitization to ATP and

silica via the NLRP3 inflammasome in the absence of microbial stimulation. *J. Immunol.* 183, 792–796.

Gross, O., Gewies, A., Finger, K., Schafer, M., Sparwasser, T., Peschel, C., Forster, I., and Ruland, J. (2006). Card9 controls a non-TLR signalling pathway for innate anti-fungal immunity. *Nature* 442, 651–656.

Gross, O., Poeck, H., Bscheider, M., Dostert, C., Hannesschlager, N., Endres, S., Hartmann, G., Tardivel, A., Schweighoffer, E., Tybulewicz, V., Mocsai, A., Tschopp, J., and Ruland, J. (2009). Syk kinase signalling couples to the Nlrp3 inflammasome for anti-fungal host defence. *Nature* 459, 433–436.

Harder, J., Franchi, L., Munoz-Planillo, R., Park, J. H., Reimer, T., and Nunez, G. (2009). Activation of the Nlrp3 inflammasome by *Streptococcus pyogenes* requires streptolysin O and NF-kappa B activation but proceeds independently of TLR signaling and P2X7 receptor. *J. Immunol.* 183, 5823–5829.

He, X., Mekasha, S., Mavrogiorgos, N., Fitzgerald, K. A., Lien, E., and Ingalls, R. R. (2010). Inflammation and fibrosis during *Chlamydia pneumoniae* infection is regulated by IL-1 and the NLRP3/ASC inflammasome. *J. Immunol.* 184, 5743–5754.

Hise, A. G., Tomalka, J., Ganesan, S., Patel, K., Hall, B. A., Brown, G. D., and Fitzgerald, K. A. (2009). An essential role for the NLRP3 inflammasome in host defense against the human fungal pathogen *Candida albicans*. *Cell Host Microbe* 5, 487–497.

Hornung, V., Bauernfeind, F., Halle, A., Samstad, E. O., Kono, H., Rock, K. L., Fitzgerald, K. A., and Latz, E. (2008). Silica crystals and aluminum salts activate the NALP3 inflammasome through phagosomal destabilization. *Nat. Immunol.* 9, 847–856.

Hornung, V., and Latz, E. (2010). Intracellular DNA recognition. *Nat. Rev. Immunol.* 10, 123–130.

Ichinohe, T., Lee, H. K., Ogura, Y., Flavell, R., and Iwasaki, A. (2009). Inflammasome recognition of influenza virus is essential for adaptive immune responses. *J. Exp. Med.* 206, 79–87.

Ichinohe, T., Pang, I. K., and Iwasaki, A. (2010). Influenza virus activates inflammasomes via its intracellular M2 ion channel. *Nat. Immunol.* 11, 404–410.

Joly, S., Ma, N., Sadler, J. J., Soll, D. R., Cassel, S. L., and Sutterwala, F. S. (2009). Cutting edge: *Candida albicans* hyphae formation triggers activation of the Nlrp3 inflammasome. *J. Immunol.* 183, 3578–3581.

Kankkunen, P., Teirila, L., Rintahaka, J., Alenius, H., Wolff, H., and

Matikainen, S. (2010). (1,3)-beta-glucans activate both dectin-1 and NLRP3 inflammasome in human macrophages. *J. Immunol.* 184, 6335–6342.

Kanneganti, T. D. (2010). Central roles of NLRs and inflammasomes in viral infection. *Nat. Rev. Immunol.* 10, 688–698.

Kanneganti, T. D., Lamkanfi, M., and Nunez, G. (2007). Intracellular NOD-like receptors in host defense and disease. *Immunity* 27, 549–559.

Kanneganti, T. D., Ozoren, N., Body-Malapel, M., Amer, A., Park, J. H., Franchi, L., Taraporewala, Z. F., Miller, D., Patton, J. T., Inohara, N., and Nunez, G. (2006a). Bacterial RNA and small antiviral compounds activate caspase-1 through cryopyrin/Nalp3. *Nature* 440, 233–236.

Kanneganti, T. D., Body-Malapel, M., Amer, A., Park, J. H., Whitfield, J., Franchi, L., Whitfield, J., Barchet, W., Colonna, M., Vandenabeele, P., Bertin, J. Coyle, A., Grant, E. P., Akira, S., and Nunez, G. (2006b). Critical role for Cryopyrin/Nalp3 in activation of caspase-1 in response to viral infection and double-stranded RNA. *J. Biol. Chem.* 281, 36560–36568.

Kumar, H., Kumagai, Y., Tsuchida, T., Koenig, P. A., Satoh, T., Guo, Z., Jang, M. H., Saitoh, T., Akira, S., and Kawai, T. (2009). Involvement of the NLRP3 inflammasome in innate and humoral adaptive immune responses to fungal beta-glucan. *J. Immunol.* 183, 8061–8067.

Lamkanfi, M., and Kanneganti, T. D. (2010). Nlrp3: an immune sensor of cellular stress and infection. *Int. J. Biochem. Cell Biol.* 42, 792–795.

Lamkanfi, M., Malireddi, R. K., and Kanneganti, T. D. (2009). Fungal zymosan and mannan activate the cryopyrin inflammasome. *J. Biol. Chem.* 284, 20574–20581.

Lo, H. J., Kohler, J. R., DiDomenico, B., Loebenberg, D., Cacciapuoti, A., and Fink, G. R. (1997). Nonfilamentous *C. albicans* mutants are avirulent. *Cell* 90, 939–949.

Mariathasan, S., Weiss, D. S., Newton, K., McBride, J., O'Rourke, K., Roose-Girma, M., Lee, W. P., Weinrauch, Y., Monack, D. M., and Dixit, V. M. (2006). Cryopyrin activates the inflammasome in response to toxins and ATP. *Nature* 440, 228–232.

McElvania Tekippe, E., Allen, I. C., Hulseberg, P. D., Sullivan, J. T., McCann, J. R., Sandor, M., Braunstein, M., and Ting, J. P. (2010). Granuloma formation and host defense in chronic *Mycobacterium tuberculosis* infection requires PYCARD/ASC but not NLRP3 or caspase-1. *PLoS ONE* 5, e12320. doi: 10.1371/journal.pone.0012320

Meissner, F., Seger, R. A., Moshous, D., Fischer, A., Reichenbach, J., and Zychlinsky, A. (2010). Inflammasome activation in NADPH oxidase defective mononuclear phagocytes from patients with chronic granulomatous disease. *Blood* 116, 1570–1573.

Mencacci, A., Bacci, A., Cenci, E., Montagnoli, C., Fiorucci, S., Casagrande, A., Flavell, R. A., Bistoni, F., and Romani, L. (2000). Interleukin 18 restores defective Th1 immunity to *Candida albicans* in caspase 1-deficient mice. *Infect. Immun.* 68, 5126–5131.

Miao, E. A., Alpuche-Aranda, C. M., Dors, M., Clark, A. E., Bader, M. W., Miller, S. I., and Aderem, A. (2006). Cytoplasmic flagellin activates caspase-1 and secretion of interleukin 1beta via Ipaf. *Nat. Immunol.* 7, 569–575.

Munoz-Planillo, R., Franchi, L., Miller, L. S., and Nunez, G. (2009). A critical role for hemolysins and bacterial lipoproteins in *Staphylococcus aureus*-induced activation of the Nlrp3 inflammasome. *J. Immunol.* 183, 3942–3948.

Muruve, D. A., Petrilli, V., Zaiss, A. K., White, L. R., Clark, S. A., Ross, P. J., Parks, R. J., and Tschopp, J. (2008). The inflammasome recognizes cytosolic microbial and host DNA and triggers an innate immune response. *Nature* 452, 103–107.

Nakagawa, I., Amano, A., Mizushima, N., Yamamoto, A., Yamaguchi, H., Kamimoto, T., Nara, A., Funao, J., Nakata, M., Tsuda, K., Hamada, S., and Yoshimori, T. (2004). Autophagy defends cells against invading group A *Streptococcus*. *Science* 306, 1037–1040.

North, R. A. (2002). Molecular physiology of P2X receptors. *Physiol. Rev.* 82, 1013–1067.

Palsson-McDermott, E. M., and O'Neill, L. A. (2007). Building an immune system from nine domains. *Biochem. Soc. Trans.* 35, 1437–1444.

Petrilli, V., Papin, S., Dostert, C., Mayor, A., Martinon, F., and Tschopp, J. (2007). Activation of the NALP3 inflammasome is triggered by low intracellular potassium concentration. *Cell Death Differ.* 14, 1583–1589.

Poeck, H., and Ruland, J. (2010). SYK kinase signaling and the NLRP3 inflammasome in antifungal immunity. *J. Mol. Med.* 88, 745–752.

Romani, L. (2004). Immunity to fungal infections. *Nat. Rev. Immunol.* 4, 1–23.

Said-Sadier, N., Padilla, E., Langsley, G., and Ojcius, D. M. (2010). *Aspergillus fumigatus* stimulates the NLRP3 inflammasome through a pathway requiring ROS production and the Syk tyrosine kinase. *PLoS ONE* 5, e10008. doi: 10.1371/journal.pone.0010008

Schilling, W. P., Sinkins, W. G., and Estacion, M. (1999). Maitotoxin

activates a nonselective cation channel and a P2Z/P2X(7)-like cytolytic pore in human skin fibroblasts. *Am. J. Physiol.* 277, C755–C765.

Thomas, P. G., Dash, P., Aldridge, J. R. Jr., Ellebedy, A. H., Reynolds, C., Funk, A. J., Martin, W. J., Lamkanfi, M., Webby, R. J., Boyd, K. L., Doherty, P. C., and Kanneganti, T. D. (2009). The intracellular sensor NLRP3 mediates key innate and healing responses to influenza A virus via the regulation of caspase-1. *Immunity* 30, 566–575.

Toma, C., Higa, N., Koizumi, Y., Nakasone, N., Ogura, Y., McCoy, A. J., Franchi, L., Uematsu, S., Sagara, J., Taniguchi, S., Tsutsui, H., Akira, S., Tschopp, J., Nunez, G., and Suzuki, T. (2010). Pathogenic *Vibrio* activate NLRP3 inflammasome via cytotoxins and TLR/nucleotide-binding oligomerization domain-mediated NF-kappa B signaling. *J. Immunol.* 184, 5287–5297.

Unterholzner, L., Keating, S. E., Baran, M., Horan, K. A., Jensen, S. B., Sharma, S.,

Sirois, C. M., Jin, T., Latz, E., Xiao, T. S., Fitzgerald, K. A., Paludan, S. R., and Bowie, A. G. (2010). IFI16 is an innate immune sensor for intracellular DNA. *Nat. Immunol.* 11, 997–1004.

van de Veerdonk, F. L., Joosten, L. A., Devesa, I., Mora-Montes, H. M., Kanneganti, T. D., Dinarello, C. A., van der Meer, J. W., Gow, N. A., Kullberg, B. J., and Netea, M. G. (2009). Bypassing pathogen-induced inflammasome activation for the regulation of interleukin-1beta production by the fungal pathogen *Candida albicans*. *J. Infect. Dis.* 199, 1087–1096.

van de Veerdonk, F. L., Smeekens, S. P., Joosten, L. A., Kullberg, B. J., Dinarello, C. A., van der Meer, J. W., and Netea, M. G. (2010). Reactive oxygen species-independent activation of the IL-1beta inflammasome in cells from patients with chronic granulomatous disease. *Proc. Natl. Acad. Sci. U.S.A.* 107, 3030–3033.

Vonk, A. G., Netea, M. G., van Krieken, J. H., Iwakura, Y., van der Meer, J. W.,

and Kullberg, B. J. (2006). Endogenous interleukin (IL)-1 alpha and IL-1 beta are crucial for host defense against disseminated candidiasis. *J. Infect. Dis.* 193, 1419–1426.

Warren, S. E., Mao, D. P., Rodriguez, A. E., Miao, E. A., and Aderem, A. (2008). Multiple Nod-like receptors activate caspase 1 during *Listeria monocytogenes* infection. *J. Immunol.* 180, 7558–7564.

Wu, J., Fernandes-Alnemri, T., and Alnemri, E. S. (2010). Involvement of the AIM2, NLRC4, and NLRP3 inflammasomes in caspase-1 activation by *Listeria monocytogenes*. *J. Clin. Immunol.* 30, 693–702.

Zhou, R. B., Tardivel, A., Thorens, B., Choi, I., and Tschopp, J. (2010). Thioredoxin-interacting protein links oxidative stress to inflammasome activation. *Nat. Immunol.* 11, 136–140.

Conflict of Interest Statement: The authors declare that the research was con-

Macroautophagy during innate immune activation

Christian Münz*

Viral Immunobiology, Institute of Experimental Immunology, University of Zürich, Zürich, Switzerland

Innate immune activation is initiated by recognition of pathogen associated molecular patterns (PAMPs). Delivery of PAMPs to their respective receptors, regulation of receptor activity, and effector functions downstream from these receptors, which constitute part of the initiated innate immune control, are in part mediated via macroautophagy, an evolutionary conserved pathway for cytoplasmic constituent degradation in lysosomes. In this review these facets of the recently unveiled involvement of macroautophagy in innate immunity will be summarized, and aspects that need additional investigations will be high-lighted. The improved understanding of the capabilities of macroautophagy for immunity suggests that this pathway should be harnessed in immunotherapies against infectious diseases.

Keywords: Atg, TLR, NOD, inflammasome, RIG-I

INTRODUCTION

Autophagy describes a group of at least three evolutionary conserved cellular degradation processes in eukaryotes that deliver cytoplasmic constituents for lysosomal degradation (Mizushima and Klionsky, 2007). These are macro-, micro-, and chaperone mediated autophagy. During microautophagy the lysosomal membrane invaginates and buds into the lysosomal lumen taking a portion of cytoplasm with it into lysosomal break-down. This pathway, however, has so far only been described in yeast and a clear demonstration that it also occurs in higher eukaryotes is still lacking. During chaperone mediated autophagy proteins with a KFERQ recognition sequence are transported across the lysosomal membrane for degradation (Massey et al., 2006). For this purpose cytosolic chaperones, including HSC70 members, recognize KFERQ, dock to Lamp2a in the lysosomal membrane and are then assisted by another HSC70 member in the lysosomal lumen to import substrates into lysosomes. This process is also conserved in higher eukaryotes, but it remains unclear to which extent it contributes to protein turn-over in the steady-state, since it has primarily been studied under extreme starvation conditions. Macroautophagy as the third pathway is characterized by *de novo* formation of a double-membrane engulfed vesicle, the autophagosome, which then fuses with late endosomes or lysosomes for the degradation of its cargo. Autophagosomes can form from membranes of multiple sources, probably depending on the location of their cargo and initiation signals for vesicle formation. These membrane sources include the rough endoplasmic reticulum (Hayashi-Nishino et al., 2009; Yla-Anttila Vihinen et al., 2009), the Golgi apparatus (Lynch-Day et al., 2010; Yen et al., 2010), the outer nuclear membrane (English et al., 2009), the outer mitochondrial membrane (He et al., 2006; Hailey et al., 2010), and the plasma membrane (Ravikumar et al., 2010). Autophagosome formation and degradation requires more than 30 gene products, so called autophagy-related (Atg) proteins. The site

of autophagosome generation is marked by phosphatidylinositol 3-phosphate (PI3) in membranes, which is deposited there by type III PI3 kinase complexes, containing the PI3 kinase VPS34, VPS15, Atg14L, and Atg6/Beclin-1 (**Figure 1**). Extension of the autophagosomal membrane is then achieved with the help of two ubiquitin-like systems (Ohsumi, 2001). In one of them Atg12 is coupled with the help of the E1- and E2-like enzymes Atg7 and 10 to Atg5, and the complex localizes then with Atg16L1 to the outer membrane of the emerging autophagosome. There it acts as an E3-like enzyme for the conjugation of the other ubiquitin-like molecule Atg8, one homolog of which in mammalian cells is called LC3. Atg8/LC3 is prior to conjugation activated by proteolytic processing through Atg4, exposing a glycine at its C-terminus. It is then activated by the E1-like enzyme Atg7, conjugated to the E2-like enzyme Atg3 and finally ligated to phosphatidylethanolamine in the inner and outer membrane of the emerging autophagosome through the E3-like activity of Atg12–Atg5/Atg16L1. At the autophagosomal membrane it mediates recruitment of ubiquitinated substrates, like protein aggregates and cell organelles, via the ubiquitin and LC3 binding proteins p62, NBR1, and NDP52 (Bjorkoy et al., 2005; Kirkin et al., 2009; Thurston et al., 2009), and probably also membrane fusion for autophagosomal membrane elongation and vesicle completion (Nakatogawa et al., 2007). While Atg8/LC3 remains at the inner autophagosomal membrane, Atg4 cleaves it from the outer autophagosomal membrane and also the Atg12–Atg5/Atg16L1 is recycled after autophagosome completion. Fusion of the completed autophagosomes with lysosomes and late endosomes is then also mediated by Atg6/Beclin-1 containing PI3 kinase complexes with UVRAG or Rubicon replacing Atg14L (Liang et al., 2008; Matsunaga et al., 2009; Zhong et al., 2009). In addition, Rab11 is required for fusion with late endosomes (Fader et al., 2008) and Rab7 and Lamp2 for fusion with lysosomes (Tanaka et al., 2000; Jager et al., 2004). Thus the molecular machinery for macroautophagy has been
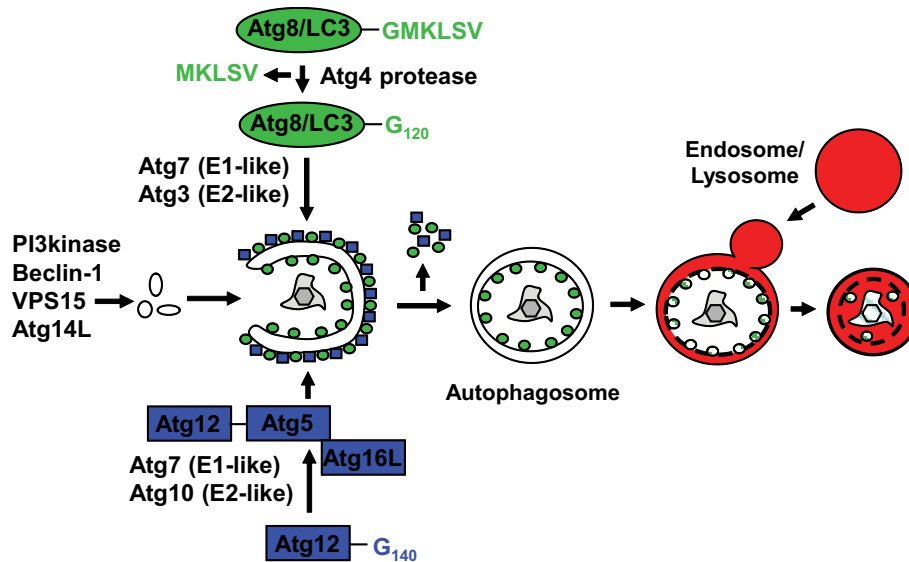
FIGURE 1 | Macroautophagy delivers cytoplasmic constituents to lysosomal degradation via the formation of an autophagosome. While PI3 generated by Beclin-1 containing complexes (PI3 kinase, Beclin-1, VPS15, and Atg14L) marks sites of autophagosome generation, two ubiquitin-like systems are required to form autophagosomes at these sites. Atg12 is coupled to Atg5 by the indicated E1-like and E2-like enzymes Atg7 and Atg10, and associates with the outer membrane of the emerging autophagosome in complex with Atg16L. The complex guides ligation as an E3-like enzyme of Atg8, whose best studied mammalian homolog is LC3, to the outer and inner autophagosomal membrane. Prior to coupling Atg8/LC3 is processed by Atg4 and activated via the two E1- and E2-like enzymes Atg7 and Atg3. Upon completion of the autophagosomes the Atgs recycle from the outer membrane, while Atg8/LC3 is degraded with the inner autophagosomal membrane as well as the autophagosome content upon fusion to lysosomes.

explored in some depth, and the gained knowledge also favored investigations of this particular autophagy pathway during innate immune responses.
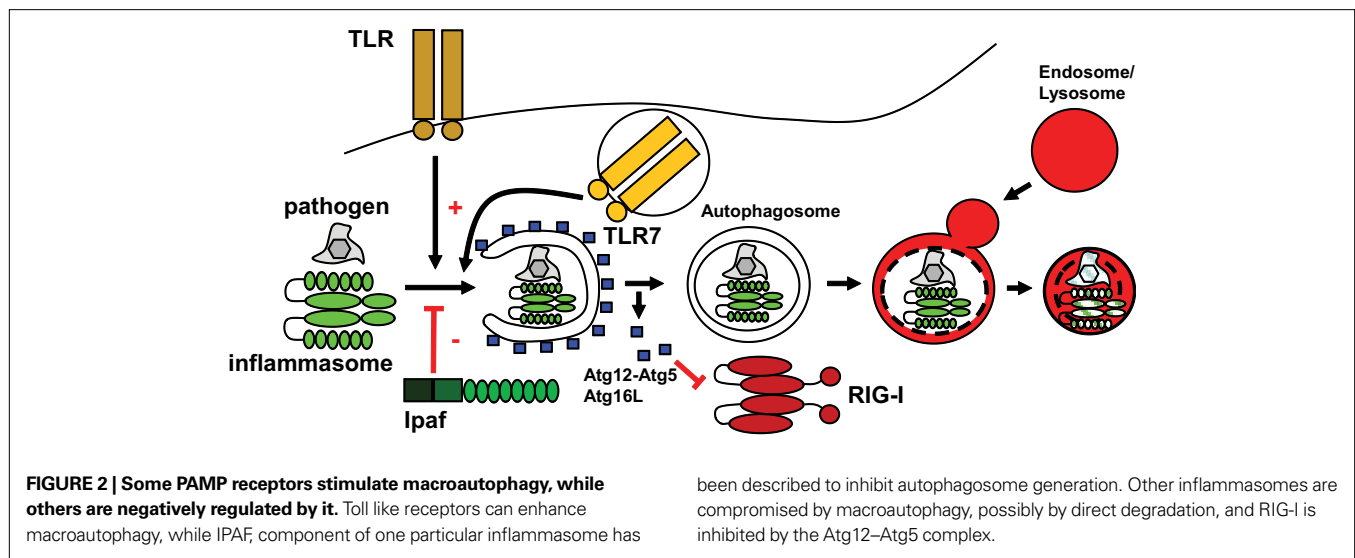
Accordingly, I will primarily summarize the evidence that innate immune recognition regulates macroautophagy, how macroautophagy modulates innate immune recognition in return, which effector functions for innate immunity can be mediated by macroautophagy and how these effector functions might restrict pathogens and commensals *in vivo* both in animal models and even in humans.

PAMP MEDIATED MACROAUTOPHAGY REGULATION

Originally macroautophagy has been described as a response to starvation, recycling cytoplasmic constituents to generate energy and macromolecular building blocks for cell survival. The signals leading to macroautophagy up-regulation in response to amino acid deprivation and growth factor withdrawal have now in part been described (He and Klionsky, 2009). In addition, more recently it has been appreciated that pathogen detection can also up-regulate macroautophagy as an innate immune effector function (Mizushima et al., 2008). Recognition of pathogen associated molecular patterns (PAMPs) or pathogen induced changes in cell organelles stimulate macroautophagy (**Figure 2**). Although intuitively one would predict from the role of macroautophagy in cytosolic organelle and protein aggregate clearance that cytosolic PAMP recognition might preferentially enhance this pathway, but PAMP recognition receptors (PRRs) recognizing extra- and intra-cellular structures have been described to up-regulate macroautophagy. This already point to functions of macroautophagy beyond just engulfment of intracellular microbes for their degradation in lysosomes, and

these additional effector functions of macroautophagy will be discussed in the fourth section of this review. With regard to surface and endosomal receptors that trigger macroautophagy mainly toll like receptors (TLRs) were so far evaluated for macroautophagy regulation (Sanjuan et al., 2007; Delgado et al., 2008; Shi and Kehrl, 2008). Among these TLR4, the receptor for bacterial lipopolysaccharide (LPS), and TLR7, a receptor for single stranded RNA, have been reported to induce the strongest autophagosome accumulation in the mouse macrophage cell line RAW264.7 (Xu et al., 2007; Delgado et al., 2008; Shi and Kehrl, 2008). However, up-regulation of macroautophagy by TLR4 has less consistently been observed with primary bone marrow derived mouse macrophages. While some investigators have reported such macroautophagy increase (Travassos et al., 2010), others have failed to observe this (Saitoh et al., 2008). TLR4 recruits upon LPS engagement the adaptor molecules MyD88 and Trif, which in turn bind ubiquitinated Atg6/Beclin-1 to enhance macroautophagy (Shi and Kehrl, 2008, 2010), possibly by initiating autophagosome formation at the cell membrane (Ravikumar et al., 2010). Therefore, TLR signaling seems to up-regulate macroautophagy in mouse phagocytes, but which PAMPs achieve this in human cells and which pathogens relevant to human disease are affected by it, remains to be determined.

In addition to TLR mediated up-regulation, cytosolic PRRs have also been demonstrated to up-regulate macroautophagy. These are the bacterial peptidoglycan receptors PGRP-LE in *Drosophila* and NOD1 and two in mammalian cells (Yano et al., 2008; Cooney et al., 2010; Travassos et al., 2010). The NOD molecules recruit Atg16L1 to the bacterial entry site for efficient degradation of invading pathogens (Travassos et al., 2010). Apart from bacterial peptidoglycan

FIGURE 2 | Some PAMP receptors stimulate macroautophagy, while others are negatively regulated by it. Toll like receptors can enhance macroautophagy, while IPAF, component of one particular inflammasome has been described to inhibit autophagosome generation. Other inflammasomes are compromised by macroautophagy, possibly by direct degradation, and RIG-I is inhibited by the Atg12–Atg5 complex.

detection, double-stranded RNA (dsRNA), a PAMP associated with viral replication, stimulated the IFN-inducible eIF2 kinase PKR (dsRNA-dependent protein kinase) to induce macroautophagy in herpesvirus infected cells (Talloczy et al., 2002). Therefore, PKR and NOD molecules seem to stimulate macroautophagy upon cytosolic pathogen recognition. In contrast, NOD-like receptors (NLRs) that give rise to inflammasomes and RIG-I like receptors (RLRs) are negatively regulated by macroautophagy, as will be discussed below.

In addition to direct macroautophagy up-regulation after PAMP recognition, cytokines that are produced in response to pathogen detection, have also been identified as modulators of macroautophagy. Initially, IFN-γ was identified to mediate *Mycobacterium tuberculosis* clearance in part via up-regulation of macroautophagy (Gutierrez et al., 2004). However, similar to PRR mediated macroautophagy modulation, this effect was more pronounced in the mouse macrophage RAW264.7 or human macrophage THP-1 and U937 cell lines, than in primary human or mouse macrophages (Harris et al., 2007). In contrast to the cell-mediated immunity supporting cytokine IFN-γ, cytokines associated primarily with humoral immune responses, like IL-4 and IL-13, seemed to rather inhibit macroautophagy (Harris et al., 2007). Furthermore, TNF-α was reported to induce macroautophagy in cells with low NF-κB activation (Djavaheri-Mergny et al., 2006). These included Ewing sarcoma cells, in which NF-κB activation was compromised and skeletal muscle cells (Keller et al., 2011). In good agreement with TNF-α induced macroautophagy in myocytes, muscle fibers of patients with inclusion body myositis, a myopathy with chronic inflammation, harbored increased numbers of autophagosomes and were surrounded by proinflammatory infiltrates. These findings suggest that pathogen recognition can up-regulate macroautophagy directly via some PRRs and indirectly via cytokines that are produced upon pathogen triggered immune activation.

Unrelated to PRRs and cytokines, macroautophagy can also be enhanced by pathogens for their benefit. The prototypic example for this is poliovirus infection, during which the viral 2BC and 3A proteins induce the accumulation of Atg12 and Atg8/LC3 dependent double-membrane vesicles for viral replication (Dales et al., 1965;

Jackson et al., 2005). Furthermore, binding of the human immunodeficiency virus (HIV) envelope protein to the chemokine receptor CXCR4 augments macroautophagy and seems to enhance bystander T cell death for immune escape (Espert et al., 2006). Finally, primarily two virus families, the herpes- and the flavi-viruses, have been described to enhance macroautophagy via the unfolded protein response (Sir et al., 2008; Lee et al., 2009; Dreux and Chisari, 2010; Lin et al., 2010), and thereby gain benefits for viral replication and latent to lytic infection transition (Dreux et al., 2009; Lee and Sugden, 2008; Lee et al., 2008). In addition to these beneficial effect of macroautophagy up-regulation for pathogens, other microbes also inhibit this pathway during infection, and these immune escape strategies from innate effector function will be discussed below.

MACROAUTOPHAGY MODIFICATION OF PAMP RECOGNITION

While TLRs and NLRs enhance macroautophagy and little is know so far about C-type lectin receptor (CLR) interaction with this pathway, other cytosolic PAMP recognition pathways seem to be negatively impacted by macroautophagy (**Figure 2**). Along these lines, dsRNA recognition by RNA helicases of the family of RLRs is augmented in macroautophagy deficient cells (Jounai et al., 2007; Tal et al., 2009). The Atg12–Atg5 complex seems to directly associate with RIG-I and its adaptor IPS-1 to inhibit viral RNA induced type I IFN production (Jounai et al., 2007). Moreover, the accumulation of damaged mitochondria in macroautophagy deficient cells and the associated increase in reactive oxygen species (ROS) production, seem to enhance RLR activity in response to viral infections (Tal et al., 2009). Thus, RLR recognition of cytosolic dsRNA is attenuated by macroautophagy.

Parallel to decreasing cytosolic RNA recognition, macroautophagy enhances delivery of viral RNA replication intermediates to TLR containing endosomes (Lee et al., 2007). TLR7 dependent recognition of these single-stranded RNAs (ssRNAs) is essential for IFN-α production by vesicular stomatitis (VSV) and Sendai virus infected plasmacytoid dendritic cells (DCs), and depends on macroautophagy (Lee et al., 2007). Similarly, endocytosed DNA seems to be targeted to TLR9 containing endosomes via macroautophagy
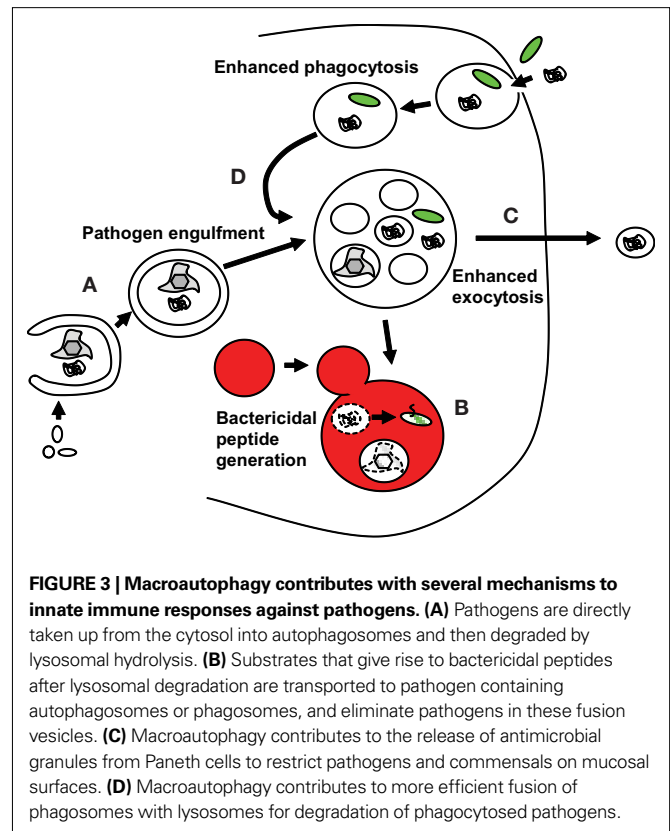
in B cells (Chaturvedi et al., 2008), possibly assisting autoantibody production in systemic lupus erythematosus patients. These findings indicate that macroautophagy enhances PAMP delivery to endosomal TLRs.

Among the NLRs, NOD1 and two seem to up-regulate macroautophagy in mouse phagocytes and human DCs as discussed above, whereas signaling through other members of this family that initiate inflammasome formation is inhibited by macroautophagy and deficiency in inflammasome components increases macroautophagy in *Shigella flexneri* infected macrophages (Suzuki et al., 2007; Saitoh et al., 2008). Indeed, loss of Atg16L1 increases IL-1β secretion by mouse macrophages, which depends on inflammasome activated caspase 1 processing. This increased IL-1β production worsens dextran sulfate sodium induced colitis in mice. The negative regulation of inflammasome dependent inflammation was suggested to be directly mediated via inflammasome degradation by macroautophagy (Harris et al., 2009), but this needs to be experimentally confirmed. In addition, it remains also unclear which inflammasomes are affected by negative regulation via macroautophagy and if the various inflammasome scaffolds are regulated differently. Thus, both RLR and inflammasome signaling in response to PAMP recognition are inhibited by macroautophagy, while the same pathway delivers RNA and DNA for endosomal PRRs.

EFFECTOR MECHANISMS OF MACROAUTOPHAGY FOR INNATE PATHOGEN RESTRICTION

PAMP recognition receptors mediated macroautophagy up-regulation is able to exert innate restriction of intracellular pathogens, both those that escape endosomes and others that condition phagosomes to become their replication compartments (Münz, 2009). The first pathogens described in these categories were group A *Streptococci* and *Mycobacterium tuberculosis*, respectively (Gutierrez et al., 2004; Nakagawa et al., 2004). Initially it was just assumed that macroautophagy delivers these pathogens for lysosomal degradation, thereby limiting pathogen burden in infected cells (**Figure 3A**). However, recently it has become apparent that macroautophagy delivers in addition substrates that are then converted into bactericidal peptides by lysosomal hydrolysis (**Figure 3B**; Alonso et al., 2007; Ponpuak et al., 2010). In both instances ubiquitin itself or proteins containing a ubiquitin-like domain, like the ribosomal protein S30 (rpS30) precursor Fau, are transported into *Mycobacterium tuberculosis* containing phagosomes to be processed to bactericides. In the case of Fau, binding of its ubiquitin-like domain to p62, which in turn recruits this ribosomal protein precursor to autophagosomes by virtue of binding to Atg8/LC3, was important to deliver this source of bactericidal activity to mycobacterial phagosomes (Ponpuak et al., 2010). Thus, macroautophagy does not only deliver pathogens for lysosomal hydrolysis, but also charges pathogen containing phagosomes with proteins that give rise to bactericidal peptides after lysosomal hydrolysis.

Apart from intracellular pathogen delivery to lysosomes and bactericidal source protein delivery to phagosomes, macroautophagy seems to also enhance degradation of phagocytosed pathogens (**Figure 3D**). A role for the molecular machinery of macroautophagy in the enhanced fusion of phagosomes with lysosomes was first identified upon TLR2 ligation in the mouse macrophage cell line RAW264.7 (Sanjuan et al., 2007). However, also *in vivo*

FIGURE 3 | Macroautophagy contributes with several mechanisms to innate immune responses against pathogens. (A) Pathogens are directly taken up from the cytosol into autophagosomes and then degraded by lysosomal hydrolysis. **(B)** Substrates that give rise to bactericidal peptides after lysosomal degradation are transported to pathogen containing autophagosomes or phagosomes, and eliminate pathogens in these fusion vesicles. **(C)** Macroautophagy contributes to the release of antimicrobial granules from Paneth cells to restrict pathogens and commensals on mucosal surfaces. **(D)** Macroautophagy contributes to more efficient fusion of phagosomes with lysosomes for degradation of phagocytosed pathogens.

processing of extracellular antigen by DCs was compromised by Atg5 deficiency in this cell subset, and this was due to diminished lysosomal hydrolase recruitment to endocytosed material (Lee et al., 2010). The enhanced phagosome maturation via Atgs seems to require either NADPH oxidase activity or diacylglycerol production at the bacterial pathogen containing endosomes in mouse myeloid cells (Huang et al., 2009; Shahnazari and Brumell, 2009). However, the nature of the quality conferred by autophagosome or just Atg recruitment to phagosomes that allows more efficient fusion with lysosomes remains to be determined.

In addition to these bactericidal effector functions in endosomes, supported by macroautophagy, the autophagic machinery seems to also support bactericidal peptide release at mucosal surfaces (**Figure 3C**). Paneth cells at the base of ileal crypts suffer aberrant packaging and reduced exocytosis of antimicrobial granules from the hypomorphic down-regulation of Atg16L1 expression (Cadwell et al., 2008). Moreover, this aberration was only observed upon co-infection with the murine norovirus, a positive strand RNA virus (Cadwell et al., 2010). Therefore, macroautophagy assistance for exocytosis of bactericidal granules seems to be mainly important during enteric infections.

An indication that these macroautophagic effector functions are interfering with pathogen replication is provided by the many mechanisms by which viruses and bacteria interfere with either autophagosome formation or fusion with lysosomes (Schmid and Münz, 2007). An exhaustive list of such pathogens and their immune escape strategies targeting macroautophagy is beyond the scope of this review and has been recently summarized elsewhere (Levine and Deretic, 2007). Thus, I will just high-light some gen-

eral pathways of immune escape from autophagy by pathogens and discuss examples below, for which *in vivo* evidence for the importance of these has been gained. While DNA viruses, like Herpesviruses, seem to preferentially inhibit autophagosome formation, RNA viruses like HIV and influenza A virus seem to block autophagosome fusion with lysosomes (Gannage et al., 2009; Kyei et al., 2009; Gannage and Münz, 2010; Taylor et al., 2011). In addition, some bacterial pathogens interfere with engulfment by macroautophagy by either blocking bacterial protein recognition or covering themselves with cytosolic host cell proteins (Ogawa et al., 2005; Yoshikawa et al., 2009). Finally, membrane pore formation and/or bacterial secretion systems, which inject modulators of cellular physiology through endosomal membranes into the cytosol, seem to be required to arrest maturation of bacteria containing amphisomes, born from phagosome fusions with autophagosomes (Celli et al., 2003; Birmingham et al., 2008). Therefore, both viral and bacterial pathogens have developed strategies to evade macroautophagy, arguing for the potency of innate restriction by this pathway and of its effector functions discussed above.

IN VIVO RESTRICTION OF PATHOGEN INFECTION BY MACROAUTOPHAGY

Due to the complexity of immune control mechanisms, it is often difficult to assign *in vivo* phenotypes to particular immune responses without underlying effects in immune compartment development and survival. Accentuated by the well documented pro-survival role of macroautophagy (Kuma et al., 2004), this is also an issue in case of infections and their immune control in mice with deficiencies in macroautophagy. Moreover, since macroautophagy assists both innate and adaptive immunity (Münz, 2009) and macroautophagy clearly assists T cell development and T cell mediated immune responses *in vivo* (Pua et al., 2007; Nedjic et al., 2008; Lee et al., 2010), the contributions of innate and adaptive pathogen restriction mechanisms via macroautophagy in vertebrates still remain to be investigated in detail in *in vivo* infections. However, two experimental systems have been analyzed in detail with respect to innate immunity to viral infections via macroautophagy in mice. These investigated neurotropic infections with the RNA virus, Sindbis Virus (SV) and the DNA virus Herpes simplex virus (HSV; Orvedahl et al., 2007, 2010). Intracerebral inoculation with SV causes autophagosome accumulation in neurons, and mortality of infected mice is increased when macroautophagy is inhibited by either delivering a dominant negative Atg5 protein with the virus, expressing cre recombinase with recombinant SV for deletion of floxed Atg5 in infected cells or neuron specific deletion of Atg5 (Orvedahl et al., 2010). Although CNS viral titers were not increased under these conditions, infection induced protein aggregate formation probably led to increased pathology and delayed virus clearance. In addition, overexpression of Atg6/Beclin-1 in neurons protects mice from lethal intracerebral SV infection (Liang et al., 1998). In contrast to increased neuropathology of SV infection without significant elevation of viral titers in the absence of macroautophagy, HSV inhibits macroautophagy to sustain high viral titers and neurovirulence (Orvedahl et al., 2007). For this inhibition HSV encodes the ICP34.5 protein, which contains a domain that inhibits macroautophagy through binding to Atg6/Beclin-1. Deletion of this domain renders HSV

neuroattenuated with decreased lethality and faster virus clearance from the brain. These data suggest that different neurotropic viruses are restricted by macroautophagy in neurons.

In addition to these mouse models, macroautophagy inhibits infection with the RNA virus VSV in flies (Shelly et al., 2009). Atg18 deficient *Drosophila* flies died rapidly after VSV infection. Furthermore, *Drosophila* uses macroautophagy to also restrict *Listeria* infection (Yano et al., 2008). These bacteria replicate to higher levels and kill their host flies after siRNA mediated silencing of Atg5. Moreover, host defense against *Salmonella typhimurium* infection in the worm *Caenorhabditis elegans* and the slime mold *Dictyostelium discoideum* depends in part on macroautophagy (Jia et al., 2009). SiRNA mediated silencing of Atg6/bec-1 and Atg8/lgg1 in worms increased their susceptibility to Salmonella infection. Similarly, deficiency in Atg1, 6, or 7 compromised survival of mold to infection by the same bacterial pathogen. In both cases diminished macroautophagy caused higher pathogen burden in the infected hosts. These invertebrate models of infectious diseases, lacking adaptive immunity, argue for a significant innate immune control function by macroautophagy *in vivo*.

ASSOCIATION BETWEEN POLYMORPHISMS IN MACROAUTOPHAGY GENES AND HUMAN DISEASE

Although animal models provide strong evidence for the importance of immune mechanisms *in vivo*, only clinical benefit upon manipulation of these pathways or genetic susceptibilities for diseases located in these pathways provide the final proof that some immune function is crucial for resistance against human pathogens. Along these lines, mutations in the essential macroautophagy gene Atg16L1, and macroautophagy stimulating genes, like immunity-related GTPase family M (IRGM) and NOD2 (Singh et al., 2006; Cooney et al., 2010; Travassos et al., 2010), have been described to be associated with inflammatory bowel disease in Crohn's disease patients (Hugot et al., 2001; Ogura et al., 2001; Hampe et al., 2007; Parkes et al., 2007; Rioux et al., 2007; McCarroll et al., 2008). The modulation of macroautophagy by the respective susceptibility alleles could facilitate the development of Crohn's disease by many mechanisms, which have been more comprehensively summarized elsewhere (Meixlsperger and Münz, 2009; Stappenbeck et al., 2011). However, with respect to the influence of macroautophagy on innate immune control of commensals in the digestive tract, whose uncontrolled invasion could trigger inflammatory bowel disease, three main mechanisms might be operational. Firstly, macroautophagy might simply restrict commensal bacteria in myeloid cells by directly targeting intracellular bacteria or assisting phagosome maturation of endocytosed extracellular commensals (Sanjuan et al., 2007; Kuballa et al., 2008; Lapaquette et al., 2010). Secondly, deficiencies in Paneth cells, caused by Atg16L1, which have been observed in Crohn's disease patients could impair innate restriction of commensals at mucosal surfaces (Cadwell et al., 2008, 2010). Thirdly, altered macroautophagy could disturb proinflammatory cytokine production in mucosal tissues by up-regulating inflammasome dependent cytokines like IL-1β and down-regulating PAMP delivery for TLR stimulation and TLR dependent cytokine production, which then in turn would limit commensal invasion (Lee et al., 2007; Saitoh et al., 2008). Although the exact mechanisms, by which the mutations in Atg16L1, IRGM, and NOD2 facilitate

the development of Crohn's disease, still need to be characterized in more detail, the association of mutations in these genes strongly argue for an immune modulating function of this ancient catabolic pathway.

CONCLUSION

The multitude of studies summarized in this review that implicate macroautophagy in pathogen restriction, escape of successful pathogens from this pathway, and genetic susceptibility to human disease due to mutations in it, suggests that macroautophagy plays a crucial role in both maintenance of our coexistence with gut commensals and resistance to infections. Apart from this roles, macroautophagy regulation could counteract consequences of aging (Zhang and Cuervo, 2008), ameliorate neurodegeneration

(Hara et al., 2006; Komatsu et al., 2006), and fight cancer (Mathew et al., 2009). Therefore, it would be desirable to develop therapeutic approaches to modulate macroautophagy in infectious and these other diseases. However at the moment we have very few pharmacologic substances like rapamycin, most of them with significant side effects, to regulate macroautophagy. If more specific modulators could be identified this pathway could be harnessed to increase innate immunity among other benefits.

REFERENCES

Alonso, S., Pethe, K., Russell, D. G., and Purdy, G. E. (2007). Lysosomal killing of *Mycobacterium* mediated by ubiquitin-derived peptides is enhanced by autophagy. *Proc. Natl. Acad. Sci. U.S.A.* 104, 6031–6036.

Birmingham, C. L., Canadien, V., Kaniuk, N. A., Steinberg, B. E., Higgins, D. E., and Brumell, J. H. (2008). Listeriolysin O allows *Listeria monocytogenes* replication in macrophage vacuoles. *Nature* 451, 350–354.

Bjorkoy, G., Lamark, T., Brech, A., Outzen, H., Perander, M., Overvatn, A., Stenmark, H., and Johansen, T. (2005). p62/SQSTM1 forms protein aggregates degraded by autophagy and has a protective effect on huntingtin-induced cell death. *J. Cell Biol.* 171, 603–614.

Cadwell, K., Liu, J. Y., Brown, S. L., Miyoshi, H., Loh, J., Lennerz, J. K., Kishi, C., Kc, W., Carrero, J. A., Hunt, S., Stone, C. D., Brunt, E. M., Xavier, R. J., Sleckman, B. P., Li, E., Mizushima, N., Stappenbeck, T. S., and Virgin, H. W. IV. (2008). A key role for autophagy and the autophagy gene Atg16l1 in mouse and human intestinal Paneth cells. *Nature* 456, 259–263.

Cadwell, K., Patel, K. K., Maloney, N. S., Liu, T. C., Ng, A. C., Storer, C. E., Head, R. D., Xavier, R., Stappenbeck, T. S., and Virgin, H. W. (2010). Virus-plus-susceptibility gene interaction determines Crohn's disease gene Atg16L1 phenotypes in intestine. *Cell* 141, 1135–1145.

Celli, J., de Chastellier, C., Franchini, D. M., Pizarro-Cerda, J., Moreno, E., and Gorvel, J. P. (2003). Brucella evades macrophage killing via VirB-dependent sustained interactions with the endoplasmic reticulum. *J. Exp. Med.* 198, 545–556.

Chaturvedi, A., Dorward, D., and Pierce, S. K. (2008). The B cell receptor governs the subcellular location of Toll-like receptor 9 leading to hyperre-

sponses to DNA-containing antigens. *Immunity* 28, 799–809.

Cooney, R., Baker, J., Brain, O., Danis, B., Pichulik, T., Allan, P., Ferguson, D. J., Campbell, B. J., Jewell, D., and Simmons, A. (2010). NOD2 stimulation induces autophagy in dendritic cells influencing bacterial handling and antigen presentation. *Nat. Med.* 16, 90–97.

Dales, S., Eggers, H. J., Tamm, I., and Palade, G. E. (1965). Electron microscopic study of the formation of poliovirus. *Virology* 26, 379–389.

Delgado, M. A., Elmaoued, R. A., Davis, A. S., Kyei, G., and Deretic, V. (2008). Toll-like receptors control autophagy. *EMBO J.* 27, 1110–1121.

Djavaheri-Mergny, M., Amelotti, M., Mathieu, J., Besancon, F., Bauvy, C., Souquere, S., Pierron, G., and Codogno, P. (2006). NF-kappaB activation represses tumor necrosis factor-alpha-induced autophagy. *J. Biol. Chem.* 281, 30373–30382.

Dreux, M., and Chisari, F. V. (2010). Viruses and the autophagy machinery. *Cell Cycle* 9, 1295–1307.

Dreux, M., Gastaminza, P., Wieland, S. F., and Chisari, F. V. (2009). The autophagy machinery is required to initiate hepatitis C virus replication. *Proc. Natl. Acad. Sci. U.S.A.* 106, 14046–14051.

English, L., Chemali, M., Duron, J., Rondeau, C., Laplante, A., Gingras, D., Alexander, D., Leib, D., Norbury, C., Lippe, R., and Desjardins, M. (2009). Autophagy enhances the presentation of endogenous viral antigens on MHC class I molecules during HSV-1 infection. *Nat. Immunol.* 10, 480–487.

Espert, L., Denizot, M., Grimaldi, M., Robert-Hebmann, V., Gay, B., Varbanov, M., Codogno, P., and Biard-Piechaczyk, M. (2006). Autophagy is involved in T cell death after binding of HIV-1 envelope proteins to CXCR4. *J. Clin. Invest.* 116, 2161–2172.

Fader, C. M., Sanchez, D., Furlan, M., and Colombo, M. I. (2008). Induction of autophagy promotes fusion of multivesicular bodies with autophagic vacuoles in k562 cells. *Traffic* 9, 230–250.

Gannage, M., Dormann, D., Albrecht, R., Dengjel, J., Torossi, T., Ramer, P. C., Lee, M., Strowig, T., Arrey, F., Conenello, G., Pypaert, M., Andersen, J., Garcia-Sastre, A., and Münz, C. (2009). Matrix protein 2 of influenza A virus blocks autophagosome fusion with lysosomes. *Cell Host Microbe* 6, 367–380.

Gannage, M., and Münz, C. (2010). MHC presentation via autophagy and how viruses escape from it. *Semin. Immunopathol.* 32, 373–381.

Gutierrez, M. G., Master, S. S., Singh, S. B., Taylor, G. A., Colombo, M. I., and Deretic, V. (2004). Autophagy is a defense mechanism inhibiting BCG and *Mycobacterium tuberculosis* survival in infected macrophages. *Cell* 119, 753–766.

Hailey, D. W., Rambold, A. S., Satpute-Krishnan, P., Mitra, K., Sougrat, R., Kim, P. K., and Lippincott-Schwartz, J. (2010). Mitochondria supply membranes for autophagosome biogenesis during starvation. *Cell* 141, 656–667.

Hampe, J., Franke, A., Rosenstiel, P., Till, A., Teuber, M., Huse, K., Albrecht, M., Mayr, G., De La Vega, F. M., Briggs, J., Gunther, S., Prescott, N. J, Onnie, C. M., Hasler, R., Sipos, B., Folsch, U. R., Lengauer, T., Platzer, M., Mathew, C. G., Krawczak, M., and Schreiber, S. (2007). A genome-wide association scan of nonsynonymous SNPs identifies a susceptibility variant for Crohn disease in ATG16L1. *Nat. Genet.* 39, 207–211.

Hara, T., Nakamura, K., Matsui, M., Yamamoto, A., Nakahara, Y., Suzuki-Migishima, R., Yokoyama, M., Mishima, K., Saito, I., Okano, H., and Mizushima, N. (2006). Suppression of basal autophagy in neural cells causes

neurodegenerative disease in mice. *Nature* 441, 885–889.

Harris, J., De Haro, S. A., Master, S. S., Keane, J., Roberts, E. A., Delgado, M., and Deretic, V. (2007). T helper 2 cytokines inhibit autophagic control of intracellular *Mycobacterium tuberculosis. Immunity* 27, 505–517.

Harris, J., Hope, J. C., and Lavelle, E. C. (2009). Autophagy and the immune response to TB. *Transbound. Emerg. Dis.* 56, 248–254.

Hayashi-Nishino, M., Fujita, N., Noda, T., Yamaguchi, A., Yoshimori, T., and Yamamoto, A. (2009). A subdomain of the endoplasmic reticulum forms a cradle for autophagosome formation. *Nat. Cell Biol.* 11, 1433–1437.

He, C., and Klionsky, D. J. (2009). Regulation mechanisms and signaling pathways of autophagy. *Annu. Rev. Genet.* 43, 67–93.

He, C., Song, H., Yorimitsu, T., Monastyrska, I., Yen, W. L., Legakis, J. E., and Klionsky, D. J. (2006). Recruitment of Atg9 to the preautophagosomal structure by Atg11 is essential for selective autophagy in budding yeast. *J. Cell Biol.* 175, 925–935.

Huang, J., Canadien, V., Lam, G. Y., Steinberg, B. E., Dinauer, M. C., Magalhaes, M. A., Glogauer, M., Grinstein, S., and Brumell, J. H. (2009). Activation of antibacterial autophagy by NADPH oxidases. *Proc. Natl. Acad. Sci. U.S.A.* 106, 6226–6231.

Hugot, J. P., Chamaillard, M., Zouali, H., Lesage, S., Cezard, J. P., Belaiche, J., Almer, S., Tysk, C., O'Morain, C. A., Gassull, M., Binder, V., Finkel, Y., Cortot, A., Modigliani, R., Laurent-Puig, P., Gower-Rousseau, C., Macry, J., Colombel, J. F., Sahbatou, M., and Thomas, G. (2001). Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* 411, 599–603.

Jackson, W. T., Giddings, T. H. Jr., Taylor, M. P., Mulinyawe, S., Rabinovitch, M., Kopito, R. R., and Kirkegaard,

K. (2005). Subversion of cellular autophagosomal machinery by RNA viruses. *PLoS Biol.* 3, e156. doi: 10.1371/journal.pbio.0030156

Jager, S., Bucci, C., Tanida, I., Ueno, T., Kominami, E., Saftig, P., and Eskelinen, E. L. (2004). Role for Rab7 in maturation of late autophagic vacuoles. *J. Cell. Sci.* 117(Pt 20), 4837–4848.

Jia, K., Thomas, C., Akbar, M., Sun, Q., Adams-Huet, B., Gilpin, C., and Levine, B. (2009). Autophagy genes protect against *Salmonella typhimurium* infection and mediate insulin signaling-regulated pathogen resistance. *Proc. Natl. Acad. Sci. U.S.A.* 106, 14564–14569.

Jounai, N., Takeshita, F., Kobiyama, K., Sawano, A., Miyawaki, A., Xin, K. Q., Ishii, K. J., Kawai, T., Akira, S., Suzuki, K., and Okuda, K. (2007). The Atg5 Atg12 conjugate associates with innate antiviral immune responses. *Proc. Natl. Acad. Sci. U.S.A.* 104, 14050–14055.

Keller, C. W., Fokken, C., Turville, S. G., Lünemann, A., Schmidt, J., Münz, C., and Lünemann, J. D. (2011). TNF-alpha induces macroautophagy and regulates MHC class II expression in human skeletal muscle cells. *J. Biol. Chem.* 286, 3970–3980.

Kirkin, V., Lamark, T., Sou, Y. S., Bjorkoy, G., Nunn, J. L., Bruun, J. A., Shvets, E., McEwan, D. G., Clausen, T. H., Wild, P., Bilusic, I., Theurillat, J. P., Overvatn, A., Ishii, T., Elazar, Z., Komatsu, M., Dikic, I., and Johansen, T. (2009). A role for NBR1 in autophagosomal degradation of ubiquitinated substrates. *Mol. Cell* 33, 505–516.

Komatsu, M., Waguri, S., Chiba, T., Murata, S., Iwata, J., Tanida, I., Ueno, T., Koike, M., Uchiyama, Y., Kominami, E., and Tanaka, K. (2006). Loss of autophagy in the central nervous system causes neurodegeneration in mice. *Nature* 441, 880–884.

Kuballa, P., Huett, A., Rioux, J. D., Daly, M. J., and Xavier, R. J. (2008). Impaired autophagy of an intracellular pathogen induced by a Crohn's disease associated ATG16L1 variant. *PLoS ONE* 3, e3391. doi: 10.1371/journal.pone.0003391

Kuma, A., Hatano, M., Matsui, M., Yamamoto, A., Nakaya, H., Yoshimori, T., Ohsumi, Y., Tokuhisa, T., and Mizushima, N. (2004). The role of autophagy during the early neonatal starvation period. *Nature* 432, 1032–1036.

Kyei, G. B., Dinkins, C., Davis, A. S., Roberts, E., Singh, S. B., Dong, C., Wu, L., Kominami, E., Ueno, T., Yamamoto, A., Federico, M., Panganiban, A., Vergne, I., and Deretic, V. (2009). Autophagy pathway intersects with HIV-1 biosynthesis and regulates viral

yields in macrophages. *J. Cell Biol.* 186, 255–268.

Lapaquette, P., Glasser, A. L., Huett, A., Xavier, R. J., and Darfeuille-Michaud, A. (2010). Crohn's disease-associated adherent-invasive *E. coli* are selectively favoured by impaired autophagy to replicate intracellularly. *Cell Microbiol.* 12, 99–113.

Lee, D. Y., Lee, J., and Sugden, B. (2009). The unfolded protein response and autophagy: herpesviruses rule! *J. Virol.* 83, 1168–1172.

Lee, D. Y., and Sugden, B. (2008). The latent membrane protein 1 oncogene modifies B-cell physiology by regulating autophagy. *Oncogene* 27, 2833–2842.

Lee, H. K., Lund, J. M., Ramanathan, B., Mizushima, N., and Iwasaki, A. (2007). Autophagy-dependent viral recognition by plasmacytoid dendritic cells. *Science* 315, 1398–1401.

Lee, H. K., Mattei, L. M., Steinberg, B. E., Alberts, P., Lee, Y. H., Chervonsky, A., Mizushima, N., Grinstein, S., and Iwasaki, A. (2010). In vivo requirement for Atg5 in antigen presentation by dendritic cells. *Immunity* 32, 227–239.

Lee, Y. R., Lei, H. Y., Liu, M. T., Wang, J. R., Chen, S. H., Jiang-Shieh, Y. F., Lin, Y. S., Yeh, T. M., Liu, C. C., and Liu, H. S. (2008). Autophagic machinery activated by dengue virus enhances virus replication. *Virology* 374, 240–248.

Levine, B., and Deretic, V. (2007). Unveiling the roles of autophagy in innate and adaptive immunity. *Nat. Rev. Immunol.* 7, 767–777.

Liang, C., Lee, J. S., Inn, K. S., Gack, M. U., Li, Q., Roberts, E. A., Vergne, I., Deretic, V., Feng, P., Akazawa, C., and Jung, J. U. (2008). Beclin1-binding UVRAG targets the class C Vps complex to coordinate autophagosome maturation and endocytic trafficking. *Nat. Cell Biol.* 10, 776–787.

Liang, X. H., Kleeman, L. K., Jiang, H. H., Gordon, G., Goldman, J. E., Berry, G., Herman, B., and Levine, B. (1998). Protection against fatal Sindbis virus encephalitis by beclin, a novel Bcl-2-interacting protein. *J. Virol.* 72, 8586–8596.

Lin, L. T., Dawson, P. W., and Richardson, C. D. (2010). Viral interactions with macroautophagy: a double-edged sword. *Virology* 402, 1–10.

Lynch-Day, M. A., Bhandari, D., Menon, S., Huang, J., Cai, H., Bartholomew, C. R., Brumell, J. H., Ferro-Novick, S., and Klionsky, D. J. (2010). Trs85 directs a Ypt1 GEF, TRAPPIII, to the phagophore to promote autophagy. *Proc. Natl. Acad. Sci. U.S.A.* 107, 7811–7816.

Massey, A. C., Zhang, C., and Cuervo, A. M. (2006). Chaperone-mediated

autophagy in aging and disease. *Curr. Top. Dev. Biol.* 73, 205–235.

Mathew, R., Karp, C. M., Beaudoin, B., Vuong, N., Chen, G., Chen, H. Y., Bray, K., Reddy, A., Bhanot, G., Gelinas, C., Dipaola, R. S., Karantza-Wadsworth, V., and White, E. (2009). Autophagy suppresses tumorigenesis through elimination of p62. *Cell* 137, 1062–1075.

Matsunaga, K., Saitoh, T., Tabata, K., Omori, H., Satoh, T., Kurotori, N., Maejima, I., Shirahama-Noda, K., Ichimura, T., Isobe, T., Akira, S., Noda, T., and Yoshimori, T. (2009). Two Beclin 1-binding proteins, Atg14L and Rubicon, reciprocally regulate autophagy at different stages. *Nat. Cell Biol.* 11, 385–396.

McCarroll, S. A., Huett, A., Kuballa, P., Chilewski, S. D., Landry, A., Goyette, P., Zody, M. C., Hall, J. L., Brant, S. R., Cho, J. H., Duerr, R. H., Silverberg, M. S., Taylor, K. D., Rioux, J. D., Altshuler, D., Daly, M. J., and Xavier, R. J. (2008). Deletion polymorphism upstream of IRGM associated with altered IRGM expression and Crohn's disease. *Nat. Genet.* 40, 1107–1112.

Meixlsperger, S., and Münz, C. (2009). Morbus Crohn – a disease of failing macroautophagy in the immune system? *Int. Immunol.* 21, 1205–1211.

Mizushima, N., and Klionsky, D. J. (2007). Protein turnover via autophagy: implications for metabolism. *Annu. Rev. Nutr.* 27, 19–40.

Mizushima, N., Levine, B., Cuervo, A. M., and Klionsky, D. J. (2008). Autophagy fights disease through cellular self-digestion. *Nature* 451, 1069–1075.

Münz, C. (2009). Enhancing immunity through autophagy. *Annu. Rev. Immunol.* 27, 423–429.

Nakagawa, I., Amano, A., Mizushima, N., Yamamoto, A., Yamaguchi, H., Kamimoto, T., Nara, A., Funao, J., Nakata, M., Tsuda, K., Hamada, S., and Yoshimori, T. (2004). Autophagy defends cells against invading group A Streptococcus. *Science* 306, 1037–1040.

Nakatogawa, H., Ichimura, Y., and Ohsumi, Y. (2007). Atg8, a ubiquitin-like protein required for autophagosome formation, mediates membrane tethering and hemifusion. *Cell* 130, 165–178.

Nedjic, J., Aichinger, M., Emmerich, J., Mizushima, N., and Klein, L. (2008). Autophagy in thymic epithelium shapes the T-cell repertoire and is essential for tolerance. *Nature* 455, 396–400.

Ogawa, M., Yoshimori, T., Suzuki, T., Sagara, H., Mizushima, N., and Sasakawa, C. (2005). Escape of intracellular *Shigella* from autophagy. *Science* 307, 727–731.

Ogura, Y., Bonen, D. K., Inohara, N., Nicolae, D. L., Chen, F. F., Ramos, R.,

Britton, H., Moran, T., Karaliuskas, R., Duerr, R. H., Achkar, J. P., Brant, S. R., Bayless, T. M., Kirschner, B. S., Hanauer, S. B., Nunez, G., and Cho, J. H. (2001). A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* 411, 603–606.

Ohsumi, Y. (2001). Molecular dissection of autophagy: two ubiquitin-like systems. *Nat. Rev. Mol. Cell Biol.* 2, 211–216.

Orvedahl, A., Alexander, D., Talloczy, Z., Sun, Q., Wei, Y., Zhang, W., Burns, D., Leib, D., and Levine, B. (2007). HSV-1 ICP34.5 confers neurovirulence by targeting the Beclin 1 autophagy protein. *Cell Host Microbe* 1, 23–35.

Orvedahl, A., MacPherson, S., Sumpter, R. Jr., Talloczy, Z., Zou, Z., and Levine, B. (2010). Autophagy protects against Sindbis virus infection of the central nervous system. *Cell Host Microbe* 7, 115–127.

Parkes, M., Barrett, J. C., Prescott, N. J., Tremelling, M., Anderson, C. A., Fisher, S. A., Roberts, R. G., Nimmo, E. R., Cummings, F. R., Soars, D., Drummond, H., Lees, C. W., Khawaja, S. A., Bagnall, R., Burke, D. A., Todhunter, C. E., Ahmad, T., Onnie, C. M., McArdle, W., Strachan, D., Bethel, G., Bryan, C., Lewis, C. M., Deloukas, P., Forbes, A., Sanderson, J., Jewell, D. P., Satsangi, J., Mansfield, J. C., Cardon, L., and Mathew, C. G. (2007). Sequence variants in the autophagy gene IRGM and multiple other replicating loci contribute to Crohn's disease susceptibility. *Nat. Genet.* 39, 830–832.

Ponpuak, M., Davis, A. S., Roberts, E. A., Delgado, M. A., Dinkins, C., Zhao, Z., Virgin, H. W. t., Kyei, G. B., Johansen, T., Vergne, I., and Deretic, V. (2010). Delivery of cytosolic components by autophagic adaptor protein p62 endows autophagosomes with unique antimicrobial properties. *Immunity* 32, 329–341.

Pua, H. H., Dzhagalov, I., Chuck, M., Mizushima, N., and He, Y. W. (2007). A critical role for the autophagy gene Atg5 in T cell survival and proliferation. *J. Exp. Med.* 204, 25–31.

Ravikumar, B., Moreau, K., Jahreiss, L., Puri, C., and Rubinsztein, D. C. (2010). Plasma membrane contributes to the formation of pre-autophagosomal structures. *Nat. Cell Biol.* 12, 747–757.

Rioux, J. D., Xavier, R. J., Taylor, K. D., Silverberg, M. S., Goyette, P., Huett, A., Green, T., Kuballa, P., Barmada, M. M., Datta, L. W., Shugart, Y. Y., Griffiths, A. M., Targan, S. R., Ippoliti, A. F., Bernard, E. J., Mei, L., Nicolae, D. L., Regueiro, M., Schumm, L. P., Steinhart, A. H., Rotter, J. I., Duerr, R. H., Cho, J. H., Daly, M. J., and Brant, S. R. (2007).

Genome-wide association study identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis. *Nat. Genet.* 39, 596–604.

Saitoh, T., Fujita, N., Jang, M. H., Uematsu, S., Yang, B. G., Satoh, T., Omori, H., Noda, T., Yamamoto, N., Komatsu, M., Tanaka, K., Kawai, T., Tsujimura, T., Takeuchi, O., Yoshimori, T., and Akira, S. (2008). Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1beta production. *Nature* 456, 264–268.

Sanjuan, M. A., Dillon, C. P., Tait, S. W., Moshiach, S., Dorsey, F., Connell, S., Komatsu, M., Tanaka, K., Cleveland, J. L., Withoff, S., and Green, D. R. (2007). Toll-like receptor signalling in macrophages links the autophagy pathway to phagocytosis. *Nature* 450, 1253–1257.

Schmid, D., and Münz, C. (2007). Innate and adaptive immunity through autophagy. *Immunity* 26, 11–21.

Shahnazari, S., and Brumell, J. H. (2009). Eating twice for the sake of immunity: a phagocytic receptor that activates autophagy. *Cell Host Microbe* 6, 297–298.

Shelly, S., Lukinova, N., Bambina, S., Berman, A., and Cherry, S. (2009). Autophagy is an essential component of *Drosophila* immunity against vesicular stomatitis virus. *Immunity* 30, 588–598.

Shi, C. S., and Kehrl, J. H. (2008). MyD88 and Trif target Beclin 1 to trigger autophagy in macrophages. *J. Biol. Chem.* 283, 33175–33182.

Shi, C. S., and Kehrl, J. H. (2010). TRAF6 and A20 regulate lysine 63-linked ubiquitination of Beclin-1 to control TLR4-induced autophagy. *Sci. Signal.* 3, ra42.

Singh, S. B., Davis, A. S., Taylor, G. A., and Deretic, V. (2006). Human IRGM induces autophagy to eliminate intracellular mycobacteria. *Science* 313, 1438–1441.

Sir, D., Chen, W. L., Choi, J., Wakita, T., Yen, T. S., and Ou, J. H. (2008). Induction of incomplete autophagic response by hepatitis C virus via the unfolded protein response. *Hepatology* 48, 1054–1061.

Stappenbeck, T. S., Rioux, J. D., Mizoguchi, A., Saitoh, T., Huett, A., Darfeuille-Michaud, A., Wileman, T., Mizushima, N., Carding, S., Akira, S., Parkes, M., and Xavier, R. J. (2011). Crohn disease: a current perspective on genetics, autophagy and immunity. *Autophagy* 7, 355–374.

Suzuki, T., Franchi, L., Toma, C., Ashida, H., Ogawa, M., Yoshikawa, Y., Mimuro, H., Inohara, N., Sasakawa, C., and Nunez, G. (2007). Differential regulation of caspase-1 activation, pyroptosis, and autophagy via Ipaf and ASC in *Shigella*-infected macrophages. *PLoS Pathog.* 3, e111. doi: 10.1371/journal.ppat.0030111

Tal, M. C., Sasai, M., Lee, H. K., Yordy, B., Shadel, G. S., and Iwasaki, A. (2009). Absence of autophagy results in reactive oxygen species-dependent amplification of RLR signaling. *Proc. Natl. Acad. Sci. U.S.A.* 106, 2770–2775.

Talloczy, Z., Jiang, W., Virgin, H. W. IV, Leib, D. A., Scheuner, D., Kaufman, R. J., Eskelinen, E. L., and Levine, B. (2002). Regulation of starvation- and virus-induced autophagy by the eIF2alpha kinase signaling pathway. *Proc. Natl. Acad. Sci. U.S.A.* 99, 190–195.

Tanaka, Y., Guhde, G., Suter, A., Eskelinen, E. L., Hartmann, D., Lullmann-Rauch, R., Janssen, P. M., Blanz, J., von Figura, K., and Saftig, P. (2000). Accumulation of autophagic vacuoles and cardiomyopathy in LAMP-2-deficient mice. *Nature* 406, 902–906.

Taylor, G. S., Mautner, J., and Münz, C. (2011). Autophagy in herpesvirus immune control and immune escape. *Herpesviridae* 2, 2.

Thurston, T. L., Ryzhakov, G., Bloor, S., von Muhlinen, N., and Randow, F. (2009). The TBK1 adaptor and autophagy receptor NDP52 restricts the proliferation of ubiquitin-coated bacteria. *Nat. Immunol.* 10, 1215–1221.

Travassos, L. H., Carneiro, L. A., Ramjeet, M., Hussey, S., Kim, Y. G., Magalhaes, J. G., Yuan, L., Soares, F., Chea, E., Le Bourhis, L., Boneca, I. G., Allaoui, A., Jones, N. L., Nunez, G., Girardin, S. E., and Philpott, D. J. (2010). Nod1 and Nod2 direct autophagy by recruiting ATG16L1 to the plasma membrane at the site of bacterial entry. *Nat. Immunol.* 11, 55–62.

Xu, Y., Jagannath, C., Liu, X. D., Sharafkhaneh, A., Kolodziejska, K. E., and Eissa, N. T. (2007). Toll-like receptor 4 is a sensor for autophagy associated with innate immunity. *Immunity* 27, 135–144.

Yano, T., Mita, S., Ohmori, H., Oshima, Y., Fujimoto, Y., Ueda, R., Takada, H., Goldman, W. E., Fukase, K., Silverman, N., Yoshimori, T., and Kurata, S. (2008). Autophagic control of listeria through intracellular innate immune recognition in *Drosophila*. *Nat. Immunol.* 9, 908–916.

Yen, W. L., Shintani, T., Nair, U., Cao, Y., Richardson, B., C., Li, Z., Hughson, F. M., Baba, M., and Klionsky, D. J. (2010). The conserved oligomeric Golgi complex is involved in double-membrane vesicle formation during autophagy. *J. Cell Biol.* 188, 101–114.

Yla-Anttila, P., Vihinen, H., Jokitalo, E., and Eskelinen, E. L. (2009). 3D tomography reveals connections between the phagophore and endoplasmic reticulum. *Autophagy* 5, 1180–1185.

Yoshikawa, Y., Ogawa, M., Hain, T., Yoshida, M., Fukumatsu, M., Kim, M., Mimuro, H., Nakagawa, I., Yanagawa, T., Ishii, T., Kakizuka, A., Sztul, E., Chakraborty, T., and Sasakawa, C. (2009). *Listeria monocytogenes* ActA-mediated escape from autophagic recognition. *Nat. Cell Biol.* 11, 1233–1240.

Zhang, C., and Cuervo, A. M. (2008). Restoration of chaperone-mediated autophagy in aging liver improves cellular maintenance and hepatic function. *Nat. Med.* 14, 959–965.

Zhong, Y., Wang, Q. J., Li, X., Yan, Y., Backer, J. M., Chait, B. T., Heintz, N., and Yue, Z. (2009). Distinct regulation of autophagic activity by Atg14L and Rubicon associated with Beclin 1-phosphatidylinositol-3-kinase complex. *Nat. Cell Biol.* 11, 468–476.

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

Julia Eitel, Norbert Suttorp and Bastian Opitz*

Department of Internal Medicine/Infectious Diseases and Pulmonary Medicine, Charité – Universitätsmedizin Berlin, Berlin, Germany

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-1β 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-κB and/or IFN regulatory factor 3 (IRF3) which direct upregulation of proinflammatory genes such as TNFα, IL-8 and pro-IL-1β, or type I IFNs, respectively. Other PRRs form protein complexes called inflammasomes that regulate production of IL-1β 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-κB 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).
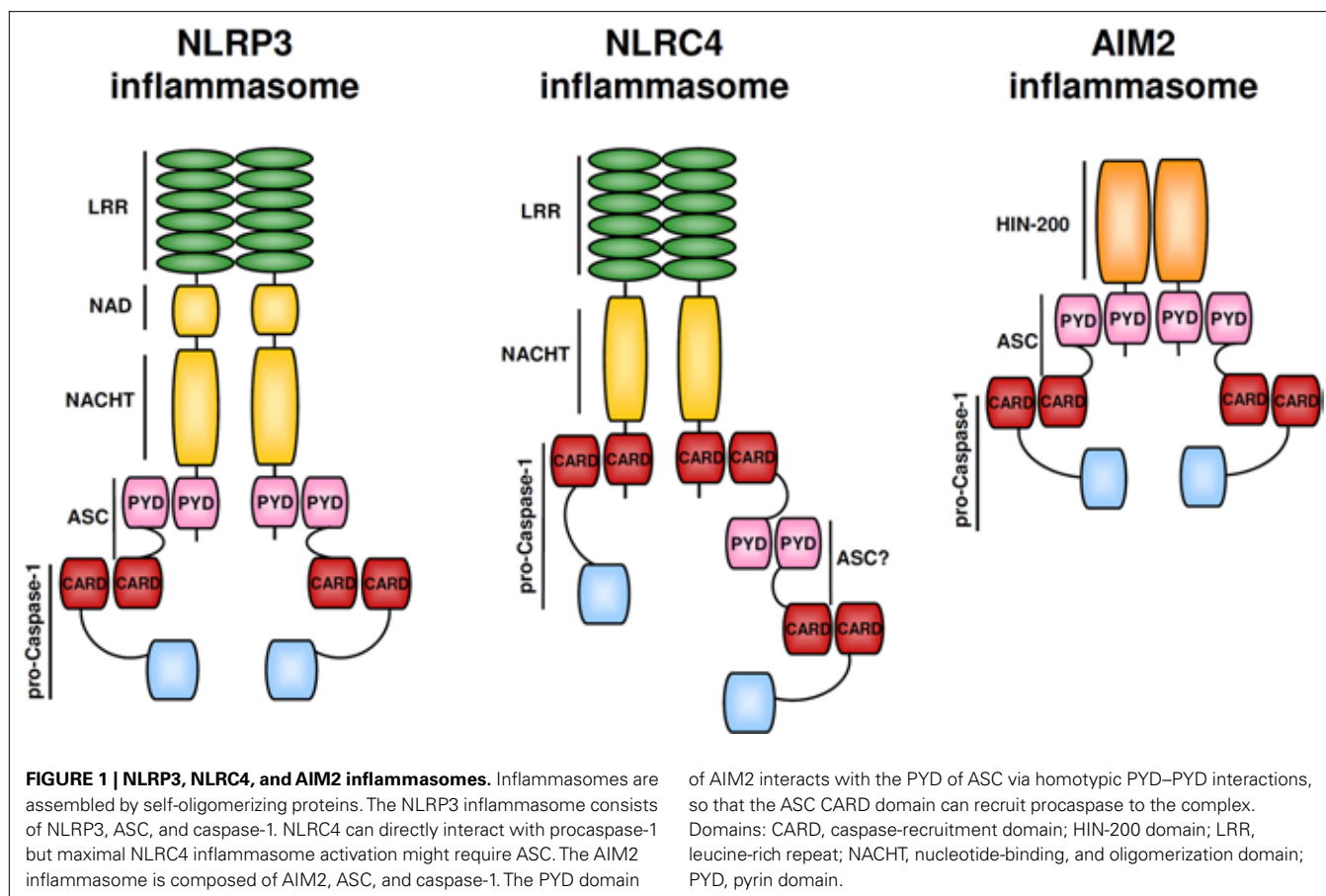
In addition to the above mentioned PRRs that mainly control immune responses to *Listeria* infection via transcriptional upregulation of inflammatory genes, some NLRs as well as other cytosolic receptors regulate production of the key proinflammatory cytokines IL-1β and IL-18 at a post-translational 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 effector 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-1β 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-1β 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-1β as well as IL-18, and to caspase-1-dependent cell death (Tsuji 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 IFNγ (Tsuji 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-1β and IL-18 (Mariathasan et al., 2006; Ozoren et al., 2006; **Figure 2**). Furthermore, mouse macrophages

FIGURE 1 | NLRP3, NLRC4, and AIM2 inflammasomes. Inflammasomes are assembled by self-oligomerizing proteins. The NLRP3 inflammasome consists of NLRP3, ASC, and caspase-1. NLRC4 can directly interact with procaspase-1 but maximal NLRC4 inflammasome activation might require ASC. The AIM2 inflammasome is composed of AIM2, ASC, and caspase-1. The PYD domain of AIM2 interacts with the PYD of ASC via homotypic PYD–PYD interactions, so that the ASC CARD domain can recruit procaspase to the complex. Domains: CARD, caspase-recruitment domain; HIN-200 domain; LRR, leucine-rich repeat; NACHT, nucleotide-binding, and oligomerization domain; PYD, pyrin domain.

infected with *L. monocytogenes* deficient for the toxin listeriolysin O (LLO) did not secrete IL-1β and IL-18 (Mariathasan et al., 2006; Ozoren et al., 2006; Hara et al., 2008). Similarly, NLRP3, ASC, and LLO were required for IL-1β 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-1β 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-1β 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-1β 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-1β 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-1β 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-1β and IL-18 secretion (Henry et al., 2007).
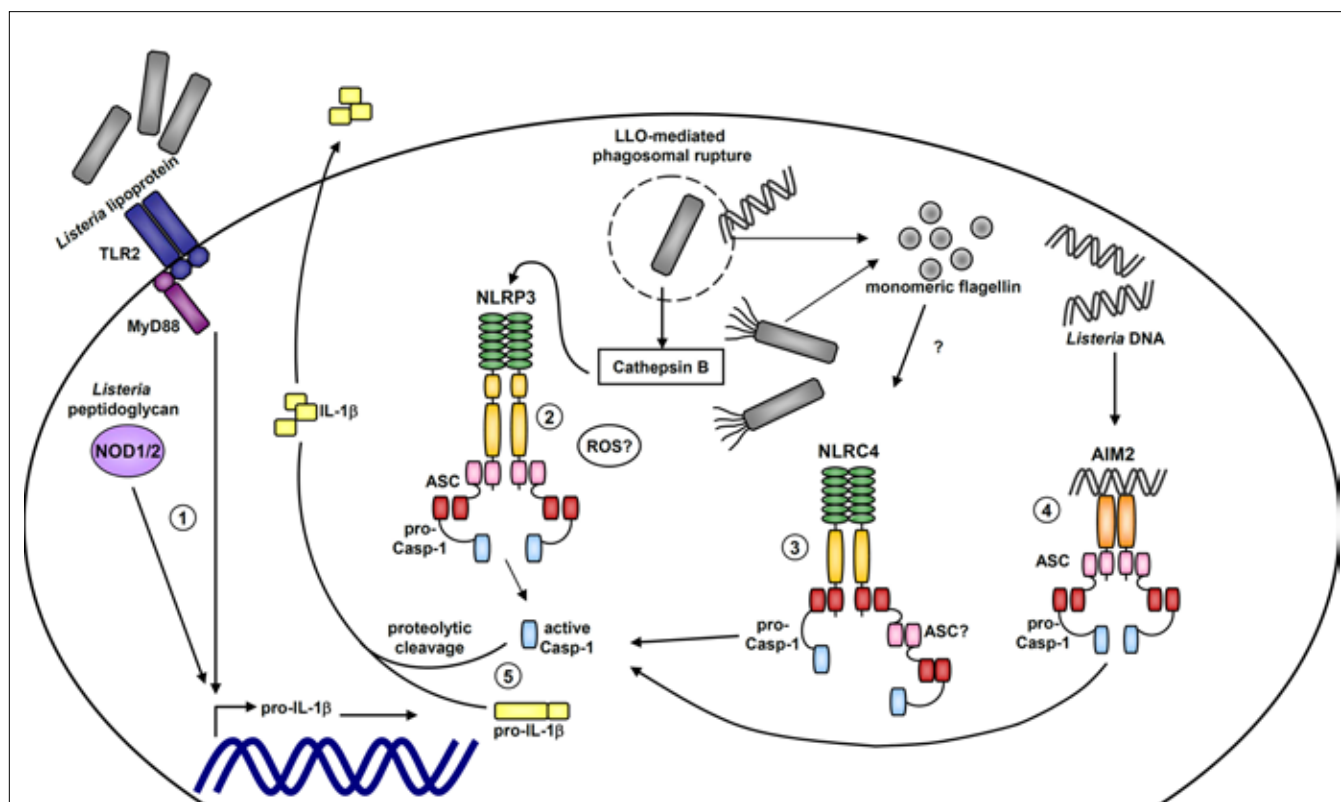
FIGURE 2 | Model of inflammasome activation by *Listeria monocytogenes*. Activation of the extracellular TLR2 pathway via listerial lipoprotein and intracellular detection of bacterial peptidoglycans by NOD1/2 lead to upregulation of pro-IL-1β transcription (1). After bacterial internalization within a membrane-bound vacuole, listeriolysin O (LLO) leads to phagosomal rupture followed by escape of *Listeria* into the cytosol as well as cathepsin B release. Lysosomal damage and cathepsin B release (and perhaps other signals such as ROS) probably activate the NLRP3 inflammasome (2). Flagellin expression by *L. monocytogenes* or monomeric flagellin in the cytosol is detected by the NLRC4 inflammasome (3). Listerial DNA in the cytosol is sensed by the AIM2 inflammasome (4). Upon inflammasome activation, caspase-1 is autoactivated and mediates processing and secretion of the proinflammatory cytokines IL-1β and IL-18 (5; IL-18 is not depicted).

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-1β 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-1β and IL-18. IL-1β and IL-18 activate via the IL-1 and IL-18 receptors a MyD88-dependent signaling and subsequent NF-κB- and MAPK-regulated gene expression. Indeed,

signals stimulated by LLO leading to IL-1β/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-1β 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.

REFERENCES

Allerberger, F., and Wagner, M. (2010). Listeriosis: a resurgent foodborne infection. *Clin. Microbiol. Infect.* 16, 16–23.

Auerbuch, V., Brockstedt, D. G., Meyer-Morse, N., O'Riordan, M., and Portnoy, D. A. (2004). Mice lacking the type I interferon receptor are resistant to *Listeria monocytogenes*. *J. Exp. Med.* 200, 527–533.

Barbuddhe, S. B., and Chakraborty, T. (2009). *Listeria* as an enteroinvasive gastrointestinal pathogen. *Curr. Top. Microbiol. Immunol.* 337, 173–195.

Bergsbaken, T., Fink, S. L., and Cookson, B. T. (2009). Pyroptosis: host cell death and inflammation. *Nat. Rev. Microbiol.* 7, 99–109.

Bielecki, J., Youngman, P., Connelly, P., and Portnoy, D. A. (1990). *Bacillus subtilis* expressing a haemolysin gene from *Listeria monocytogenes* can grow in mammalian cells. *Nature* 345, 175–176.

Birmingham, C. L., Canadien, V., Kaniuk, N. A., Steinberg, B. E., Higgins, D. E., and Brumell, J. H. (2008). Listeriolysin O allows *Listeria monocytogenes* replication in macrophage vacuoles. *Nature* 451, 350–354.

Burckstummer, T., Baumann, C., Bluml, S., Dixit, E., Durnberger, G., Jahn, H., Planyavsky, M., Bilban, M., Colinge, J., Bennett, K. L., and Superti-Furga, G. (2009). An orthogonal proteomic-genomic screen identifies AIM2 as a cytoplasmic DNA sensor for the inflammasome. *Nat. Immunol.* 10, 266–272.

Carrero, J. A., Calderon, B., and Unanue, E. R. (2004). Type I interferon sensitizes lymphocytes to apoptosis and reduces resistance to *Listeria* infection. *J. Exp. Med.* 200, 535–540.

Cervantes, J., Nagata, T., Uchijima, M., Shibata, K., and Koide, Y. (2008). Intracytosolic *Listeria monocytogenes* induces cell death through caspase-1 activation in murine macrophages. *Cell. Microbiol.* 10, 41–52.

Chin, A. I., Dempsey, P. W., Bruhn, K., Miller, J. F., Xu, Y., and Cheng, G. (2002). Involvement of receptor-interacting protein 2 in innate and adaptive immune responses. *Nature* 416, 190–194.

Corr, S. C., and O'Neill, L. A. (2009). *Listeria monocytogenes* infection in the face of innate immunity. *Cell. Microbiol.* 11, 703–709.

Domann, E., Wehland, J., Rohde, M., Pistor, S., Hartl, M., Goebel, W., Leimeister-Wachter, M., Wuenscher, M., and Chakraborty, T. (1992). A novel bacterial virulence gene in *Listeria monocytogenes* required for host cell microfilament interaction with homology to the proline-rich region of vinculin. *EMBO J.* 11, 1981–1990.

Dostert, C., Petrilli, V., Van Bruggen, R., Steele, C., Mossman, B. T., and Tschopp, J. (2008). Innate immune activation through Nalp3 inflammasome sensing of asbestos and silica. *Science* 320, 674–677.

Dramsi, S., Biswas, I., Maguin, E., Braun, L., Mastroeni, P., and Cossart, P. (1995). Entry of *Listeria monocytogenes* into hepatocytes requires expression of inIB, a surface protein of the internalin multigene family. *Mol. Microbiol.* 16, 251–261.

Duewell, P., Kono, H., Rayner, K. J., Sirois, C. M., Vladimer, G., Bauernfeind, F. G., Abela, G. S., Franchi, L., Nunez, G., Schnurr, M., Espevik, T., Lien, E., Fitzgerald, K. A., Rock, K. L., Moore, K. J., Wright, S. D., Hornung, V., and Latz, E. (2010). NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. *Nature* 464, 1357–1361.

Edelson, B. T., and Unanue, E. R. (2002). MyD88-dependent but toll-like receptor 2-independent innate immunity to *Listeria*: no role for either in macrophage listericidal activity. *J. Immunol.* 169, 3869–3875.

Fernandes-Alnemri, T., Yu, J. W., Datta, P., Wu, J., and Alnemri, E. S. (2009). AIM2 activates the inflammasome and cell death in response to cytoplasmic DNA. *Nature* 458, 509–513.

Franchi, L., Amer, A., Body-Malapel, M., Kanneganti, T. D., Ozoren, N., Jagirdar, R., Inohara, N., Vandenabeele, P., Bertin, J., Coyle, A., Grant, E. P., and Nunez, G. (2006). Cytosolic flagellin requires Ipaf for activation of caspase-1 and interleukin 1beta in salmonella-infected macrophages. *Nat. Immunol.* 7, 576–582.

Franchi, L., Kanneganti, T. D., Dubyak, G. R., and Nunez, G. (2007). Differential requirement of P2X7 receptor and intracellular K+ for caspase-1 activation induced by intracellular and extracellular bacteria. *J. Biol. Chem.* 282, 18810–18818.

Franchi, L., Warner, N., Viani, K., and Nunez, G. (2009). Function of Nod-like receptors in microbial recognition and host defense. *Immunol. Rev.* 227, 106–128.

Gekara, N. O., Dietrich, N., Lyszkiewicz, M., Lienenklaus, S., and Weiss, S. (2009). Signals triggered by a bacterial pore-forming toxin contribute to toll-like receptor redundancy in Gram-positive bacterial recognition. *J. Infect. Dis.* 199, 124–133.

Grundling, A., Burrack, L. S., Bouwer, H. G., and Higgins, D. E. (2004). *Listeria monocytogenes* regulates flagellar motility gene expression through MogR, a transcriptional repressor required for virulence. *Proc. Natl. Acad. Sci. U.S.A.* 101, 12318–12323.

Halle, A., Hornung, V., Petzold, G. C., Stewart, C. R., Monks, B. G., Reinheckel, T., Fitzgerald, K. A., Latz, E., Moore, K. J., and Golenbock, D. T. (2008). The NALP3 inflammasome is involved in the innate immune response to amyloid-beta. *Nat. Immunol.* 9, 857–865.

Hamon, M., Bierne, H., and Cossart, P. (2006). *Listeria monocytogenes*: a multifaceted model. *Nat. Rev. Microbiol.* 4, 423–434.

Hara, H., Tsuchiya, K., Nomura, T., Kawamura, I., Shoma, S., and Mitsuyama, M. (2008). Dependency of caspase-1 activation induced in macrophages by *Listeria monocytogenes* on cytolysin, listeriolysin O, after evasion from phagosome into the cytoplasm. *J. Immunol.* 180, 7859–7868.

Havell, E. A., Moldawer, L. L., Helfgott, D., Kilian, P. L., and Sehgal, P. B. (1992). Type I IL-1 receptor blockade exacerbates murine listeriosis. *J. Immunol.* 148, 1486–1492.

Hayashi, F., Smith, K. D., Ozinsky, A., Hawn, T. R., Yi, E. C., Goodlett, D. R., Eng, J. K., Akira, S., Underhill, D. M., and Aderem, A. (2001). The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. *Nature* 410, 1099–1103.

Henry, T., Brotcke, A., Weiss, D. S., Thompson, L. J., and Monack, D. M. (2007). Type I interferon signaling is required for activation of the inflammasome during *Francisella* infection. *J. Exp. Med.* 204, 987–994.

Hirsch, E., Irikura, V. M., Paul, S. M., and Hirsh, D. (1996). Functions of interleukin 1 receptor antagonist in gene knockout and overproducing mice. *Proc. Natl. Acad. Sci. U.S.A.* 93, 11008–11013.

Hornung, V., Ablasser, A., Charrel-Dennis, M., Bauernfeind, F., Horvath, G., Caffrey, D. R., Latz, E., and Fitzgerald, K. A. (2009). AIM2 recognizes cytosolic dsDNA and forms a caspase-1-activating inflammasome with ASC. *Nature* 458, 514–518.

Hornung, V., Bauernfeind, F., Halle, A., Samstad, E. O., Kono, H., Rock, K. L., Fitzgerald, K. A., and Latz, E. (2008). Silica crystals and aluminum salts activate the NALP3 inflammasome through phagosomal destabilization. *Nat. Immunol.* 9, 847–856.

Ishikawa, H., Ma, Z., and Barber, G. N. (2009). STING regulates intracellular DNA-mediated, type I interferon-dependent innate immunity. *Nature* 461, 788–792.

Kanneganti, T. D., Ozoren, N., Body-Malapel, M., Amer, A., Park, J. H., Franchi, L., Whitfield, J., Barchet, W., Colonna, M., Vandenabeele, P., Bertin, J., Coyle, A., Grant, E. P., Akira, S., and Nunez, G. (2006). Bacterial RNA and small antiviral compounds activate caspase-1 through cryopyrin/Nalp3. *Nature* 440, 233–236.

Kim, S., Bauernfeind, F., Ablasser, A., Hartmann, G., Fitzgerald, K. A., Latz, E., and Hornung, V. (2010). *Listeria monocytogenes* is sensed by the NLRP3 and AIM2 inflammasome. *Eur. J. Immunol.* 40, 1545–1551.

Kobayashi, K., Inohara, N., Hernandez, L. D., Galan, J. E., Nunez, G., Janeway, C. A., Medzhitov, R., and Flavell, R. A. (2002). RICK/Rip2/CARDIAK mediates signalling for receptors of the innate and adaptive immune systems. *Nature* 416, 194–199.

Kobayashi, K. S., Chamaillard, M., Ogura, Y., Henegariu, O., Inohara, N., Nunez, G., and Flavell, R. A. (2005). Nod2-dependent regulation of innate and adaptive immunity in the intestinal tract. *Science* 307, 731–734.

Kocks, C., Gouin, E., Tabouret, M., Berche, P., Ohayon, H., and Cossart, P. (1992). *L. monocytogenes*-induced actin assembly requires the actA gene product, a surface protein. *Cell* 68, 521–531.

Krull, M., Nost, R., Hippenstiel, S., Domann, E., Chakraborty, T., and Suttorp, N. (1997). *Listeria monocytogenes* potently induces up-regulation of endothelial adhesion

molecules and neutrophil adhesion to cultured human endothelial cells. *J. Immunol.* 159, 1970–1976.

Labow, M., Shuster, D., Zetterstrom, M., Nunes, P., Terry, R., Cullinan, E. B., Bartfai, T., Solorzano, C., Moldawer, L. L., Chizzonite, R., and McIntyre, K. W. (1997). Absence of IL-1 signaling and reduced inflammatory response in IL-1 type I receptor-deficient mice. *J. Immunol.* 159, 2452–2461.

Leber, J. H., Crimmins, G. T., Raghavan, S., Meyer-Morse, N. P., Cox, J. S., and Portnoy, D. A. (2008). Distinct TLR- and NLR-mediated transcriptional responses to an intracellular pathogen. *PLoS Pathog.* 4, e6. doi: 10.1371/journal.ppat.0040006

Lippmann, J., Rothenburg, S., Deigendesch, N., Eitel, J., Meixenberger, K., van Laak, V., Slevogt, H., N'guessan, P. D., Hippenstiel, S., Chakraborty, T., Flieger, A., Suttorp, N., and Opitz, B. (2008). IFNbeta responses induced by intracellular bacteria or cytosolic DNA in different human cells do not require ZBP1 (DLM-1/DAI). *Cell. Microbiol.* 10, 2579–2588.

Machata, S., Tchatalbachev, S., Mohamed, W., Jansch, L., Hain, T., and Chakraborty, T. (2008). Lipoproteins of *Listeria monocytogenes* are critical for virulence and TLR2-mediated immune activation. *J. Immunol.* 181, 2028–2035.

Mariathasan, S., Newton, K., Monack, D. M., Vucic, D., French, D. M., Lee, W. P., Roose-Girma, M., Erickson, S., and Dixit, V. M. (2004). Differential activation of the inflammasome by caspase-1 adaptors ASC and Ipaf. *Nature* 430, 213–218.

Mariathasan, S., Weiss, D. S., Newton, K., McBride, J., O'Rourke, K., Roose-Girma, M., Lee, W. P., Weinrauch, Y., Monack, D. M., and Dixit, V. M. (2006). Cryopyrin activates the inflammasome in response to toxins and ATP. *Nature* 440, 228–232.

Martinon, F., Burns, K., and Tschopp, J. (2002). The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. *Mol. Cell* 10, 417–426.

Martinon, F., Petrilli, V., Mayor, A., Tardivel, A., and Tschopp, J. (2006). Gout-associated uric acid crystals activate the NALP3 inflammasome. *Nature* 440, 237–241.

McCaffrey, R. L., Fawcett, P., O'Riordan, M., Lee, K. D., Havell, E. A., Brown, P. O., and Portnoy, D. A. (2004). A specific gene expression program triggered by Gram-positive bacteria in the cytosol. *Proc. Natl. Acad. Sci. U.S.A.* 101, 11386–11391.

Meixenberger, K., Pache, F., Eitel, J., Schmeck, B., Hippenstiel, S., Slevogt,

H., N'Guessan, P., Witzenrath, M., Netea, M. G., Chakraborty, T., Suttorp, N., and Opitz, B. (2010). *Listeria monocytogenes*-infected human peripheral blood mononuclear cells produce IL-1beta, depending on listeriolysin O and NLRP3. *J. Immunol.* 184, 922–930.

Mengaud, J., Ohayon, H., Gounon, P., Mege, R.-M., and Cossart, P. (1996). E-cadherin is the receptor for internalin, a surface protein required for entry of *L. monocytogenes* into epithelial cells. *Cell* 84, 923–932.

Miao, E. A., Alpuche-Aranda, C. M., Dors, M., Clark, A. E., Bader, M. W., Miller, S. I., and Aderem, A. (2006). Cytoplasmic flagellin activates caspase-1 and secretion of interleukin 1beta via Ipaf. *Nat. Immunol.* 7, 569–575.

Miao, E. A., Mao, D. P., Yudkovsky, N., Bonneau, R., Lorang, C. G., Warren, S. E., Leaf, I. A., and Aderem, A. (2010). Innate immune detection of the type III secretion apparatus through the NLRC4 inflammasome. *Proc. Natl. Acad. Sci. U.S.A.* 107, 3076–3080.

Mosa, A., Trumstedt, C., Eriksson, E., Soehnlein, O., Heuts, F., Janik, K., Klos, A., Dittrich-Breiholz, O., Kracht, M., Hidmark, A., Wigzell, H., and Rottenberg, M. E. (2009). Nonhematopoietic cells control the outcome of infection with *Listeria monocytogenes* in a nucleotide oligomerization domain 1-dependent manner. *Infect. Immun.* 77, 2908–2918.

O'Connell, R. M., Saha, S. K., Vaidya, S. A., Bruhn, K. W., Miranda, G. A., Zarnegar, B., Perry, A. K., Nguyen, B. O., Lane, T. F., Taniguchi, T., Miller, J. F., and Cheng, G. (2004). Type I interferon production enhances susceptibility to *Listeria monocytogenes* infection. *J. Exp. Med.* 200, 437–445.

O'Connell, R. M., Vaidya, S. A., Perry, A. K., Saha, S. K., Dempsey, P. W., and Cheng, G. (2005). Immune activation of type I IFNs by *Listeria monocytogenes* occurs independently of TLR4, TLR2, and receptor interacting protein 2 but involves TNFR-associated NF kappa B kinase-binding kinase 1. *J. Immunol.* 174, 1602–1607.

O'Riordan, M., Yi, C. H., Gonzales, R., Lee, K. D., and Portnoy, D. A. (2002). Innate recognition of bacteria by a macrophage cytosolic surveillance pathway. *Proc. Natl. Acad. Sci. U.S.A.* 99, 13861–13866.

Opitz, B., Eitel, J., Meixenberger, K., and Suttorp, N. (2009). Role of Toll-like receptors, NOD-like receptors and RIG-I-like receptors in endothelial cells and systemic infections. *Thromb. Haemost.* 102, 1103–1109.

Opitz, B., Puschel, A., Beermann, W., Hocke, A. C., Forster, S., Schmeck, B., van Laak, V., Chakraborty, T., Suttorp, N., and Hippenstiel, S. (2006). *Listeria monocytogenes* activated p38 MAPK and induced IL-8 secretion in a nucleotide-binding oligomerization domain 1-dependent manner in endothelial cells. *J. Immunol.* 176, 484–490.

Ozoren, N., Masumoto, J., Franchi, L., Kanneganti, T. D., Body-Malapel, M., Erturk, I., Jagirdar, R., Zhu, L., Inohara, N., Bertin, J., Coyle, A., Grant, E. P., and Nunez, G. (2006). Distinct roles of TLR2 and the adaptor ASC in IL-1beta/IL-18 secretion in response to *Listeria monocytogenes*. *J. Immunol.* 176, 4337–4342.

Park, J. H., Kim, Y. G., McDonald, C., Kanneganti, T. D., Hasegawa, M., Body-Malapel, M., Inohara, N., and Nunez, G. (2007). RICK/RIP2 mediates innate immune responses induced through Nod1 and Nod2 but not TLRs. *J. Immunol.* 178, 2380–2386.

Pfeffer, K., Matsuyama, T., Kundig, T. M., Wakeham, A., Kishihara, K., Shahinian, A., Wiegmann, K., Ohashi, P. S., Kronke, M., and Mak, T. W. (1993). Mice deficient for the 55 kd tumor necrosis factor receptor are resistant to endotoxic shock, yet succumb to *L. monocytogenes* infection. *Cell* 73, 457–467.

Rathinam, V. A., Jiang, Z., Waggoner, S. N., Sharma, S., Cole, L. E., Waggoner, L., Vanaja, S. K., Monks, B. G., Ganesan, S., Latz, E., Hornung, V., Vogel, S. N., Szomolanyi-Tsuda, E., and Fitzgerald, K. A. (2010). The AIM2 inflammasome is essential for host defense against cytosolic bacteria and DNA viruses. *Nat. Immunol.* 11, 395–402.

Roberts, T. L., Idris, A., Dunn, J. A., Kelly, G. M., Burnton, C. M., Hodgson, S., Hardy, L. L., Garceau, V., Sweet, M. J., Ross, I. L., Hume, D. A., and Stacey, K. J. (2009). HIN-200 proteins regulate caspase activation in response to foreign cytoplasmic DNA. *Science* 323, 1057–1060.

Sauer, J. D., Witte, C. E., Zemansky, J., Hanson, B., Lauer, P., and Portnoy, D. A. (2010). *Listeria monocytogenes* triggers AIM2-mediated pyroptosis upon infrequent bacteriolysis in the macrophage cytosol. *Cell Host Microbe* 7, 412–419.

Schroder, K., and Tschopp, J. (2010). The inflammasomes. *Cell* 140, 821–832.

Schubert, W. D., Urbanke, C., Ziehm, T., Beier, V., Machner, M. P., Domann, E., Wehland, J., Chakraborty, T., and Heinz, D. W. (2002). Structure of internalin, a major invasion protein of *Listeria monocytogenes*, in complex

with its human receptor E-cadherin. *Cell* 111, 825–836.

Seki, E., Tsutsui, H., Tsuji, N. M., Hayashi, N., Adachi, K., Nakano, H., Futatsugi-Yumikura, S., Takeuchi, O., Hoshino, K., Akira, S., Fujimoto, J., and Nakanishi, K. (2002). Critical roles of myeloid differentiation factor 88-dependent proinflammatory cytokine release in early phase clearance of *Listeria monocytogenes* in mice. *J. Immunol.* 169, 3863–3868.

Shen, Y., Naujokas, M., Park, M., and Ireton, K. (2000). InIB-dependent internalization of *Listeria* is mediated by the Met receptor tyrosine kinase. *Cell* 103, 501–510.

Stetson, D. B., and Medzhitov, R. (2006). Recognition of cytosolic DNA activates an IRF3-dependent innate immune response. *Immunity* 24, 93–103.

Stockinger, S., Materna, T., Stoiber, D., Bayr, L., Steinborn, R., Kolbe, T., Unger, H., Chakraborty, T., Levy, D. E., Muller, M., and Decker, T. (2002). Production of type I IFN sensitizes macrophages to cell death induced by *Listeria monocytogenes*. *J. Immunol.* 169, 6522–6529.

Stockinger, S., Reutterer, B., Schaljo, B., Schellack, C., Brunner, S., Materna, T., Yamamoto, M., Akira, S., Taniguchi, T., Murray, P. J., Muller, M., and Decker, T. (2004). IFN regulatory factor 3-dependent induction of type I IFNs by intracellular bacteria is mediated by a TLR- and Nod2-independent mechanism. *J. Immunol.* 173, 7416–7425.

Stutz, A., Golenbock, D. T., and Latz, E. (2009). Inflammasomes: too big to miss. *J. Clin. Invest.* 119, 3502–3511.

Takeuchi, O., and Akira, S. (2010). Pattern recognition receptors and inflammation. *Cell* 140, 805–820.

Tilney, L. G., and Portnoy, D. A. (1989). Actin filaments and the growth, movement, and spread of the intracellular bacterial parasite, *Listeria monocytogenes*. *J. Cell Biol.* 109, 1597–1608.

Torres, D., Barrier, M., Bihl, F., Quesniaux, V. J., Maillet, I., Akira, S., Ryffel, B., and Erard, F. (2004). Toll-like receptor 2 is required for optimal control of *Listeria monocytogenes* infection. *Infect. Immun.* 72, 2131–2139.

Tsuchiya, K., Hara, H., Kawamura, I., Nomura, T., Yamamoto, T., Daim, S., Dewamitta, S. R., Shen, Y., Fang, R., and Mitsuyama, M. (2010). Involvement of absent in melanoma 2 in inflammasome activation in macrophages infected with *Listeria monocytogenes*. *J. Immunol.* 185, 1186–1195.

Tsuji, N. M., Tsutsui, H., Seki, E., Kuida, K., Okamura, H., Nakanishi, K., and Flavell, R. A. (2004). Roles of caspase-1 in *Listeria* infection in mice. *Int. Immunol.* 16, 335–343.

Unanue, E. R. (1997). Studies in listeriosis show the strong symbiosis between the innate cellular system and the T-cell response. *Immunol. Rev.* 158, 11–25.

Veiga, E., and Cossart, P. (2005). *Listeria* hijacks the clathrin-dependent endocytic machinery to invade mammalian cells. *Nat. Cell Biol.* 7, 894–900.

Warren, S. E., Armstrong, A., Hamilton, M. K., Mao, D. P., Leaf, I. A., Miao, E. A., and Aderem, A. (2010). Cutting edge: cytosolic bacterial DNA activates the inflammasome via Aim2. *J. Immunol.* 185, 818–821.

Warren, S. E., Mao, D. P., Rodriguez, A. E., Miao, E. A., and Aderem, A. (2008).

Multiple Nod-like receptors activate caspase 1 during *Listeria monocytogenes* infection. *J. Immunol.* 180, 7558–7564.

Way, S. S., Thompson, L. J., Lopes, J. E., Hajjar, A. M., Kollmann, T. R., Freitag, N. E., and Wilson, C. B. (2004). Characterization of flagellin expression and its role in *Listeria monocytogenes* infection and immunity. *Cell. Microbiol.* 6, 235–242.

Wu, J., Fernandes-Alnemri, T., and Alnemri, E. S. (2010). Involvement of the AIM2, NLRC4, and NLRP3 inflammasomes in caspase-1 activation by *Listeria monocytogenes*. *J. Clin. Immunol.* 30, 693–702.

Zhou, R., Yazdi, A. S., Menu, P., and Tschopp, J. (2010). A role for mitochondria in NLRP3 inflammasome activation. *Nature.* doi:10.1038/nature09663. [Epub ahead of print].

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Role of inflammasomes in *Salmonella* infection

Luigi Franchi*

Department of Pathology and Comprehensive Cancer Center, University of Michigan Medical School, Ann Arbor, MI, USA

Pattern recognition receptors (PRRs) play a crucial role in both the detection of pathogens and the activation of the innate immune system. Nod-like receptors (NLR) family members are cytosolic PRRs that sense bacterial products or endogenous danger signals. Recent evidence suggests that NLRs contribute to the detection of *Salmonella* through the activation of inflammasomes, molecular platforms that promotes the maturation of the proinflammatory cytokines IL-1β and IL-18. During enteric *Salmonella* infection the activation of caspase-1 and the production of IL-1β and IL-18 result in a protective host response. In macrophages, the activation of caspase-1 induced by *Salmonella* is mainly mediated by the NLR family member NLRC4 that senses cytosolic flagellin. Recent data suggest that an effective innate immune response against *Salmonella* requires the engagement of multiple inflammasomes in both hematopoietic and non-hematopoietic cell lineages. Further understanding of the innate immune response mediated by inflammasomes should provide new insights into the mechanisms of host defense and the pathogenesis of inflammatory diseases.

Keywords: *Salmonella*, inflammation, innate immunity, inflammasome, IL-1β

SALMONELLA

Salmonella enterica consists of more than 2500 serovars. *S. enterica* serovar Typhi and Paratyphi cause typhoid in humans, a disease characterized by fever, systemic infection, and gastroenteritis. *S. enterica* serovar Typhi and Paratyphi are estimated to affect more than 16 million people worldwide and cause 600,000 deaths/year. Several non-typhoidal *S. enterica* serovars (NTS) can cause enterocolitis in humans. In infants and immunocompromised patients, NTS can disseminate and cause potentially lethal bacteremia. In the United States, NTS are the leading cause of foodborne disease with an estimated cost of 2 billion dollars/year (Grassl et al., 2008; Santos et al., 2009).

MOUSE MODEL OF SALMONELLA INFECTIONS

Salmonella enterica serovar Typhimurium (herein referred to as *Salmonella*) causes enterocolitis in humans. In mice, *Salmonella* cause a systemic infection that resembles human typhoid fever. During the natural infection, *Salmonella* enters the host via the oral route and overcome the defense provided by mucus and the endogenous microbiota. Then *Salmonella* crosses the epithelial barrier, mainly by invading M cells (Hase et al., 2009; Knoop et al., 2009), an intestinal epithelial cell type specialized in the transport of particles from the gut lumen to the lamina propria. Once in the lamina propria, *Salmonella* can be taken up by dendritic cells (Uematsu and Akira, 2009; DC) and transported to Peyer patches and other gut-associated lymphoid tissues, from which *Salmonella* disseminates to other organs such as the liver and/or spleen where they can survive within phagocytic cells. An alternative route for *Salmonella* uptake via DCs that sample the intestinal lumen has been described (Rescigno et al., 2001; Niess et al., 2005). In resistant mouse strains, *Salmonella* causes chronic infections (Monack et al., 2004; Grassl et al., 2008) while in susceptible strains *Salmonella* is lethal. Commensal bacteria have an important role in preventing *Salmonella* from colonizing the intestine and causing acute intestinal inflammation (Hapfelmeier and Hardt, 2005). Pretreatment with a single dose of the antibiotic streptomycin reduces the presence of commensal bacteria and renders otherwise resistant mice susceptible to *Salmonella* (Hapfelmeier and Hardt, 2005). These findings have opened the way to studies aimed at investigating both the bacterial virulence factors and the host response in the acute intestinal inflammation triggered by *Salmonella* in mice.

BACTERIA VIRULENCE FACTORS

Bacterial secretion systems are molecular machineries used by pathogenic bacteria to deliver effectors proteins inside the host cell and manipulate cell behavior. *Salmonella* encodes two type III protein secretion systems which manipulate host cell behavior through the delivery of specific effectors proteins. One of the systems is encoded within the *Salmonella* pathogenicity island 1 (SPI-1) and is important in the initial phase of infection (Grassl and Finlay, 2008); in agreement effectors proteins encoded within the SPI-1 have been implicated in cytoskeletal rearrangement responsible for bacterial uptake by epithelial cells, a step required for the bacteria to breach the intestinal barrier (Grassl and Finlay, 2008). The other is encoded within the SPI-2 and has an essential role in bacterial survival during the systemic phase of infection (Monack et al., 2004). Effector proteins encoded within the SPI-2 have been shown to be essential for intracellular growth and survival inside macrophages (Grassl and Finlay, 2008).

THE HOST RESPONSE

Initial studies aimed at identifying host factors responsible for susceptibility to pathogens identified natural resistance-associated macrophage protein 1 (*NRAMP1*) as a crucial gene in natural resistance to infection by *Salmonella*, *Mycobacterium tuberculosis*, and *Leishmania donovani*. Successive studies have greatly expanded our knowledge about the host factors responsible for defense against *Salmonella*, a topic that has been reviewed in detail elsewhere

(Raupach and Kaufmann, 2001). Here, I will focus on the role of pattern recognition receptors (PRRs) belonging to the Toll-like receptors (TLR) and Nod-like receptors (NLR) in mounting protective host responses against *Salmonella*.

ROLE OF TLR4 AND TLR5 IN *SALMONELLA* INFECTION

The initial detection of microbes is achieved though the detection of conserved microbial structures by PRRs. PRRs comprise an array of sensors present in different cell types and cellular location that control the activation of signaling pathways ultimately aimed at the elimination of invading microbes (Takeuchi and Akira, 2010). TLRs localize either at the cell surface or within endosomes, in contrast to NLRs that are located in the host cytosol.

Toll-like receptor 4 recognizes bacterial lipopolysaccharide (LPS), a major constituent of the outer membrane of Gram-negative bacteria (Takeuchi and Akira, 2010), such as *Salmonella*. The recognition of LPS by TLR4 is assisted by several proteins such as CD14, LPS-binding protein (LBP), and MD-2. Knockout mice revealed a crucial role of TLR4, LBP, and CD14 in host defense during the systemic phase of *Salmonella* infection (Bernheiden et al., 2001). The increased susceptibility to *Salmonella* was attributed to a delayed recruitment of neutrophils allowing increased bacterial growth in liver macrophages (Vazquez-Torres et al., 2004), and not to defects in activation of the adaptive immune response (Ko et al., 2009).

Toll-like receptor 5 is a sensor of extracellular flagellin, the main component of the flagella, an appendage that is used by bacteria for movement. TLR5 is expressed on the basal surface of intestinal epithelial cells and in a specific population of intestinal DCs (Uematsu et al., 2006). Initial experiments using intestinal epithelial cell lines indicate that flagellin, but not LPS, is a powerful inducer of the proinflammatory cytokine IL-8 (Zeng et al., 2003). Experiments with polarized intestinal epithelial cell demonstrate that stimulation with flagellin induces an inflammatory response only when in contact with the basolateral surface (Gewirtz et al., 2001a,b). Together these data suggest that in the intestine, flagellin can activate TLR5 only if it breaches the intestinal epithelial barrier, thus potentially preventing the induction of an inflammatory response against flagellated intestinal commensal bacteria (Vijay-Kumar et al., 2008).

While there is evidence that flagellin can trigger the recruitment of immature DCs and other leukocytes through TLR5 in human epithelial cell lines (Sierro et al., 2001), the expression of TLR5 in mouse intestinal epithelial cells is low. This is in contrast to specific populations of lamina propria DCs (CD11c^{hi} CD11b^{hi}) isolated from the small intestine that express high levels of TLR5 (Uematsu et al., 2006). Importantly, while conventional DCs, macrophages, and lamina propria macrophages do not express TLR5 and fail to respond to flagellin, lamina propria DCs stimulated with flagellin produce elevated levels of IL-6 and IL-12 in a TLR5-dependent manner (Uematsu et al., 2006). The response of lamina propria DCs was specific for flagellin, in that lamina propria DCs did not respond to stimulation with other TLR ligands such as LPS (Uematsu et al., 2006). So it seems that some lamina propria DCs are equipped to mount a proinflammatory response against infectious flagellated bacteria such as *Salmonella*. Surprisingly, TLR5-deficient mice are less susceptible than wild-type mice to

infection with *Salmonella* (Uematsu et al., 2006). Given that DCs are important for transporting bacteria from the lamina propria to the mesenteric lymph nodes (Uematsu and Akira, 2009), it has been suggested that in response to *Salmonella* infection TLR5 plays an important role in the maturation of lamina propria DCs and subsequent migration to mesenteric lymph nodes (Uematsu et al., 2006). These data suggest that during *Salmonella* infection the host response mediated by TLR5 in lamina propria DCs is hijacked by the bacterium using intestinal DCs as Trojan horses to cause systemic infection.

ROLE OF NLRs IN *SALMONELLA* INFECTION

Nucleotide-binding oligomerization domain 1 (NOD1) and NOD2 sense bacterial molecules produced during the synthesis, degradation, and remodeling of peptidoglycan (PGN). NOD2 is expressed in a variety of different cells types including macrophages, DCs, and Paneth cells. NOD2 senses muramyl dipeptide (MDP), a moiety found in PGN of nearly all Gram-positive and Gram-negative organisms. Genetic studies revealed that several NOD2 variants are associated with susceptibility to Crohn's disease (CD), a chronic disorder characterized by transmural inflammation of the intestine, particularly in the distal ileum. NOD1 is ubiquitously expressed and recognizes γ-D-glutamyl-meso-diaminopimelic acid that is found in PGN from most Gram-negative bacteria and certain Gram-positive bacteria. Stimulation of NOD1 or NOD2 results in the activation of the adaptor protein receptor-interacting protein 2 (RIP2; also called RICK) which promotes the activation of NF-κB and MAPKs pathway leading to transcription of numerous genes involved in inflammation and host defense. NOD1 and NOD2 are involved in the sensing of several pathogenic bacteria, including *Salmonella*, and has been reviewed in detail elsewhere (Inohara and Nunez, 2003; Franchi et al., 2009b). The first attempt to characterize the role of NOD2 in *Salmonella* infection was performed in human DCs carrying a homozygous loss-of-function NOD2 L1007fs mutation and showed a decreased production of proinflammatory cytokines compared to DCs from healthy individuals (Salucci et al., 2008). Subsequent studies investigated the role of NOD1 and NOD2 in epithelial cells and found that the proinflammatory response induced by *Salmonella* was dependent on the SPI-1 and a set of effectors proteins (including SopE and SopE2), but was independent of Rip2 (Bruno et al., 2009). However, recent evidence suggests that NOD1 and NOD2 may restrict bacterial replication independent of NF-κB activation. For example, NOD1 and NOD2 can sense certain bacteria through the stimulation of the autophagic response in a Rip2- and NF-κB independent fashion (Travassos et al., 2010). In addition, exogenous stimulation of NOD2 via MDP further enhances both the autophagic response and *Salmonella* killing (Homer et al., 2010). In mice, infection with wild-type *Salmonella* failed to reveal a major role for NOD1 or NOD2 in host defense (Le Bourhis et al., 2009; Geddes et al., 2010). However, a role for NOD1 and NOD2 was revealed when mice were infected with a bacterial mutant deficient in SPI-1 or when *Salmonella* was cultured in a manner that favors the expression of SPI-2 (Le Bourhis et al., 2009; Geddes et al., 2010), suggesting that the contribution of NOD1 and NOD2 in the host defense response against *Salmonella* is dependent on the expression of *Salmonella* virulence factors.

INFLAMMASOMES IN *SALMONELLA* INFECTION

Inflammasomes are molecular complexes that regulate the activation of the proteolytic enzyme caspase-1 (Franchi et al., 2009a). Four different inflammasome has been functionally characterized. The NLRP1 inflammasome is activated by MDP and anthrax lethal toxin. The NLRC4 is activated by cytosolic flagellin. The NLRP3-inflammasome is activated by a variety of stimuli of microbial origins as well as particulate matter and danger signals. The AIM2 inflammasome is activated by cytosolic DNA (Franchi et al., 2009a).

In macrophages, *Salmonella* induces the activation of caspase-1 necessary for the maturation of the proinflammatory cytokines IL-1β and IL-18. The activation of caspase-1 also induces a form of cell death called pyroptosis that has feature of both apoptosis (such as the activation of caspases) and necrosis (such as the permeabilization of the cellular membrane; Fink and Cookson, 2007) and that may play a role in host defense independent of cytokine production (Miao et al., 2010a).

Initial studies aimed at identifying the inflammasome responsible for the activation of caspase-1 in macrophages infected with *Salmonella* revealed a crucial role of NLRC4 (also called IPAF; Mariathasan et al., 2004). Subsequently, we and others have found that NLRC4 senses cytosolic flagellin. In fact, macrophages infected with *Salmonella* mutants lacking flagellin possess an impaired ability to induce caspase-1 activation, IL-1β production, and cell death (Amer et al., 2006; Franchi et al., 2006; Miao et al., 2006). In addition, macrophages infected with a *Salmonella* mutant carrying a point mutation in the C-terminal region of flagellin are impaired in their ability to induce the activation of the NLRC4-inflammasome (Franchi et al., 2006). Furthermore, cytosolic delivery of purified flagellin, but not other microbial molecules, activates caspase-1 in an NLRC4-dependent manner (Amer et al., 2006; Franchi et al., 2006; Miao et al., 2006). Consistent with the role of cytosolic flagellin, TLR5-deficient macrophages show no impairment in the activation of caspase-1 induced by *Salmonella* or cytosolic flagellin (Franchi et al., 2006; Miao et al., 2006). Subsequent studies using macrophages infected with *Legionella pneumophila* (Amer et al., 2006; Zamboni et al., 2006) or *Pseudomonas aeruginosa* (Franchi et al., 2007; Miao et al., 2008) and their mutants lacking flagellin confirmed the role of NLRC4 in the activation of caspase-1 induced by cytosolic flagellin. In the case of *L. pneumophila*, but not *P. aeruginosa* or *Salmonella*, the recognition of flagellin by NLRC4 has been suggested to be mediated by the NLR family member NAIP5 (Lightfield et al., 2008). However NAIP5 can restrict *L. pneumophila* growth independently of caspase-1 activation suggesting that the host defense response mediated by NAIP5 proceeds independently of the NLRC4-inflammasome (Lamkanfi et al., 2007). NLRC4 is also important in the activation of caspase-1 induced by *Shigella*, a non-flagellated bacteria, indicating that the existence of a flagellin-independent pathway that leads to the activation of the NLRC4-inflammasome (Suzuki et al., 2007). There is also evidence suggesting that other proteins of the type III secretion system which share homology with flagellin can stimulate the NLRC4-inflammasome (Miao et al., 2010b).

The activation of caspase-1 induced by *Salmonella*, *L. pneumophila*, or *P. aeruginosa* requires a functional bacterial secretion system (Franchi et al., 2008). The observation that during *Salmonella* infection the type III secretion system encoded by SPI-1 is required for both the translocation of flagellin into the cytosol and the activation of caspase-1 (Sun et al., 2007), suggests that small amounts of flagellin may inadvertently leak into the cytosol when bacterial secretion systems puncture cellular membranes.

The role of inflammasomes in *Salmonella* infection *in vivo* has been investigated in caspase-1 deficient mice. Initial work with caspase-1 deficient mice in a B10.RIII background revealed a dramatic phenotype suggesting that *Salmonella* require caspase-1 for bacterial dissemination (Monack et al., 2000). These results are at odds with the role of caspase-1 in other models of bacterial infection (Franchi et al., 2008). More recently, the role of caspase-1 in *Salmonella* infection has been reexamined in caspase-1 deficient mice in the B6 background (Lara-Tejero et al., 2006; Raupach et al., 2006). These experiments showed an increased susceptibility of caspase-1 knockout mice which was associated with increased bacterial loads in the spleen and the mesenteric lymph nodes (Lara-Tejero et al., 2006; Raupach et al., 2006). In agreement with a role for caspase-1 in the systemic phase of the infection, caspase-1 deficient mice were more susceptible than wild-type mice when challenged with *Salmonella* intraperitoneally (Raupach et al., 2006). Using IL-1β and IL-18 knockout mice, IL-18 was revealed as the main cytokine responsible for host defense (Raupach et al., 2006). While caspase-1 has a protective role against *Salmonella* infection, there is also evidence that caspase-1 has a detrimental role in septic shock. Furthermore, in a mouse model in which septic shock was induced by intraperitoneal injection of high doses of an attenuated *Salmonella* strain, caspase-1 and IL-18 deficient mice, but not IL-1β deficient mice, were protected (Raupach et al., 2006). Furthermore, NLRC4-deficient mice have been shown to be equally susceptible as wild-type mice (Lara-Tejero et al., 2006), a result that is not surprising considering that *Salmonella* is known to down regulate the expression of flagellin during the systemic phase of the infection (Alaniz et al., 2006). In contrast, NLRC4 plays an important role in the host defense response toward *Salmonella* mutants that express flagellin during the systemic phase of the infection (Miao et al., 2010a).

Another inflammasome involved in both microbial and non-microbial signaling pathways is the NLRP3-inflammasome. The mechanism of activation of the NLRP3-inflammasome is complex, in that its activation requires two different signals. One signal provided by microbial ligands or endogenous cytokines is necessary to prime macrophages through the upregulation of NLRP3 and pro-IL-1β. The second signal directly triggers caspase-1 activation, and can be mediated by at least four separate pathways that include ATP-P2X7R-pannexin-1, Syk signaling, particulate matters (such as silica and urate crystals), and bacterial exotoxins (Franchi et al., 2009a, 2010). Although disruption of the lysosomal membrane and/or production of reactive oxygen species (ROS) and/or decrease in K+ cytosolic concentration have been implicated the activation of the NLRP3-inflammasome, the molecular mechanisms responsible for the activation of the NLRP3-inflammasome remain elusive.

A role of the NLRP3-inflammasome in the control of *Salmonella* infection was initially excluded based on the fact that NLRP3-deficient mice were as susceptible as wild-type mice to *Salmonella* infection (Lara-Tejero et al., 2006). More recently, Broz et al. (2010) investigated whether the lack of phenotype in

NLRC4- and NLRP3-deficient mice was due to redundant functions of these two inflammasomes and found that mice deficient in both NLRP3 and NLRC4 are more susceptible than wild-type mice to *Salmonella* infection similar to caspase-1 deficient mice. Using a combination of bacterial mutants and specific experimental procedures to maximize the expression of SPI-2, Broz et al. found that in macrophages infected with *Salmonella* that do not express SPI-1 the activation of caspase-1 occurs via the NLRP3-inflammasome and is dependent on SPI-2. These data suggest the existence of two different pathways (**Figure 1**), one dependent on SPI-1, NLRC4, and flagellin that triggers a robust activation of caspase-1 within minutes after infection, and a second pathway dependent on SPI-2 and NLRP3 that triggers the activation of caspase-1 with slower kinetics. The molecular mechanisms by which SPI-2 activates the NLRP3-inflammasome remain unknown. Surprisingly Apoptosis-associated Speck-like protein containing a CARD (ASC), a common adaptor protein used by both NLRC4 and NLRP3 to activate caspase-1 and to trigger the production of IL-1β, has no effect on susceptibility to *Salmonella* infection (Lara-Tejero et al., 2006).
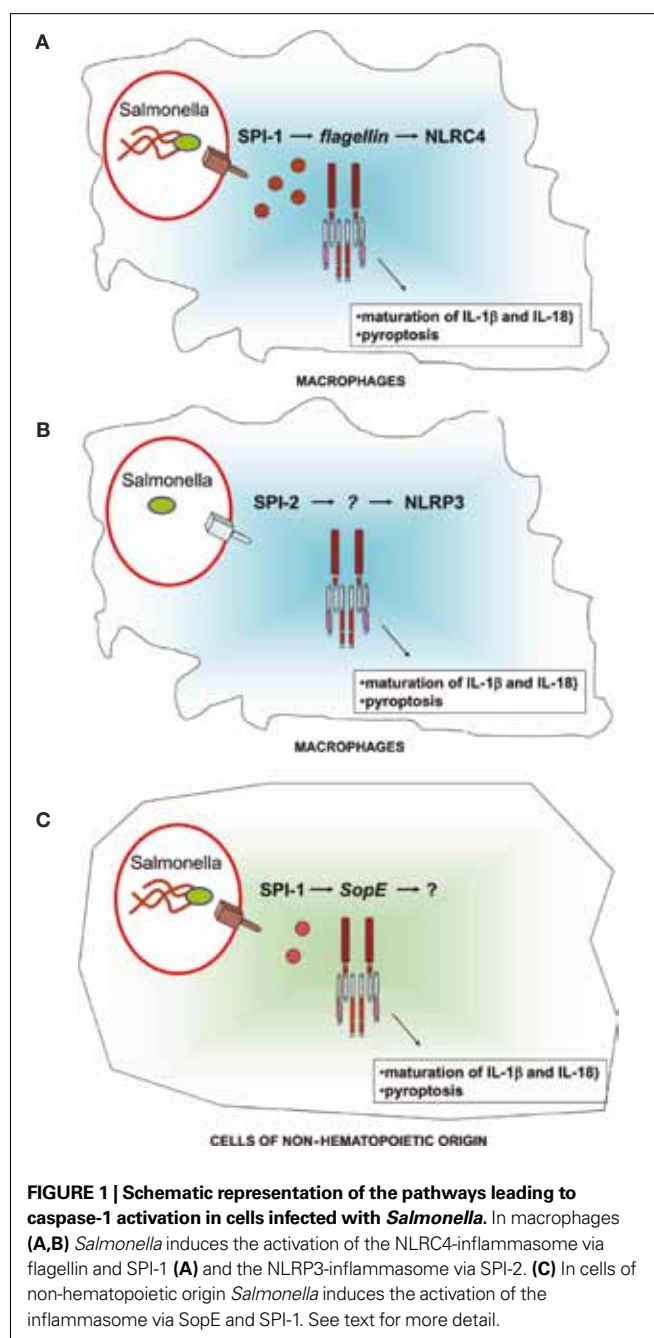
A third pathway (**Figure 1**) of caspase-1 activation has recently been identified. Experiments using epithelial cell lines suggest that the virulence factor SopE encoded by the SPI-1 is able to induce caspase-1 activation (Muller et al., 2009). Furthermore, bone-marrow chimera experiments showed that *Salmonella* mutants that selectively express the virulence factor SopE could induce the activation of caspase-1 in cells of non-hematopoietic origin (Muller et al., 2009). The SopE-dependent activation of caspase-1 in cells of non-hematopoietic origin was found to play a significant role in gut inflammation, but was not sufficient to restrict bacterial replication (Muller et al., 2009). It remains to be determined whether the SopE-dependent activation of caspase-1 is NLRC4 or NLRP3 dependent.

CONCLUDING REMARKS

In this review, I have summarized recent advances in our understanding of the role of NLRs in the innate immune response to *Salmonella*. The available data suggest that the main role of inflammasomes is to restrict the replication of the bacteria during the systemic phase of the infection. In macrophages, the activation of the NLRC4-inflammasome requires SPI-1 and cytosolic flagellin. In macrophages infected with bacteria that do not express SPI-1 and flagellin a second pathway dependent on NLRP3 and the expression of SPI-2 is revealed. A third pathway that can lead to the activation of caspase-1 in non-hematopoietic cells is dependent on SPI-1 and the virulence factor SopE. *In vivo* both the NLRC4- and NLRP3-inflammasome are required for the innate immune response to *Salmonella*. It will be interesting in future studies to explore the role of inflammasomes in the activation of adaptive immune responses elicited by *Salmonella*.

FIGURE 1 | Schematic representation of the pathways leading to caspase-1 activation in cells infected with *Salmonella*. In macrophages **(A,B)** *Salmonella* induces the activation of the NLRC4-inflammasome via flagellin and SPI-1 **(A)** and the NLRP3-inflammasome via SPI-2. **(C)** In cells of non-hematopoietic origin *Salmonella* induces the activation of the inflammasome via SopE and SPI-1. See text for more detail.

REFERENCES

Alaniz, R. C., Cummings, L. A., Bergman, M. A., Rassoulian-Barrett, S. L., and Cookson, B. T. (2006). *Salmonella typhimurium* coordinately regulates FliC location and reduces dendritic cell activation and antigen presentation to CD4+ T cells. *J. Immunol.* 177, 3983–3993.

Amer, A., Franchi, L., Kanneganti, T. D., Body-Malapel, M., Ozoren, N., Brady, G., Meshinchi, S., Jagirdar, R., Gewirtz, A., Akira, S., and Nunez, G. (2006). Regulation of *Legionella* phagosome maturation and infection through flagellin and host Ipaf. *J. Biol. Chem.* 281, 35217–35223.

Bernheiden, M., Heinrich, J. M., Minigo, G., Schutt, C., Stelter, F., Freeman, M., Golenbock, D., and Jack, R. S. (2001). LBP, CD14, TLR4 and the murine innate immune response to a peritoneal *Salmonella* infection. *J. Endotoxin Res.* 7, 447–450.

Broz, P., Newton, K., Lamkanfi, M., Mariathasan, S., Dixit, V. M., and Monack, D. M. (2010). Redundant roles for inflammasome receptors

NLRP3 and NLRC4 in host defense against *Salmonella*. *J. Exp. Med.* 207, 1745–1755.

Bruno, V. M., Hannemann, S., Lara-Tejero, M., Flavell, R. A., Kleinstein, S. H., and Galan, J. E. (2009). *Salmonella typhimurium* type III secretion effectors stimulate innate immune responses in cultured epithelial cells. *PLoS Pathog.* 5, e1000538. doi: 10.1371/journal.ppat.1000538

Fink, S. L., and Cookson, B. T. (2007). Pyroptosis and host cell death responses during *Salmonella* infection. *Cell. Microbiol.* 9, 2562–2570.

Franchi, L., Amer, A., Body-Malapel, M., Kanneganti, T. D., Ozoren, N., Jagirdar, R., Inohara, N., Vandenabeele, P., Bertin, J., Coyle, A., Grant, E. P., and Nunez, G. (2006). Cytosolic flagellin requires Ipaf for activation of caspase-1 and interleukin 1beta in *Salmonella*-infected macrophages. *Nat. Immunol.* 7, 576–582.

Franchi, L., Eigenbrod, T., Munoz-Planillo, R., and Nunez, G. (2009a). The inflammasome: a caspase-1-activation platform that regulates immune responses and disease pathogenesis. *Nat. Immunol.* 10, 241–247.

Franchi, L., Warner, N., Viani, K., and Nunez, G. (2009b). Function of Nod-like receptors in microbial recognition and host defense. *Immunol. Rev.* 227, 106–128.

Franchi, L., Munoz-Planillo, R., Reimer, T., Eigenbrod, T., and Nunez, G. (2010). Inflammasomes as microbial sensors. *Eur. J. Immunol.* 40, 611–615.

Franchi, L., Park, J. H., Shaw, M. H., Marina-Garcia, N., Chen, G., Kim, Y. G., and Nunez, G. (2008). Intracellular NOD-like receptors in innate immunity, infection and disease. *Cell. Microbiol.* 10, 1–8.

Franchi, L., Stoolman, J., Kanneganti, T. D., Verma, A., Ramphal, R., and Nunez, G. (2007). Critical role for Ipaf in *Pseudomonas aeruginosa*-induced caspase-1 activation. *Eur. J. Immunol.* 37, 3030–3039.

Geddes, K., Rubino, S., Streutker, C., Cho, J. H., Magalhaes, J. G., Le Bourhis, L., Selvanantham, T., Girardin, S. E., and Philpott, D. J. (2010). Nod1 and Nod2 regulation of inflammation in the *Salmonella* colitis model. *Infect. Immun.* 78, 5107–5115.

Gewirtz, A. T., Navas, T. A., Lyons, S., Godowski, P. J., and Madara, J. L. (2001a). Cutting edge: bacterial flagellin activates basolaterally expressed TLR5 to induce epithelial proinflammatory gene expression. *J. Immunol.* 167, 1882–1885.

Gewirtz, A. T., Simon, P. O. Jr., Schmitt, C. K., Taylor, L. J., Hagedorn, C. H., O'Brien, A. D., Neish, A. S., and Madara, J. L. (2001b). *Salmonella typhimurium* translocates flagellin across intestinal epithelia, inducing a proinflammatory response. *J. Clin. Invest.* 107, 99–109.

Grassl, G. A., and Finlay, B. B. (2008). Pathogenesis of enteric *Salmonella* infections. *Curr. Opin. Gastroenterol.* 24, 22–26.

Grassl, G. A., Valdez, Y., Bergstrom, K. S., Vallance, B. A., and Finlay, B. B. (2008). Chronic enteric *Salmonella* infection in mice leads to severe and persistent intestinal fibrosis. *Gastroenterology* 134, 768–780.

Hapfelmeier, S., and Hardt, W. D. (2005). A mouse model for *S. typhimurium*-induced enterocolitis. *Trends Microbiol.* 13, 497–503.

Hase, K., Kawano, K., Nochi, T., Pontes, G. S., Fukuda, S., Ebisawa, M., Kadokura, K., Tobe, T., Fujimura, Y., Kawano, S., Yabashi, A., Waguri, S., Nakato, G., Kimura, S., Murakami, T., Iimura, M., Hamura, K., Fukuoka, S., Lowe, A. W., Itoh, K., Kiyono, H., and Ohno, H. (2009). Uptake through glycoprotein 2 of FimH+ bacteria by M cells initiates mucosal immune response. *Nature* 462, 226–230.

Homer, C. R., Richmond, A. L., Rebert, N. A., Achkar, J. P., and McDonald, C. (2010). ATG16L1 and NOD2 interact in an autophagy-dependent antibacterial pathway implicated in Crohn's disease pathogenesis. *Gastroenterology* 139, 1630–1641.

Inohara, N., and Nunez, G. (2003). NODs: intracellular proteins involved in inflammation and apoptosis. *Nat. Rev. Immunol.* 3, 371–382.

Knoop, K. A., Kumar, N., Butler, B. R., Sakthivel, S. K., Taylor, R. T., Nochi, T., Akiba, H., Yagita, H., Kiyono, H., and Williams, I. R. (2009). RANKL is necessary and sufficient to initiate development of antigen-sampling M cells in the intestinal epithelium. *J. Immunol.* 183, 5738–5747.

Ko, H. J., Yang, J. Y., Shim, D. H., Yang, H., Park, S. M., Curtiss, R., 3rd, and Kweon, M. N. (2009). Innate immunity mediated by MyD88 signal is not essential for induction of lipopolysaccharide-specific B cell responses but is indispensable for protection against *Salmonella enterica* serovar Typhimurium infection. *J. Immunol.* 182, 2305–2312.

Lamkanfi, M., Amer, A., Kanneganti, T. D., Munoz-Planillo, R., Chen, G., Vandenabeele, P., Fortier, A., Gros, P., and Nunez, G. (2007). The Nod-like receptor family member Naip5/Birc1e restricts *Legionella pneumophila* growth independently of caspase-1 activation. *J. Immunol.* 178, 8022–8027.

Lara-Tejero, M., Sutterwala, F. S., Ogura, Y., Grant, E. P., Bertin, J., Coyle, A. J., Flavell, R. A., and Galan, J. E. (2006). Role of the caspase-1 inflammasome in *Salmonella typhimurium* pathogenesis. *J. Exp. Med.* 203, 1407–1412.

Le Bourhis, L., Magalhaes, J. G., Selvanantham, T., Travassos, L. H., Geddes, K., Fritz, J. H., Viala, J., Tedin, K., Girardin, S. E., and Philpott, D. J. (2009). Role of Nod1 in mucosal dendritic cells during *Salmonella* pathogenicity island 1-independent *Salmonella enterica* serovar Typhimurium infection. *Infect. Immun.* 77, 4480–4486.

Lightfield, K. L., Persson, J., Brubaker, S. W., Witte, C. E., J. von Moltke, Dunipace, E. A., Henry, T., Sun, Y. H., Cado, D., Dietrich, W. F., Monack, D. M., Tsolis, R. M., and Vance, R. E. (2008). Critical function for Naip5 in inflammasome activation by a conserved carboxy-terminal domain of flagellin. *Nat. Immunol.* 9, 1171–1178.

Mariathasan, S., Newton, K., Monack, D. M., Vucic, D., French, D. M., Lee, W. P., Roose-Girma, M., Erickson, S., and Dixit, V. M. (2004). Differential activation of the inflammasome by caspase-1 adaptors ASC and Ipaf. *Nature* 430, 213–218.

Miao, E. A., Alpuche-Aranda, C. M., Dors, M., Clark, A. E., Bader, M. W., Miller, S. I., and Aderem, A. (2006). Cytoplasmic flagellin activates caspase-1 and secretion of interleukin 1beta via Ipaf. *Nat. Immunol.* 7, 569–575.

Miao, E. A., Ernst, R. K., Dors, M., Mao, D. P., and Aderem, A. (2008). Pseudomonas aeruginosa activates caspase 1 through Ipaf. *Proc. Natl. Acad. Sci. U.S.A.* 105, 2562–2567.

Miao, E. A., Leaf, I. A., Treuting, P. M., Mao, D. P., Dors, M., Sarkar, A., Warren, S. E., Wewers, M. D., and Aderem, A. (2010a). Caspase-1-induced pyroptosis is an innate immune effector mechanism against intracellular bacteria. *Nat. Immunol.* 11, 1136–1142.

Miao, E. A., Mao, D. P., Yudkovsky, N., Bonneau, R., Lorang, C. G., Warren, S. E., Leaf, I. A., and Aderem, A. (2010b). Innate immune detection of the type III secretion apparatus through the NLRC4 inflammasome. *Proc. Natl. Acad. Sci. U.S.A.* 107, 3076–3080.

Monack, D. M., Bouley, D. M., and Falkow, S. (2004). *Salmonella typhimurium* persists within macrophages in the mesenteric lymph nodes of chronically infected Nramp1+ mice and can be reactivated by IFNgamma neutralization. *J. Exp. Med.* 199, 231–241.

Monack, D. M., Hersh, D., Ghori, N., Bouley, D., Zychlinsky, A., and Falkow, S. (2000). *Salmonella* exploits caspase-1 to colonize Peyer's patches in a murine typhoid model. *J. Exp. Med.* 192, 249–258.

Muller, A. J., Hoffmann, C., Galle, M., Van Den Broeke, A., Heikenwalder, M., Falter, L., Misselwitz, B., Kremer, M., Beyaert, R., and Hardt, W. D. (2009). The S. Typhimurium, effector SopE induces caspase-1 activation in stromal cells to initiate gut inflammation. *Cell Host Microbe* 6, 125–136.

Niess, J. H., Brand, S., Gu, X., Landsman, L., Jung, S., McCormick, B. A., Vyas, J. M., Boes, M., Ploegh, H. L., Fox, J. G., Littman, D. R., and Reinecker, H. C. (2005). CX3CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance. *Science* 307, 254–258.

Raupach, B., and Kaufmann, S. H. (2001). Bacterial virulence, proinflammatory cytokines and host immunity: how to choose the appropriate *Salmonella* vaccine strain? *Microbes Infect.* 3, 1261–1269.

Raupach, B., Peuschel, S. K., Monack, D. M., and Zychlinsky, A. (2006). Caspase-1-mediated activation of interleukin-1beta (IL-1beta) and IL-18 contributes to innate immune defenses against *Salmonella enterica* serovar Typhimurium infection. *Infect. Immun.* 74, 4922–4926.

Rescigno, M., Urbano, M., Valzasina, B., Francolini, M., Rotta, G., Bonasio, R., Granucci, F., Kraehenbuhl, J. P., and Ricciardi-Castagnoli, P. (2001). Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nat. Immunol.* 2, 361–367.

Salucci, V., Rimoldi, M., Penati, C., Sampietro, G. M., van Duist, M. M., Matteoli, G., Saibeni, S., Vecchi, M., Ardizzone, S., Porro, G. B., and Rescigno, M. (2008). Monocyte-derived dendritic cells from Crohn patients show differential NOD2/CARD15-dependent immune responses to bacteria. *Inflamm. Bowel Dis.* 14, 812–818.

Santos, R. L., Raffatellu, M., Bevins, C. L., Adams, L. G., Tukel, C., Tsolis, R. M., and Baumler, A. J. (2009). Life in the inflamed intestine, *Salmonella* style. *Trends Microbiol.* 17, 498–506.

Sierro, F., Dubois, B., Coste, A., Kaiserlian, D., Kraehenbuhl, J. P., and Sirard, J. C. (2001). Flagellin stimulation of intestinal epithelial cells triggers CCL20-mediated migration of dendritic cells. *Proc. Natl. Acad. Sci. U.S.A.* 98, 13722–13727.

Sun, Y. H., Rolan, H. G., and Tsolis, R. M. (2007). Injection of flagellin into the host cell cytosol by *Salmonella enterica* serotype Typhimurium. *J. Biol. Chem.* 282, 33897–33901.

Suzuki, T., Franchi, L., Toma, C., Ashida, H., Ogawa, M., Yoshikawa, Y., Mimuro, H., Inohara, N., Sasakawa, C., and Nunez, G. (2007). Differential regulation of

caspase-1 activation, pyroptosis, and autophagy via Ipaf and ASC in Shigella-infected macrophages. *PLoS Pathog.* 3, e111. doi: 10.1371/journal.ppat.0030111

Takeuchi, O., and Akira, S. (2010). Pattern recognition receptors and inflammation. *Cell* 140, 805–820.

Travassos, L. H., Carneiro, L. A., Ramjeet, M., Hussey, S., Kim, Y. G., Magalhaes, J. G., Yuan, L., Soares, F., Chea, E., Le Bourhis, L., Boneca, I. G., Allaoui, A., Jones, N. L., Nunez, G., Girardin, S. E., and Philpott, D. J. (2010). Nod1 and Nod2 direct autophagy by recruiting ATG16L1 to the plasma membrane at the site of bacterial entry. *Nat. Immunol.* 11, 55–62.

Uematsu, S., and Akira, S. (2009). Immune responses of TLR5+ lamina propria dendritic cells in enterobacterial infection. *J. Gastroenterol.* 44, 803–811.

Uematsu, S., Jang, M. H., Chevrier, N., Guo, Z., Kumagai, Y., Yamamoto, M., Kato, H., Sougawa, N., Matsui, H., Kuwata, H., Hemmi, H., Coban, C., Kawai, T., Ishii, K. J., Takeuchi, O., Miyasaka, M., Takeda, K., and Akira, S. (2006). Detection of pathogenic intestinal bacteria by Toll-like receptor 5 on intestinal CD11c+ lamina propria cells. *Nat. Immunol.* 7, 868–874.

Vazquez-Torres, A., Vallance, B. A., Bergman, M. A., Finlay, B. B., Cookson, B. T., Jones-Carson, J., and Fang, F. C. (2004). Toll-like receptor 4 dependence of innate and adaptive immunity to *Salmonella*: importance of the Kupffer cell network. *J. Immunol.* 172, 6202–6208.

Vijay-Kumar, M., Aitken, J. D., and Gewirtz, A. T. (2008). Toll like receptor-5: protecting the gut from enteric microbes. *Semin. Immunopathol.* 30, 11–21.

Zamboni, D. S., Kobayashi, K. S., Kohlsdorf, T., Ogura, Y., Long, E. M., Vance, R. E., Kuida, K., Mariathasan, S., Dixit, V. M., Flavell, R. A., Dietrich, W. F., and Roy, C. R. (2006). The Birc1e cytosolic pattern-recognition receptor contributes to the detection and control of *Legionella pneumophila* infection. *Nat. Immunol.* 7, 318–325.

Zeng, H., Carlson, A. Q., Guo, Y., Yu, Y., Collier-Hyams, L. S., Madara, J. L., Gewirtz, A. T., and Neish, A. S. (2003). Flagellin is the major proinflammatory determinant of enteropathogenic *Salmonella*. *J. Immunol.* 171, 3668–3674.

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Mycobacterium tuberculosis infection and inflammation: what is beneficial for the host and for the bacterium?

Smitha J. Sasindran and Jordi B. Torrelles*

Center for Microbial Interface Biology, Division of Infectious Diseases, Department of Internal Medicine, The Ohio State University, Columbus, OH, USA

Edited by:
Amal Amer, The Ohio State University, USA

Reviewed by:
Jose A. Bengoechea, Fundacion Caubet-CIMERA Illes Balears, Spain
Andrey P. Anisimov, State Research Center for Applied Microbiology and Biotechnology, Russia

*Correspondence:
Jordi B. Torrelles, Center for Microbial Interface Biology, Division of Infectious Diseases, Department of Medicine, The Ohio State University, 460 West 12th Avenue, Biomedical Research Tower, Room 1016, Columbus, OH 43210, USA.
e-mail: jordi.torrelles@osumc.edu

Tuberculosis is still a major health problem in the world. Initial interactions between *Mycobacterium tuberculosis* and the host mark the pathway of infection and the subsequent host inflammatory response. This inflammatory response is tightly regulated by both the host and the bacterium during different stages of infection. As infection progresses, the initial intense pro-inflammatory response observed is regulated by suppressive mediators balancing inflammation. In this environment, *M. tuberculosis* battles to survive interfering with the host inflammatory response. In this review we discuss the major effector molecules involved in inflammation in relation to the different stages of *M. tuberculosis* infection.

Keywords: tuberculosis, inflammation, *Mycobacterium tuberculosis*, pattern recognition receptors

INTRODUCTION

Over the past 15 years as a result of a rigorous approach to treatment endorsed by the World Health Organization, close to 36 million people have been cured of tuberculosis (TB). However, although a great deal of effort has occurred for handling the TB pandemic around the world, there are still 1.8 million people dying from TB each year. Indeed, there are too many inherent factors involved in the surveillance of *Mycobacterium tuberculosis* (*M.tb*, the etiological agent of TB) including the nature of the spread of *M.tb* infection, the difficultly in obtaining the right drug treatment, and a complex therapeutic regime which still makes TB one of the major health challenges in the world. HIV and *M.tb* co-infection and the emergence of multi-drug and extensively drug resistant strains of *M.tb* are also adding to the burden of TB clinical cases in the world.

Tuberculosis pathogenesis is driven by a complex interplay between the host immune system and the survival strategies of the bacterium. The inflammatory response to *M.tb* infection is tightly regulated by both the host and the bacterium. The primary goal of this review is to discuss the major effector molecules involved in inflammation in relation to the different stages of *M.tb* infection.

STAGES OF *MYCOBACTERIUM TUBERCULOSIS* INFECTION

Mycobacterium tuberculosis is mainly considered to be an airborne pathogen. The infection process of *M.tb* can be divided into three different but interrelated stages. The first stage is the aerosol transmission of droplets containing *M.tb* from an infected individual to a healthy individual. Once within the lungs, *M.tb* enters and resides within alveolar macrophages (AMs) and dendritic cells (DCs; Cooper, 2009). Though the AM ingests bacilli and often kills them, the bactericidal capacity of the AM is still not very well defined. In a given *M.tb* infection, the initial containment of the infection depends partially on the genetics of the human population (i.e., defined by the intrinsic microbicidal capacity of host phagocytes) and also on the inhaled *M.tb* strain (i.e., defined by innate virulent factors in each *M.tb* strain). In the primary infection *M.tb* multiplies in the lungs and causes mild inflammation. Although AMs are thought to be an effective barrier to contain pathogens, *M.tb* has evolved various mechanisms to evade the host immune response and survive in these cells. These survival mechanisms include triggering an anti-inflammatory response, blocking reactive oxygen and nitrogen intermediate (ROIs and RNIs, respectively) production, and reducing the acidification of the *M.tb*-containing phagosome (Flynn and Chan, 2001; Fenton et al., 2005; Cooper, 2009).

The next stage of infection is characterized by the emergence of cell-mediated immunity and the formation of granulomas (described below). *M.tb* bacilli that escape the bactericidal effects of the AM, will multiply and result in destruction of AMs. This will in turn attract blood monocytes and other inflammatory cells (i.e., neutrophils) to the site of infection. Monocytes mature to become antigen presenting AMs and DCs and ingest, but not effectively kill the bacteria. At this stage, *M.tb* grows under limited tissue damage. By 6–8 weeks post-infection, antigen presenting DCs have traveled to lymph nodes where T lymphocytes are activated and recruited. Activated T lymphocytes that migrate to the site of infection proliferate forming an early stage granuloma, where macrophages become activated to kill intracellular *M.tb* (Ulrichs and Kaufmann, 2006). However, continuing T cell activation leads to formation of granulomas that mark the persistence stage of the infection (latency), where the growth and spread of bacteria into additional tissue sites are limited. At this stage more than 90% of infected people remain asymptomatic, but *M.tb* may survive within AMs.

The third and final stage is when latent and controlled *M.tb* infection is reactivated. There are two main reasons described for a reactivation event to occur, a decline in the host's immunity due to genetic or environmental cause; and a failure to develop and maintain immune signals. Under these circumstances, the granuloma structure disrupts and results in lung cavitation and pulmonary disease (Kaplan et al., 2003; Dheda et al., 2005; Ulrichs and Kaufmann, 2006; Russell, 2007). Among the genetic causes described that make a subject susceptible to TB are mutations in specific host C-type lectins, cytokines, chemokines, and their specific receptors disrupting critical signaling pathways involved in the immune response against *M.tb*. Compromised immune surveillance for reasons such as co-infection with HIV, where a host becomes immuno-compromised especially for CD4 T cells (the cell target for HIV), is the most important environmental or exogenous cause of susceptibility to TB (Geldmacher et al., 2010). The reactivation of *M.tb* infection can also be due to changes in host cytokine/chemokine networks, implicated in the inflammatory response against *M.tb* infection, that are a consequence of stress and/or old age (Turner, 2011). Earlier studies have also suggested that exogenous re-infection with another strain of *M.tb* (Sonnenberg et al., 2001; Behr, 2004) is an additional factor leading to active disease.
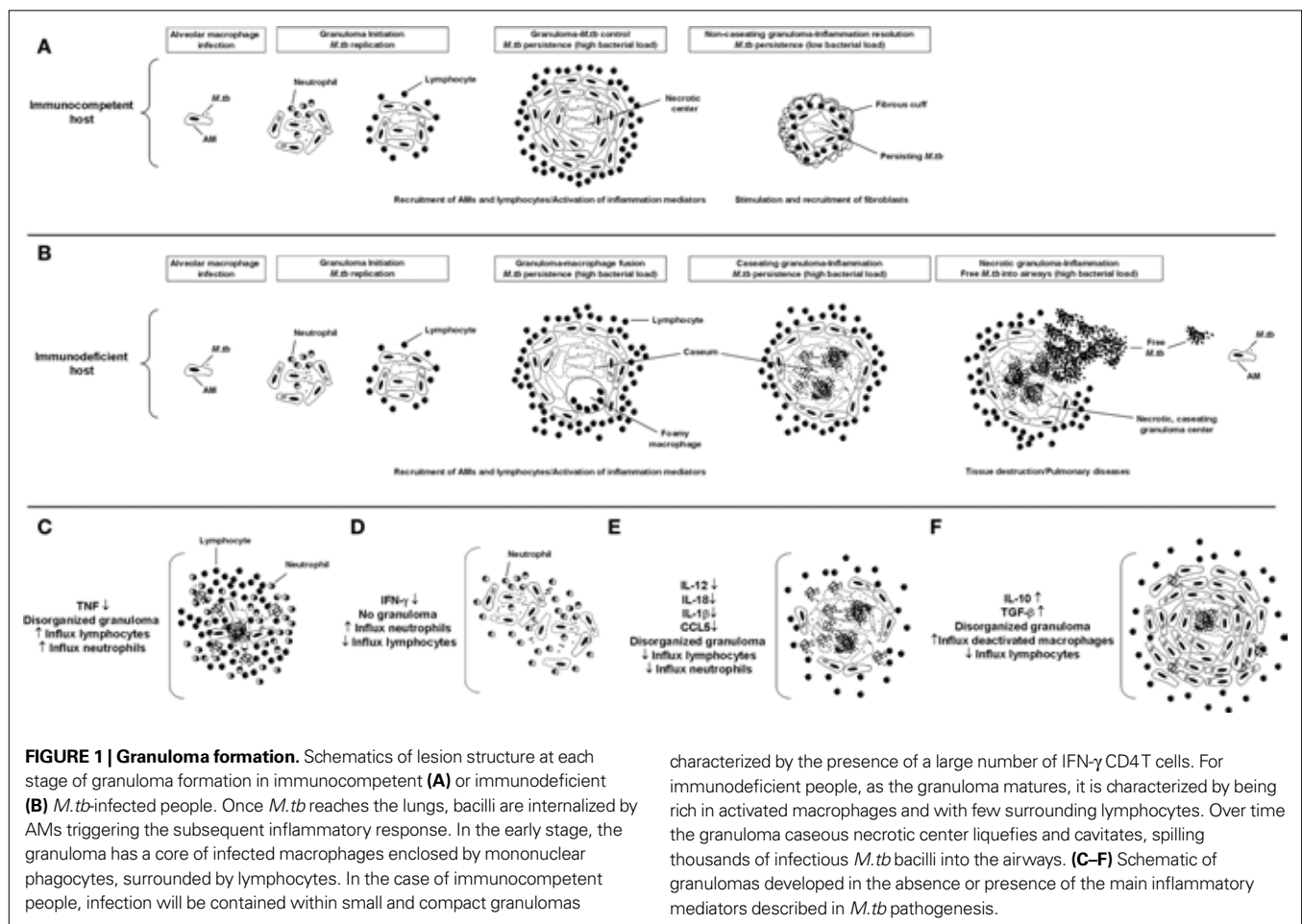
DEVELOPMENT OF THE *M.tb* GRANULOMA

The hallmark in *M.tb* infection is the presence of granulomas within the lung. From the perspective of the bacterium, *M.tb*-induced granulomas are a collection of well organized immune cells that provide a safe microenvironment to establish latency. From the host perspective, granuloma formation restricts the spread of *M.tb* infection. The formation of a granuloma starts with a transient influx of neutrophils to the site of the infection, followed by activated macrophages and lymphocytes (reviewed in detail in Ulrichs and Kaufmann, 2006; Russell, 2007). An established granuloma is composed of infected AMs and epithelioid cells that form a necrotic central core which provides nutritional support to *M.tb*. The central necrotic core of the granuloma is composed by both host and *M.tb* factors. Surrounding this necrotic center there are activated macrophages and layers of CD4+ and CD8+ T cells defining a dense cellular wall that restricts the spread of the *M.tb* (Saunders and Cooper, 2002). In immunocompetent *M.tb*-infected people (**Figure 1A**), *M.tb*-containing granulomas are small, compact, and characterized by the presence of a large number of IFN-γ CD4-T cells; however, in immunodeficient *M.tb*-infected people (**Figure 1B**), granulomas are characterized by being large, rich in activated macrophages, and with few surrounding lymphocytes (Ulrichs et al., 2005). The main cause for tissue injury and clinical manifestation is the presence of large caseating granulomas and fibrotic scarring due to granulomatous inflammation (Daley, 2010); where the host Th1 response serves to try to contain the infection and prevent the development of active disease yet cannot eliminate *M.tb* (Saunders et al., 1999).

The mechanism and the products influencing granuloma formation are not very well established. In response to *M.tb* interaction with AMs and DCs, there is a release of inflammatory cytokines such as tumor necrosis factor-α (TNF), interleukin-(IL)-12, and IL-23 along with a variety of chemokines including (C-C) motif ligand 2 (CCL2), CCL5, and (C-X-C) motif ligand 8 (CXCL8).

Production of IL-12 (Seder et al., 1993) and IL-23 (Oppmann et al., 2000) by DCs primes the Th1 T cell response, which is important for granuloma assembly. This flow of inflammatory events is regulated by the production of IFN-γ and IL-2 by activated T cells that reach the infection site (Cooper, 2009). Studies using CD4 deficient mice demonstrate that CD4 T cells are required for the recruitment of mononuclear cells to the infection site and the organization that is required for the long term survival of the host (Saunders and Cooper, 2002). Along with CD4 T cells, CD8 T cells (Saunders and Britton, 2007) and CD1-restricted NKT-cells (Chackerian et al., 2002; Co et al., 2004) are also key components of developing granuloma. A significant characteristic common between mouse and human pulmonary TB is also the presence of B-cell lymphocyte clusters (Turner et al., 2001; Ulrichs et al., 2004; Tsai et al., 2006). Later studies indicate that B-cell aggregates may serve different purposes in both species as in mice macrophages encircle B-cells; however, in humans B-cell aggregates recruit T cells that are evenly distributed in the clusters (Tsai et al., 2006). Interestingly, studies looking at the ratio between the necrotic core and the neighboring layers of dense leukocytes suggest that the necrotic core expands at the expense of the surrounding layers of cells and not because of leukocyte infiltration (Ulrichs and Kaufmann, 2006).

Although it seems contradictory, it is understood that the initial inflammatory response to *M.tb* is crucial for granuloma generation but also for *M.tb* long term survival within the host. In this context, pro-inflammatory cytokines reduce the bacterial burden, regulating the activity of other cytokines and chemokines, and generating and maintaining organized granulomas. Infection of macrophages by *M.tb* primarily induces the production of TNF, IFN-γ, IL-12, and RNIs and ROIs (Flynn and Chan, 2001; Cooper, 2009), which are considered to be key regulatory factors in the formation and/or maintenance of the granuloma structure. The importance of TNF in granuloma formation has been shown by using TNF neutralizing antibodies and TNF deficient mice *in vivo*. These studies showed that TNF and its receptor are critical in granuloma formation and subsequent protection against *M.tb* infection, as well as their involvement in RNI production by macrophages during early infection (Flynn et al., 1995a; Kaneko et al., 1999). TNF can also induce macrophages to release chemokines that guide cells to the site of infection and prevent their migration away from the site (Flynn and Chan, 2005). Recent studies performed in *Cynomolgus* macaques using TNF neutralizing drugs before *M.tb* infection demonstrated uncontrolled, disseminated infection by 8 weeks of infection. This lack of TNF also induced a high rate of reactivation TB among macaques with latent infection. Importantly, the granuloma architecture in non-human primates after treatment with neutralizing TNF agents was similar to that seen in active TB in humans (Capuano III et al., 2003; Lin et al., 2010). Histological studies performed on human lung biopsies from patients receiving TNF blockade reconfirm the role of TNF in granuloma formation by showing granuloma disorganization due to extensive lymphocytic infiltrations (Keane et al., 2001; **Figure 1C**). In addition to TNF, IFN-γ is also important in the development of granulomas (Flynn et al., 1993). IFN-γ deficient mice were found to be incapable of developing granulomas following aerosol infection and their lungs were found to be infiltrated with neutrophils resulting in cellular necrosis instead of granuloma formation (Cooper et al., 1993; Pearl et al., 2001; **Figure 1D**).
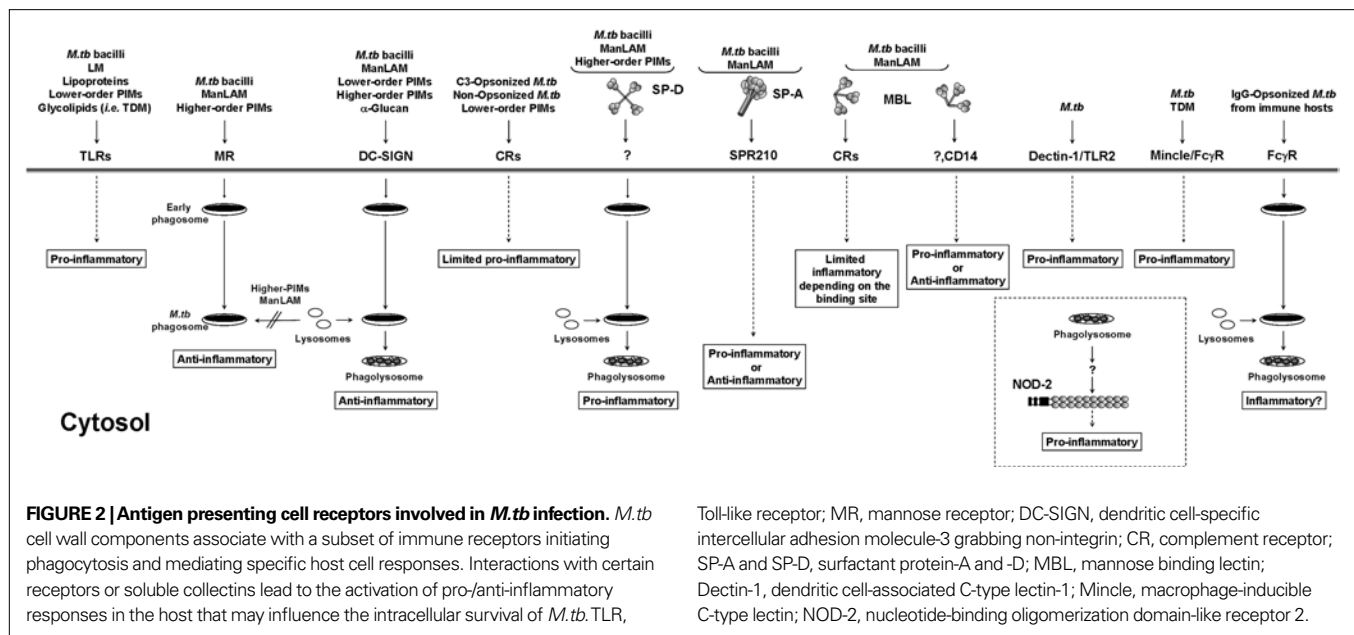
FIGURE 1 | Granuloma formation. Schematics of lesion structure at each stage of granuloma formation in immunocompetent **(A)** or immunodeficient **(B)** *M.tb*-infected people. Once *M.tb* reaches the lungs, bacilli are internalized by AMs triggering the subsequent inflammatory response. In the early stage, the granuloma has a core of infected macrophages enclosed by mononuclear phagocytes, surrounded by lymphocytes. In the case of immunocompetent people, infection will be contained within small and compact granulomas characterized by the presence of a large number of IFN-γ CD4 T cells. For immunodeficient people, as the granuloma matures, it is characterized by being rich in activated macrophages and with few surrounding lymphocytes. Over time the granuloma caseous necrotic center liquefies and cavitates, spilling thousands of infectious *M.tb* bacilli into the airways. **(C–F)** Schematic of granulomas developed in the absence or presence of the main inflammatory mediators described in *M.tb* pathogenesis.

Interleukin-12 is also related to granuloma formation by promoting the Th1 response and inducing IFN-γ positive CD4 T cells (Seder et al., 1993). Studies in mice established that neutralization of IL-12 by specific monoclonal antibodies results in a reduction in granuloma integrity and slowing of the capacity of the animal to control *M.tb* growth (Cooper et al., 1995; **Figure 1E**). Later human studies corroborated the importance of IL-12 in granuloma maintenance, where specific mutations in either the IL-12 p40 or IL-12Rβ1 (IL-12 receptor) gene showed reduced levels of IL-12 and IL-23, and subsequent low IFN-γ induced T cell responses in subjects susceptible to mycobacterial infections (Remus et al., 2001; Fieschi and Casanova, 2003). Other cytokines have also been implicated in the establishment of the granuloma; however, their specific role is still uncertain. This is the case for IL-10, where recent *in vivo* studies using mice over-expressing human IL-10 in their lungs found an increase in macrophage presence and bacterial burden in *M. avium*-containing granulomas (Feng et al., 2002; **Figure 1F**). This was related to a decrease in the levels of pro-inflammatory cytokines like TNF and IL-12 indicating that IL-10 may employ a suppressive effect on the control of mycobacterial infection by negatively controlling macrophage activation. Another regulatory molecule that has been indirectly implicated in granuloma formation is IL-27, which has been shown to activate naïve T cells after their encounter with macrophages and DCs (Owaki et al., 2005). In this context, IL-27 deficient mice have severe lung pathology due to the uncontrolled spread of *M.tb* infection (Holscher et al., 2005).

The exact role of chemokines in granuloma formation is still not very clear. Studies have shown some role for CC chemokine subfamily members particularly CCL5, CCL10, and CCL21 in granuloma formation (Mendez-Samperio, 2008). *In vivo* studies using mice have established that there is a certain level of redundancy in this model system. More recent studies showed the importance of CCL5 and CCL4 and their receptors in the establishment of early granulomas in mice by directly controlling the migration of IFN-γ positive CD4 T cells to the site of the infection (Vesosky et al., 2010). However in humans, chemokines like CCL2, CCL5, and CXCL10 may participate in the protection mechanism against *M.tb* infection (Algood et al., 2003).

Inflammasome-related cytokines like IL-1β (Juffermans et al., 2000; Sugawara et al., 2001) and IL-18 (Sugawara et al., 1999; Schneider et al., 2010) have also been shown to be involved in promoting granuloma formation and organization. In this context, the apoptotic speck-like protein containing a CARD domain (PYCARD), defined as an adaptor protein involved in IL-1β and IL-18 activation through caspase-1 processing, has been shown to play a major role in maintaining granuloma integrity during chronic *M.tb* infection (McElvania et al., 2010).

HOST RECEPTORS AND INFLAMMATION IN *M.tb* INFECTION

The initial interaction between *M.tb* and the host dictates the pathway and outcome of infection (**Figure 2**). On the host side, there are specific receptors capable of recognizing *M.tb*. Normally this

FIGURE 2 | Antigen presenting cell receptors involved in *M.tb* infection. *M.tb* cell wall components associate with a subset of immune receptors initiating phagocytosis and mediating specific host cell responses. Interactions with certain receptors or soluble collectins lead to the activation of pro-/anti-inflammatory responses in the host that may influence the intracellular survival of *M.tb*. TLR, Toll-like receptor; MR, mannose receptor; DC-SIGN, dendritic cell-specific intercellular adhesion molecule-3 grabbing non-integrin; CR, complement receptor; SP-A and SP-D, surfactant protein-A and -D; MBL, mannose binding lectin; Dectin-1, dendritic cell-associated C-type lectin-1; Mincle, macrophage-inducible C-type lectin; NOD-2, nucleotide-binding oligomerization domain-like receptor 2.

recognition is beneficial for the host by activating innate immunity; however, there are specific receptors that favor *M.tb* uptake, bypassing the pro-inflammatory response and leading to intracellular survival. In this section we will discuss host receptors involved in *M.tb* recognition and the subsequent inflammatory response.

TOLL-LIKE RECEPTORS

Toll-like receptor (TLR) signaling is essential for immunity to various intracellular pathogens. TLRs are a set of pattern recognition receptors (PRRs) that are expressed on many cell types but their function on antigen presenting cells (APCs) is particularly important (reviewed in Kawai and Akira, 2010). On macrophages TLRs are either expressed on the surface (like TLR2 and 4) or inside cell compartments (like TLR8 and 9; Kawai and Akira, 2010). TLRs detect a wide range of structures on *M.tb* (**Figure 2**), which aid in the activation of innate immunity and enhance adaptive immunity by mediating the secretion of various pro-inflammatory cytokines along with other anti-bacterial effector molecules. The relationship between TLRs and *M.tb* is a bit more complex than with other bacteria. Various studies performed indicate that different components of the bacteria interact with different TLRs. Among TLRs the key players in TB immunity are TLR2 (alone or as a heterodimer with TLR1 or TLR6), TLR9, and probably TLR4 (Harding and Boom, 2010). For TLR2 alone or in association with TLR1 or TLR6, several groups have reported a strong pro-inflammatory response induced by mycobacterial cell wall components. This is the case for the 19 KDa lipoprotein, phospho-*myo*-inositol-capped or non-capped lipoarabinomannan (PILAM and AraLAM respectively, both produced by less pathogenic mycobacteria), lower- and higher-order phosphatidyl-*myo*-inositol mannosides (PIM_1, PIM_2, and PIM_6 families), lipomannan, and trehalose dimycolate (reviewed in Jo et al., 2007). A combination of studies using several *M.tb* ligands and mouse macrophages deficient in TLR2 and MyD88 (myeloid differentiation primary-response protein 88) determined conclusively that the strong pro-inflammatory response to *M.tb* infection

observed via TLR2 signaling is mediated through its adaptor protein MyD88 (Quesniaux et al., 2004), where engagement of TLR2 triggers a nuclear factor kappa-light chain-enhancer of activated B-cell (NFκB) signaling cascade through the recruitment of MyD88 and TIRAP (toll-interleukin 1 receptor [TIR] domain containing adaptor protein; Kawai and Akira, 2010). Of no surprise is that the response observed depends on the mycobacterial ligand and the nature of the host cell type studied. As an example, uncapped AraLAM was shown to induce cell activation via TLR2 (Underhill et al., 1999) leading to *M.tb* killing in both murine and human macrophages but in a nitric oxide dependent and independent manner, respectively (Thoma-Uszynski et al., 2001).

Interestingly, an overwhelming stimulation of the TLR2 pathway may also be advantageous for *M.tb*. Recent studies have indicated that prolonged TLR2 signaling induced by *M.tb* lipoproteins LpqH, LpRG, and LpRA inhibit the expression of major histocompatibility complex (MHC) class II thereby decreasing antigen presentation in macrophages infected with *M.tb* (Harding and Boom, 2010). Related to this, more recent studies using mice also suggest that TLR2 may be one of many pathways exploited by *M.tb* to inhibit MHC-I antigen cross processing and presentation to CD8+ T cells (Harding and Boom, 2010). Studies performed on infected DCs also resulted in TLR2-dependent inhibition of TLR9-dependent IFN-α/β expression, leading to a decrease in induction of IFN-α/β-dependent MHC-I cross processing (Simmons et al., 2010). In this regard, during *M.tb* infection *in vivo*, it is still uncertain whether TLR2 dependent inhibition of the TLR9/IFN-α/β-dependent MHC-I processing pathway is of benefit to the host by limiting the harmful effects of excessive inflammation or to *M.tb*, as it may provide a path for *M.tb* to escape the host immune response. Overall, a negative regulatory feedback mechanism among TLR signaling networks during *M.tb* chronic infection may benefit the host by preventing detrimental effects of excessive inflammation (Simmons et al., 2010). In this context, studies performed to understand the role of TLR signaling *in vivo* using TLR deficient mice indicate

that a balance in the TLR signaling network is necessary to control inflammation. This is indicated by studies where the exclusion of the TLR2 signaling pathway with a moderate dose of *M.tb in vivo* results in an exaggerated inflammatory response which may be detrimental for the host (Drennan et al., 2004).

TLR9 recognizes unmethylated CpG (cytosine phosphate guanosine motif) found in mycobacterial DNA (Kawai and Akira, 2010). As mentioned above, activation of TLR9 induces IFNα/β and MHC-I antigen cross processing (Simmons et al., 2010). Mycobacterial ligands for TLR4 are still undetermined; although recently, recombinant *M.tb* heat shock protein (hsp) 65 has been described as a ligand for TLR4 inducing NFκB via MyD88, TIRAP, TRIF (TIR-domain-containing adapter-inducing interferon-β), and TRAM (TRIF-related adaptor molecule)-dependent signaling pathways (Bulut et al., 2005). Studies using TLR4 deficient mice infected with *M. bovis* Bacillus Calmette–Guérin (BCG), showed that these mice are capable of controlling the infection; however, mice eventually showed body weight loss and increased local inflammation indicating that TLR4 plays a defined role in modulating inflammation in mycobacterial infections (Fremond et al., 2003).

THE MANNOSE RECEPTOR

The cell envelope of pathogenic mycobacteria like *M.tb* is particularly rich in mannose-containing biomolecules, including ManLAM, LM, higher-order PIMs, arabinomannan, mannan, and mannosylated glycoproteins. ManLAM, LM, and higher-order PIMs are incorporated into the *M.tb* plasma membrane and also exposed on its cell surface acting as ligands for host cell receptors contributing to the pathogenesis of *M.tb* (Torrelles and Schlesinger, 2010). ManLAM and higher-order PIMs contain terminal mannosyl units ideally located to interact with the MR on macrophages and DCs (Schlesinger et al., 1994; Torrelles and Schlesinger, 2010; **Figure 2**). The MR is defined as a homeostatic/clearance/immunomodulatory receptor for endogenous serum glycoproteins with *N*-linked high-mannose content normally elevated during inflammation (Martinez-Pomares et al., 2001). Evidence in the literature suggests that *M.tb* may exploit its mannosylated cell surface components to survive within the host by binding the MR. In this context, we recently demonstrated that ManLAM/MR and higher-order PIMs/MR phagocytic pathways lead to phagosome maturation arrest (Kang et al., 2005; Torrelles et al., 2006). Recognition of mannose residues by the MR has also been shown to reduce the microbicidal activities of the macrophage by inhibiting the production of nitric oxide, oxygen radicals, and pro-inflammatory cytokines and by blocking *M.tb*-induced apoptosis through modifying Ca^{2+}-dependent signaling pathways (reviewed in Torrelles et al., 2008a). In addition, several studies have shown that ManLAM generates an anti-inflammatory response inhibiting the production of TNF and IL-12, and inducing the production of IL-10 and transforming growth factor β (TGF-β; Astarie-Dequeker et al., 1999; Nigou et al., 2001; Chieppa et al., 2003). Recent studies have also established an MR-specific signaling pathway in the pathogenesis of *M.tb* (Rajaram et al., 2010). Infection of human macrophages with virulent *M.tb* or the addition of ManLAM up-regulates a transcription factor named peroxisome proliferator-activated receptor-gamma (PPAR-γ), an important molecule that regulates the macrophage

inflammatory response. PPAR-γ up-regulation via the MR leads to the simultaneous increase in the production of IL-8 (or CXCL-8), expression of cyclooxygenase 2 (COX_2), and production of prostaglandin 2 (PGE_2; Rajaram et al., 2010). This study also revealed that PPAR-γ deficiency in human macrophages leads to increased levels of TNF and decreased bacterial load, indicating that the MR negatively regulates protective macrophage inflammatory responses to *M.tb* infection. In murine macrophages, inhibition of PGE_2 by *M.tb* infection prevents apoptosis and leads the infected cell to a necrotic pathway favoring the spread of the infection (Divangahi et al., 2010). In these type of studies we should carefully consider differences between mouse and human macrophages as well as the existence of receptor-dependent/specific regulatory mechanism(s) in these complex signaling networks. In this context, in addition to *M.tb* ManLAM blocking TNF production via the MR, unpublished data by Rajaram and Schlesinger identify a novel molecular and cellular mechanism underlying the ability of another major *M.tb* cell wall component, LM, to block TLR2 induced biosynthesis of TNF in macrophages, thereby allowing *M.tb* to subvert the host immune response and potentially increase its virulence (personal communication).

DENDRITIC CELL-SPECIFIC ICAM-3-GRABBING NON-INTEGRIN

Dendritic cell-specific ICAM-3-grabbing non-integrin (DC-SIGN) expressed on DCs (Geijtenbeek et al., 2000), strongly binds to *M.tb* through its surface-exposed mannose-containing molecules ManLAM, LM, and PIMs (reviewed in Ehlers, 2009). Recently, α-glucan found on the *M.tb* surface was also found to act as a ligand for DC-SIGN (Geurtsen et al., 2009). DC-SIGN actively participates in the phagocytosis of *M.tb* by DCs leading to bacterial killing by acidification of the *M.tb*-containing phagosome (Geijtenbeek et al., 2003) (**Figure 2**). However, the role of DC-SIGN in inflammation is controversial. Association of *M.tb*, α-glucan or its mannosylated cell wall components with DC-SIGN has been shown to induce production of anti-inflammatory mediators like IL-10 (Ehlers, 2009; Geurtsen et al., 2009). Recent *in vivo* studies using wild-type mice expressing human DC-SIGN homologs (Park et al., 2001; McGreal et al., 2005; Powlesland et al., 2006) or transgenic mice expressing human DC-SIGN, consider the possibility that DC-SIGN may act to dampen the immune response thereby promoting host protection by limiting tissue damage (Wieland et al., 2007; Schaefer et al., 2008; Tanne et al., 2009). Conversely, using genetically engineered *M. marinum* strains lacking essential mannosylated components on their cell surface revealed that the ManLAM–PIM/DC-SIGN pathway may not be significantly involved in the regulation of cytokine secretion (Appelmelk et al., 2008). However, as *M. marinum* does not reflect the overall mannosylation pattern observed in *M.tb* laboratory strains (where *M.tb* clinical isolates also present different degrees of mannosylation; Torrelles et al., 2008b), the direct functional consequences of DC-SIGN ligation in *M.tb* pathogenesis are still unclear.

COMPLEMENT RECEPTORS

Complement receptors (CRs) are expressed on all mononuclear phagocytes and mediate the phagocytosis of a diverse group of intracellular pathogens. Several studies have established the role of C3 opsonization and the contribution of CR1, CR3, and CR4

in the phagocytosis of *M.tb* (Fenton et al., 2005; **Figure 2**). C3 deposition on *M.tb* happens quickly and is initiated by activation of the classical (in low serum concentrations) and/or alternative pathways (in high serum concentrations) via covalent linkages to *M.tb* surface targets in the forms of C3b and C3bi (Ferguson et al., 2004). However, it is still unknown if C3 opsonization varies in form and amount among different stages of *M.tb* infection or tissue sites. *M.tb* surface-exposed polysaccharides and lower-order PIMs (i.e., PIM_2) have also been shown to interact directly with the lectin domain of CR3 (Villeneuve et al., 2005) and thus, presumably mediate *M.tb* uptake by macrophages. Although CR3 mediates both opsonic and non-opsonic uptake of *M.tb* by macrophages, its role during human infection remains unknown. *In vitro* and *in vivo* studies did not show an altered phenotype between CR3 deficient and wild-type mice in bacterial burden and pathology (Hu et al., 2000). In this context, several studies have shown that CR4 is particularly abundant on cells that are involved in the uptake of *M.tb* leading to the conclusion that in the naïve host, CR4 may be the major player mediating *M.tb* uptake in the early stages of infection (Hirsch et al., 1994; Zaffran et al., 1998). This may correlate with the fact that CR4 and the MR are the most highly expressed receptors on AMs (Schlesinger et al., 2008).

COLLECTINS AND THEIR SPECIFIC RECEPTORS

There are three major collectins within the C-type lectin family that recognize specific sugar moieties on the *M.tb* surface increasing the interaction between the host and the bacteria. They are surfactant proteins (SP)-A and SP-D, and mannose binding lectin (MBL). SP-A and SP-D are mainly located in the lung, being secreted by alveolar epithelial type II cells into the surfactant (Wright, 2005). Various studies have provided evidence suggesting a dual role model for SP-A and SP-D, as they help maintain a balance between pro- and anti-inflammatory responses to *M.tb* in the lung environment. In this context, SP-A may be considered to be the perfect liaison for *M.tb*. Apart from being characterized for its capacity to act as an opsonin, a regulator for cell receptor activity enhancing phagocytosis by macrophages, and a mediator of inflammation by regulating the synthesis of ROIs and RNIs and cytokine secretion (reviewed in Torrelles et al., 2008a; **Figure 2**); recent studies show that SP-A may be a contributor to the establishment of a successful *M.tb* infection. In this context, SP-A is capable of opsonizing *M.tb* and after being recognized by its specific receptor, SP-R210, leads to the secretion of anti-inflammatory cytokines like IL-10 and TGF-β mediating suppression of cell-mediated immunity against *M.tb* (Samten et al., 2008). Linked to this, is the establishment that SP-A also up-regulates macrophage expression of the MR (a receptor that leads to *M.tb* intracellular survival within the macrophage by limiting phagosome maturation; Beharka et al., 2002; Torrelles et al., 2008a). SP-A also regulates TLR surface expression and activity in human macrophages (Henning et al., 2008), where despite its ability to specifically up-regulate TLR2 expression, SP-A seems to dampen TLR2 and TLR4 signaling in these cells, and thus situates SP-A as a critical mediator in regulating lung inflammatory responses through TLRs (Henning et al., 2008). SP-D has been recently shown to bind in high avidity to *M.tb* ManLAM and PIMs (Ferguson et al., 1999; Carlson et al., 2009). SP-D opsonizes and subsequently aggregates *M.tb* thereby reducing its phagocytosis (Ferguson et al., 1999).

Conversely, SP-D opsonized *M.tb* that is phagocytosed undergoes increased phagosome–lysosome fusion resulting in limited intracellular growth (Ferguson et al., 2006; **Figure 2**). Another soluble collectin considered in the establishment of *M.tb* infection is the MBL. MBL binds to *M.tb* promoting activation of the complement lectin pathway leading to C3bi deposition, as well as complement-independent phagocytosis which induces pro-inflammation with the release of TNF, IL-1β, and IL-6 (reviewed in Dommett et al., 2006; **Figure 2**). However, controversy regarding the model system (i.e., cell types and species specificity) studied still exists for determining if MBL is overall ultimately beneficial for *M.tb* or the host (Torrelles et al., 2008a).

OTHER MACROPHAGE SURFACE RECEPTORS

Other receptors (**Figure 2**) involved in *M.tb* recognition and inflammation are CD14 (Khanna et al., 1996) and the scavenger receptors SR-A (Zimmerli et al., 1996), which participate in the uptake of non-opsonized bacilli by tissue-specific macrophages. However, their role in inflammation varies depending on the species-specific cell type used. Dectin-1, a β-glucan receptor, in combination with TLR2 has also been shown to participate in the immune response against *M.tb* by inducing TNF production in macrophages only infected with attenuated *M.tb* strains (Yadav and Schorey, 2006). Recently, macrophage-inducible C-type lectin (Mincle; Yamasaki et al., 2008) on the macrophage surface, has been shown to recognize *M.tb* trehalose dimycolate, and working together with the Fcγ receptor transmembrane segment induces pro-inflammation (Ishikawa et al., 2009; Schoenen et al., 2010). Conversely, Fcγ receptors do not play a role in the phagocytosis of *M.tb* in the absence of specific antibody (Schlesinger et al., 1990).

CYTOSOLIC RECEPTORS: NOD2

Cytosolic regulators of the pro-inflammatory response known as nucleotide-binding oligomerization domain (NOD)-like receptors (Franchi et al., 2008) have been recently described to play a role during *M.tb* infection. NOD2, found in epithelial cells and APCs (Ogura et al., 2001; Gutierrez et al., 2002; Inohara and Nunez, 2003), regulates the production of inflammatory mediators in response to bacterial peptidoglycan components such as muramyl dipeptide (Inohara and Nunez, 2003). Human studies in patients infected with *M.tb* or *M. leprae* have linked a polymorphism in NOD2 to susceptibility to mycobacterial infections (Austin et al., 2008; Zhang et al., 2009). However, the role of NOD2 in the early stages of *M.tb* infection seems to be dependent on the model system studied. In this context, results obtained from *in vitro* and *in vivo* studies using the mouse model dispute the significance of NOD2 in controlling *M.tb* growth during the early stages of infection (Gandotra et al., 2007; Divangahi et al., 2008). Conversely, *in vitro* studies using human macrophages infected with *M.tb* have established that, in accordance with the human polymorphism studies, NOD2 plays a role in controlling pro-inflammation and *M.tb* intracellular growth (Brooks et al., 2010; **Figure 2**). How NOD2 intersects with signaling/trafficking networks is still unexplored. Although, NOD2 can synergize with other signaling pathways like TLRs, enhancing pro-inflammation (Ferwerda et al., 2005), its capability to interfere/associate with phagocytic receptor trafficking networks is not established. As cytosolic NOD2 appears to be associated with

intracellular vesicles (Brooks et al., 2010), its role in triggering pro-inflammation may depend on vesicular fusion events controlled during *M.tb* phagocytosis.

The outcome of *M.tb* infection and the subsequent inflammatory response depends on the initial interaction(s) between *M.tb* and APCs. These interactions are mainly based on two factors: The nature and distribution of recognition receptors on the APC surface, and on the biochemical nature of the cell wall constituents of the *M.tb* bacilli recognized by APCs. As described above, published studies define the role of each individual receptor (or a combination of them) during infection *in vitro* or *in vivo* using different means. It still is unclear though, which receptors and how many of them are involved in the initial *M.tb*–host interaction *in vivo*. Our *in vitro* studies using single cell suspensions of *M.tb* and human macrophages show that *M.tb* has a predilection for infection of specific subpopulations of APCs. Do these APC subpopulations preferentially express a specific receptor(s) beneficial for *M.tb* recognition/infection? Does the genetic background of the host (man) predispose for surface expression of specific receptors on APCs that favor *M.tb* infection? If the *M.tb* cell wall determines the impact of the infection; why do different virulent strains of *M.tb* present different motifs on their surface? Are biochemical differences on the *M.tb* cell wall surface favoring *M.tb* long term persistence within the host? Conversely, are we accounting for how disruption of a specific receptor alters the complex inflammatory network *in vivo*? Human studies addressing receptor polymorphisms in TB patients may help address these still unanswered questions.

CHEMOKINES IN *M.tb* INFECTION

Due to their assistance in cell migration and subsequently granuloma formation, chemokines are essential but undefined players in the inflammatory response to *M.tb* infection. Their main function is to direct cell migration and immune homeostasis in the host. Current literature already has established a role for CC and CXC chemokines in the protective and immune host response to TB (reviewed in Mendez-Samperio, 2008). *In vitro* studies using *M.tb*-infected murine and human cells demonstrate that CC chemokines like CCL2, CCL3, CCL4, CCL5, CCL19, CCL20, CCL21, and CCL22 and their related chemokine receptors (i.e., CCR2 [for CCL2], CCR4 [for CCL2, CCL3, CCL5, and CCL22], CCR5 [for CCL2 to CCL5], CCR6 [for CCL20], and CCR7 [for CCL19, CCL21]) regulate migration and activation of various immune cells like monocytes, macrophages, DCs and T lymphocytes by chemotaxis (Mendez-Samperio, 2008). In particular, the use of CCL5 deficient mice confirmed the role of this chemokine in *M.tb* infection. CCL5 deficient mice infected with a low dose of aerosolized *M.tb* are described to present a decrease in recruitment of lymphocytes to TB granulomas site along with an impaired early acquired immunity (Vesosky et al., 2010). Human studies involving bronchoalveolar lavage fluid and tissue from TB patients also demonstrate the involvement of CXCL8 (or IL-8), CCL2, and CCL5 during the acute phase of this disease (Kurashima et al., 1997).

Three major cellular receptor-dependent intracellular signaling pathways regulating chemokine secretion in response to mycobacterial infections have been described (reviewed in detail in Mendez-Samperio, 2008). Mitogen-activated protein kinases (MAPKs) p38 and extracellular signal-regulated kinases (ERK)1/2 are shown to

be phosphorylated by a variety of stimuli leading to a cell type and stimulus-specific dependent production of chemokines (Roach and Schorey, 2002). NFκB, a key molecule in inflammation regulation, is also involved in the regulation of chemokine gene transcription (Mendez-Samperio, 2008) and subsequent secretion. In this context, specific heat-stable cell wall components of *M.tb* are described to induce chemokine secretion in specific cell types differentially expressing defined receptors (Jones et al., 2001). This is the case for ManLAM, which induced chemokine secretion in monocytes, but not in AMs (Barnes et al., 1992). The fact that the MR, a major receptor for ManLAM, is present on AMs but not in monocytes, may favor ManLAM signaling through TLR2 and TLR4 in monocytes leading to chemokine secretion. Recent contradictory studies also established a role for TNF as a controller for chemokine secretion depending of the cell type and the mycobacterial strain (stimulus-specific) studied (Juffermans et al., 1999; Mendez-Samperio et al., 2003). For example, chemokine production induced by *M. bovis* BCG does not seem to be controlled by TNF (Mendez-Samperio et al., 2003), however, *M.tb*-infected macrophages reduce inflammatory chemokine secretion after TNF neutralization (Algood et al., 2004). The controversy also appears when the debate is who regulates who. Are cytokines regulating chemokines or contrary to the current view, are chemokines controlling the host response to infection? Kinetic studies closely evaluating levels of cytokines and chemokines in *M.tb*-infected host are necessary to address this issue. In this context, a recent study using *M. bovis* BCG showed that both cytokine and chemokine secretions seem to be tightly regulated, where a relationship between CCL2, CCL3, and TNF production exist in early stages of infection; and the same is described for CXCL10 and IL6 and IL10 in intermediate phases, and for CCL22 and IFN-γ in late stages of infection (Mendez-Samperio, 2008). As CCL2 has been recently shown to inhibit the pro-inflammatory cytokine IL-12p40 during *M.tb* infection (Flores-Villanueva et al., 2005), the regulatory role of chemokines controlling inflammation may dictate the establishment and outcome of *M.tb* infection. Current literature supporting this concept indicates that several chemokines trigger the activation of signaling cascades like MAPK/c-Jun N-terminal kinase (MAPK/JNK) and PI3K pathways associated with cell mitogenesis, chemotaxis, and cell activation (Mendez-Samperio, 2008). This is described for CCL2, which interacts with CCR2 activating different MAPK-ERK1/2 cascades involved in CCL2-associated cell immune responses against mycobacterial infections (Ashida et al., 2001). The importance of these regulatory pathways are emphasized in a recent study where MAPK-p38 and ERK1/2 activities can control intracellular mycobacterial replication within the macrophage independently of TNF and/or IL-10 mediated effects (Klug et al., 2010).

Other members of the CXC chemokine family are also implicated in controlling the inflammatory conditions following infection by *M.tb*. This is the case for CXCL1, CXCL7, CXCL8, CXCL9, and CXCL10 (reviewed in detail in Mendez-Samperio, 2008). In all cases, these CXC chemokines are, as in the case for CC chemokines, secreted depending on the stimulus and cell-type. Probably among the CXC chemokines CXCL8 (or IL-8) is the most studied in the regulation of *M.tb* infection. CXCL8 is secreted by different types of host cells in response to virulent *M.tb* infection recruiting inflammatory cells (i.e., neutrophils, lymphocytes, monocytes) to

the infection site, and stimulating bactericidal non-oxidative mechanisms (Nibbering et al., 1993; Pace et al., 1999). Thus, chemokines triggered by *M.tb* infection regulate the innate immune events laying the foundation for the establishment of the subsequent host adaptive immune response.

DECOY RECEPTORS

These rare receptors are structurally similar to conventional chemokine receptors but without the intrinsic capability to trigger signaling events. Although only three of these receptors have been identified in mammals, D6 has been intensely studied due to its capacity to be recognized by multiple chemokines during inflammation (reviewed in Di et al., 2009). In this context, studies challenging D6 deficient mice with complete Freund's adjuvant described an extensive inflammation characterized by leukocyte infiltration with localized areas of severe necrosis (Martinez de la et al., 2005). *M.tb*-infected D6 deficient mice presented a similar phenotype, also characterized by a substantial leukocyte infiltration causing vast local and systemic production and accumulation of inflammatory chemokines and cytokines resulting in an overwhelming inflammation followed by severe tissue damage (Di et al., 2008). These findings support the initial concept that D6 is a receptor assisting with control of inflammation by scavenging circulating pro-inflammatory CC chemokines, but its action is dependent on the inflammatory conditions in the host environment. Another decoy receptor gaining interest in the field of TB research is Tir8. Tir8 is described to participate in the tight regulation of the immune response by inhibiting TLR/IL-1 NFκB activation (Polentarutti et al., 2003). *In vivo* studies using Tir8 deficient mice showed that, like D6 deficient mice, Tir8 play a role in damping the immure response against *M.tb* infection (Di et al., 2009).

PROTECTIVE PRO-INFLAMMATORY MEDIATORS

Inflammation and immune reaction in response to most pathogens is partly mediated by a group of secreted polypeptides known as cytokines. Not only is the inflammatory response generated by cytokines against *M.tb* helping in the control of the infection, but they also play an important role during the chronic infection stage dictating the pathogenesis of the disease (Flynn and Chan, 2001; Cooper, 2009). The fate of *M.tb* infection is determined by the interplay between various cytokines released at different time points of infection and the effect of these cytokines on the host cell and *M.tb*. In this section, we will present the main cytokines involved in chronic and cell-mediated inflammatory responses to *M.tb* infection.

TUMOR NECROSIS FACTOR

Tumor necrosis factor, an important component of innate immune mechanisms of the host against pathogens, is shown to be critical in the control of *M.tb*. TNF is an autocrine cytokine produced by macrophages, DCs and T cells, and performs functions like chemotaxis and granuloma formation. TNF, if not regulated can lead to tissue damage and cause immunopathology. The most widely studied models to understand the role of TNF *in vivo* are human TNF transgenic and TNF gene deficient mice. Studies using TNF and lymphotoxin-α (LT-α) deficient mice demonstrated that complete disruption of TNF and LT-α reduces host resistance to

mycobacterial infection due to a delay and deficient granuloma formation leading to a widespread dissemination of *M.tb* (Bean et al., 1999; Jacobs et al., 2000). In this regard, TNF deficient mice are characterized for containing immature lymphocyte cells concentrated in the perivascular and peribronchial areas surrounding granulomas. Moreover, a deficiency in chemokine induction and cellular recruitment has also been established in this animal model system (Roach et al., 2002). Thus, disrupting the TNF balance leads to tissue damage and necrosis observed in many pathological features of disseminated TB. This TNF balance is critical to maintain as other studies showed that continued presence of TNF has also growth promoting effects on *M.tb* (Byrd, 1997).

LYMPHOTOXIN

Studies done so far have been inconclusive about the unique role played by lymphotoxin in the host immune response to *M.tb*. However, a recent study done using both conventional and neo-free LT-α deficient mice indicates a non-essential role for LT-α in presence of unperturbed TNF expression in host defense to *M.tb* infection (Allie et al., 2010).

IL-12 FAMILY

This family is composed of IL-12p40 (homodimer with p40 + p40 subunits), IL-12 or IL-12p70 (heterodimer with p40 + p35 subunits), IL-23 (heterodimer with p40 + p19 subunits), and IL-27 (heterodimer cytokine with Epstein–Barr virus-induced gene 3 [EBI3 or IL-27B] + p28 [known as IL-30]). IL-12Rβ1/IL-12Rβ2 complex is the receptor for IL-12, IL-12β1/IL-23R complex is the receptor for IL-23 while gp130/IL-27α complex is the receptor for IL-27 (Trinchieri et al., 2003; Hunter, 2005). Experimental models and human population studies have established that IL-12 plays an important role in both innate and adaptive responses against *M.tb* infection. IL-12 is shown to bind to its receptor IL-12R-β2 and activate the JAK-STAT pathway inducing IFN-γ to differentiate CD4^{+} T cells in Th1 effectors (Mendez-Samperio, 2010). Recent studies established a great level of complexity on the IL-12 family cytokines in their involvement in the immunological control of *M.tb* infection (reviewed in detail in Cooper et al., 2007). IL-12 is induced in macrophages and DCs after activation by microbial TLR ligands and other cytokines. Experiments using IL-12 deficient mice show an increase in bacterial burden and a drastic reduction in host survival (Flynn et al., 1995b; Cooper et al., 1997). These studies established IL-12 as a crucial cytokine in the development and maintenance of the type-1 cellular response to *M.tb* infection. IL-23 induces IL-17 production by memory T cells inducing the inflammatory response by Th17 cells (Khader and Cooper, 2008). IL-23 like IL-12 generates protective cellular responses but at lesser degree, however, IL-27 is shown to moderate inflammation by inducing the early Th1 differentiation and generation of IL-10-producing regulatory T cells, which results in limited tissue destruction but subsequently limited bacterial control (Khader and Cooper, 2008). Thus, the IL-12 family is implicated in maintaining the balance between inflammation and bacterial killing to minimize tissue damage. A new role for IL-12p35 has been recently described, where this cytokine induces inflammation by suppressing TGF-β and stimulating NKT cells (Park et al., 2010). Other recent studies also support this new role showing that IL-12p35 alone also

drives neutrophil and CD4+ T cell infiltrations by increasing the levels of CCL2, CXCL2, CXCL3, and the angiogenic factor vascular endothelial growth factor (VEGF; Frank et al., 2010). Recently, it has been shown that the p35 subunit of IL-12 is forming a heterodimer with EBI3 resulting in IL-35. IL-35 is defined as an IL-12 family cytokine that is produced only by regulatory T cells contributing to immune suppression (Collison et al., 2007). The role of IL-35 in *M.tb* infection is still to be determined.

INTERFERON FAMILY

The IFN are a heterogeneous family of cytokines divided in type I and type II on basis of their structure, function, and cell of origin. While type I IFN (IFN-α and IFN-β) is secreted by various cell types and through innate immune receptors, type II (IFN-γ) is mainly produced as a result of stimulation of T lymphocytes and NK cells.

Type I IFN regulates different aspects of the immune response, inducing cell-mediated immunity. Upon infection with *M.tb*, immune cells and receptors induce the production of IFN-α and INF-β promoting the priming of CD8+ and CD4+ T cells (Cho et al., 2002; Remoli et al., 2002). The effect of IFN-α/β may be both favorable and unfavorable to the infected host cell. Studies using exogenous administration of IFN-α/β have shown that increased production of IFN-α/β could contribute to the host susceptibility to *M.tb* infection due to an overwhelming inflammatory response (Manca et al., 2001; Bouchonnet et al., 2002). However, a recent study has demonstrated that *M.tb* inhibits the production of IFN-α/β in response to TLR9 signaling suggesting an evasion mechanism to control the immune response against *M.tb* that is beneficial to the host by controlling an excessive immune response (Simmons et al., 2010).

Type II IFN-γ released by AMs, CD4 T cells, CD8 T cells, NKT cells, γδ T cells, and NK cells (Boehm et al., 1997; Fenton et al., 1997; Wang et al., 1999; Vesosky et al., 2004) is considered as the key cytokine involved in the control of *M.tb* (Flynn and Chan, 2001; Cooper, 2009). IFN-γ activates macrophages enhancing their production of pro-inflammatory cytokines, and up-regulating their surface expression of cytokine/chemokine receptors, costimulatory and adhesion molecules, and MHC-I and II molecules enhancing macrophage antigen presentation to T cells (Boehm et al., 1997). IFN-γ also controls cellular immunity by regulating T cell-mediated apoptosis (Li et al., 2007) as well as accumulation of Th$_{17}$ cells (Sher and Coffman, 1992), thus protecting the host from a massive T cell influx to the infection site. IFN-γ is defined as a correlate for host resistance to *M.tb* infection. In this scenario, antigen-specific IFN-γ is low in patients with clinical cases of active TB, when compared to latent TB (Zhang et al., 1995; Lin et al., 1996). It has also been reported that individuals defective for IFN-γ or IFN-γ receptors (IFNGR) present increased susceptibility to infection with severe disease manifestations (de Jong et al., 1998), suggesting that insufficient IFN-γ is associated with TB disease progression. Two independent studies using a sub-lethal infection with *M.tb* showed that IFN-γ deficient mice failed to produce nitric oxide due to inadequate *iNOS* expression, and exhibited unrestricted bacterial growth and tissue necrosis with mice succumbing to disease faster (Cooper et al., 1993; Flynn et al., 1993). These studies prove the essentiality of IFN-γ

in protective cellular immunity to TB infection, where cessation of bacterial growth directly correlates with the release of IFN-γ (Cooper et al., 1993).

OTHER PRO-INFLAMMATORY CYTOKINES

Other cytokines like IL-1, IL-6, IL-17, and IL-32 have been shown to be implicated to different degrees in *M.tb* infection control. IL-1β is a pro-inflammatory cytokine produced by monocytes, macrophages, DCs, B cells, and NK cells (Dinarello, 2009).

Interleukin-1β up-regulates essential mediators necessary for the control *M.tb* infection including *iNOS* and subsequent NO production (Chan et al., 2001), phagosomal acidification and maturation (Horsburgh Jr., 1991), adhesion molecules (Dinarello, 2009), and enzymatic activities like phospholipase A$_2$ and cyclooxygenase (Hernandez-Pando et al., 2006; Yang et al., 2009). Human studies (Gomez et al., 2006; Hawn et al., 2006; Settas et al., 2007) and *in vivo* studies using IL-1Ra (receptor antagonist) and IL-1α/IL-1β deficient mice (Juffermans et al., 2000; Yamada et al., 2000; Fremond et al., 2007) established that the continuous effect of IL-1 is required for maintaining resistance to *M.tb* infection. IL-6 is mainly considered to be a pro-inflammatory cytokine involved in macrophage and cytotoxic T cell differentiation. Infection of IL-6 deficient mice with *M.tb* indicate that IL-6 is important for the control of *M.tb* infection, however, in humans it is considered a correlate for disease progression due to its role in inflammation and tissue damage (Appelberg, 1994; Ladel et al., 1997; Casarini et al., 1999; Tsao et al., 1999; Ilonidis et al., 2006).

Interleukin-17 is produced abundantly by Th17 CD4 T cells during early stages of *M.tb* infection (Lockhart et al., 2006). Studies using IL-17 deficient mice demonstrate that IL-17 is not essential to control *M.tb* infection, however, this cytokine plays a role in granuloma formation (Umemura et al., 2007). In this context, IL-17 has been implicated in the recruitment of Th$_1$ cells (Happel et al., 2005; Khader et al., 2005; Wozniak et al., 2006), which produce antigen-specific IFN-γ, and inhibit *M.tb* growth (Khader et al., 2007). Human studies show that IL-17 is produced by TB patients and IL-17 is also induced by BCG vaccination, however, its protective role against *M.tb* infection in human remains to be elucidated.

Interleukin-32 is a cell-associated proinflammatory cytokine, which is specifically stimulated by mycobacteria through a caspase-1- and IL-18-dependent production of IFNγ (Netea et al., 2006).

IMMUNOMODULATORY CYTOKINES
INTERLEUKIN-10

Cell types producing IL-10 include monocytes, macrophages, DCs, regulatory CD4 T cells, and CD8 T cells (Sabat, 2010). The role of IL-10 is complicated to decipher as its regulatory properties depend on the cell type and infection model studied. For *M.tb* infection, IL-10 is thought to limit inflammation. In this scenario, IL-10 inhibits pro-inflammatory cytokines like IL-1, IL-6, IL-12, IL-18, and IFN-γ (Bogdan et al., 1991; de Waal Malefyt et al., 1991, 1993; Fiorentino et al., 1991; D'Andrea et al., 1993; Gruber et al., 1994; Aste-Amezaga et al., 1998); and chemokines like CCL3, CCL4, and CCL5 affecting cell recruitment, as well as blocking the generation of ROIs and NOIs (Bogdan et al., 1991;

Cunha et al., 1992; Niiro et al., 1992; Cenci et al., 1993; Kuga et al., 1996; Roilides et al., 1997) that are important for *M.tb* control. In this process IL-10 is thought to work by interfering with intracellular signaling cascades such as suppressor of cytokine signaling-3 (SOCS3; Cassatella et al., 1999) and NFκB (Wang et al., 1995; Romano et al., 1996; Schottelius et al., 1999; Berrebi et al., 2003). IL-10 is also implicated in blocking antigen processing and presentation in different APCs, therefore diminishing T cell responses (Moore et al., 2001). Experimental data using the mouse model show that IL-10 alone plays a pivotal role during the chronic/latent stage of pulmonary TB potentially contributing to TB reactivation (Turner et al., 2002; Beamer et al., 2008). In this context, induction of IL-10 may be considered as preventing tissue damage during chronic *M.tb* infection. Studies using transgenic mice support this funding where induction of IL-10 is considered to prevent tissue damage but also contributes to *M.tb* growth (Murray et al., 1997). Studies using IL-10 deficient mice show no effect (North, 1998) or a protective effect when IL-10 is absent, which is also supported by studies blocking the action of IL-10 receptor (Beamer et al., 2008; Redford et al., 2010). These murine studies are supported by recent human population studies where presence of IL-10 correlates with susceptibility to mycobacterial infections (Boussiotis et al., 2000; de la Barrera et al., 2004). Recent *in vitro* reports suggest that IL-10 may interfere with *M.tb* persistence by stalling phagosome maturation in human macrophages (O'Leary et al., 2010). Thus, the benefits of IL-10 are controversial, as at the same time that IL-10 seems to limit tissue damage by suppressing inflammation, IL-10 also contributes to the host environment that allows *M.tb* to persist, and thus IL-10 directly contributes to reactivation of TB.

TRANSFORMING GROWTH FACTOR β

Transforming growth factor β is defined as a pluripotent cytokine that modulates the immune response by down-regulating acquired immunity and de-activating macrophages. TGF-β is secreted by a large number of cell types including monocytes, macrophages, DCs, and CD4+ regulatory T cells (Aung et al., 2005; Latchumanan et al., 2005). TGF-β is shown to synergize with IL-10 to promote immune tolerance and limit pathological inflammation (Oswald et al., 1992; Zeller et al., 1999; Clegg and Hughes, 2002; Chen et al., 2003). Similar to IL-10, TGF-β also suppresses APCs costimulatory molecules (Strobl and Knapp, 1999), NOS production, and indirectly down-regulates T cell function and proliferation by inhibiting cytokine production (Lee et al., 1997; Nakao et al., 1997; Bright and Sriram, 1998; Gorham et al., 1998). Studies have shown up-regulation of TGF-β in monocytes and macrophages in granuloma from patients with active TB (Toossi et al., 1995), and high levels of TGF-β is directly associated with severe stages of the disease. As TGF-β is considered an anti-inflammatory cytokine that helps to reduce the harmful inflammatory effects associated with T cell immunity to *M.tb* infection, regulating TGF-β effects may be critical to control *M.tb* infection. In this scenario, using TGF-β blockers like latency associated protein (Saharinen and Keski-Oja, 2000) and decorin (Hildebrand et al., 1994), which form inactive complexes with TGF-β should reduce TGF-β anti-inflammatory effects on T cells and macrophages, and may be a way to control inflammation and prevent *M.tb* infection spread.

Overall, the local response to *M.tb* infection is defined by an initial intense pro-inflammatory response followed by the production of anti-inflammatory mediators that serve to regulate tissue damage. Within this local environment *M.tb* battles to survive by interfering with the host inflammatory networks. It remains unclear whether the neutralization of anti-inflammatory mediators will benefit the host in contrast to the current goals of boosting Th1 mediated protective immunity.

CONCLUSION REMARKS

The interplay between *M.tb* and the host inflammatory response depends on many factors. From the bacterial side, *M.tb* strains differ in the cell wall components exposed on their surface. Virulent laboratory strains $H_{37}R_v$ and Erdman are defined to be hypermannosylated on their surface; however, clinical isolates, some of them related to cases of hypervirulence and clinical outbreaks (i.e., HN878), present a more hydrophobic cell wall surface (Torrelles and Schlesinger, 2010). In this context, hypervirulent strains are characterized by the presence of other surface-exposed cell wall components (i.e., phenolic glycolipid, triacylglycerols), which regulate the cytokine response, and demonstrate rapid intracellular growth and marked tissue damage. These hypervirulent strains are associated with an unusual high proportion of active cases of disease and a high frequency of extrapulmonary disease. This has been directly attributed to host immune subversion. In contrast, *M.tb* strain CDC1551 is related to a low number of cases of active disease, followed by an unusually high rate of seroconversion, inducing a more rigorous immunologic response (reviewed in Torrelles and Schlesinger, 2010). Even within the same *M.tb* strain, it is likely not the case that all bacilli are identical replicas, and thus bacilli may differentially interact with the host. Thus, at an *M.tb* infection site we may find a mixture of events, where simplistically speaking, depending on its cell wall surface phenotype an *M.tb* bacillus may interact with a host cell receptor triggering pro-inflammation that promotes bacterial killing, or may interact with a host cell receptor triggering anti-inflammation and bacterial intracellular survival. From the host (man) perspective, genetic predisposition and environmental living conditions dictate the predilection for an *M.tb* infection. Even in the context of the same host (man) and within the same host cell population, differences between host cells in receptor expression, signaling and innate and adaptive immune functions exist (and we can extend these differences to the different model systems used to study *M.tb* infection *in vitro* and *in vivo*). Moreover, within the same host cell population (i.e., AMs), there are likely to be different host cell subpopulation(s) that differentially trigger the inflammatory response (i.e., AMs expressing more TLR2 vs. AMs expressing more MR). With these questions in mind, is the establishment of a successful *M.tb* infection just a question of chance or are we looking at the evolutionarily developed perfect storm of elements? In this review we have presented the major players involved in the tight regulation of the inflammatory response during *M.tb* infection. Some favor infection and others do not. However, upon the successful establishment of an *M.tb* infection, the ultimate goal of the host is to reduce inflammation and tissue destruction, and in this scenario, *M.tb* has learned to survive. Efforts to define the *M.tb* cell wall components produced *in vivo* as well as

the properties of the *M.tb* host cell reservoir *in vivo* will establish the optimal *M.tb* and host cell phenotype/genotype combination that allows for the successful establishment of an *M.tb* infection *in vivo*. In this scenario, the understudied microenvironments that *M.tb* encounters during infection may be key elements to consider in future *M.tb* pathogenesis studies.

REFERENCES

Algood, H. M., Chan, J., and Flynn, J. L. (2003). Chemokines and tuberculosis. *Cytokine Growth Factor Rev.* 14, 467–477.

Algood, H. M., Lin, P. L., Yankura, D., Jones, A., Chan, J., and Flynn, J. L. (2004). TNF influences chemokine expression of macrophages in vitro and that of CD11b+ cells in vivo during *Mycobacterium tuberculosis* infection. *J. Immunol.* 172, 6846–6857.

Allie, N., Keeton, R., Court, N., Abel, B., Fick, L., Vasseur, V., Vacher, R., Olleros, M. L., Drutskaya, M. S., Guler, R., Nedospasov, S. A., Garcia, I., Ryffel, B., Quesniaux, V. F., and Jacobs, M. (2010). Limited role for lymphotoxin alpha in the host immune response to *Mycobacterium tuberculosis*. *J. Immunol.* 185, 4292–4301.

Appelberg, R. (1994). Protective role of interferon gamma, tumor necrosis factor alpha and interleukin-6 in *Mycobacterium tuberculosis* and *M. avium* infections. *Immunobiology* 191, 520–525.

Appelmelk, B. J., den Dunnen, J., Driessen, N. N., Ummels, R., Pak, M., Nigou, J., Larrouy-Maumus, G., Gurcha, S. S., Movahedzadeh, F., Geurtsen, J., Brown, E. J., Eysink Smeets, M. M., Besra, G. S., Willemsen, P. T., Lowary, T. L., van, K. Y., Maaskant, J. J., Stoker, N. G., van der, L. P., Puzo, G., Vandenbroucke-Grauls, C. M., Wieland, C. W., Van Der, P. T., Geijtenbeek, T. B., van der Sar, A. M., and Bitter, W. (2008). The mannose cap of mycobacterial lipoarabinomannan does not dominate the *Mycobacterium*–host interaction. *Cell. Microbiol.* 10, 930–944.

Ashida, N., Arai, H., Yamasaki, M., and Kita, T. (2001). Distinct signaling pathways for MCP-1-dependent integrin activation and chemotaxis. *J. Biol. Chem.* 276, 16555–16560.

Astarie-Dequeker, C., N'Diaye, E. N., Le Cabec, V., Rittig, M. G., Prandi, J., and Maridonneau-Parini, I. (1999). The mannose receptor mediates uptake of pathogenic and nonpathogenic mycobacteria and bypasses bactericidal responses in human macrophages. *Infect. Immun.* 67, 469–477.

Aste-Amezaga, M., Ma, X., Sartori, A., and Trinchieri, G. (1998). Molecular mechanisms of the induction of IL-12 and its inhibition by IL-10. *J. Immunol.* 160, 5936–5944.

Aung, H., Wu, M., Johnson, J. L., Hirsch, C. S., and Toossi, Z. (2005). Bioactivation of latent transforming growth factor beta1 by *Mycobacterium tuberculosis* in human mononuclear phagocytes. *Scand. J. Immunol.* 61, 558–565.

Austin, C. M., Ma, X., and Graviss, E. A. (2008). Common nonsynonymous polymorphisms in the NOD2 gene are associated with resistance or susceptibility to tuberculosis disease in African Americans. *J. Infect. Dis.* 197, 1713–1716.

Barnes, P. F., Chatterjee, D., Abrams, J. S., Lu, S., Wang, E., Yamamura, M., Brennan, P. J., and Modlin, R. L. (1992). Cytokine production induced by *Mycobacterium tuberculosis* lipoarabinomannan. Relationship to chemical structure. *J. Immunol.* 149, 541–547.

Beamer, G. L., Flaherty, D. K., Assogba, B. D., Stromberg, P., Gonzalez-Juarrero, M., de Waal, M. R., Vesosky, B., and Turner, J. (2008). Interleukin-10 promotes *Mycobacterium tuberculosis* disease progression in CBA/J mice. *J. Immunol.* 181, 5545–5550.

Bean, A. G. D., Roach, D. R., Briscoe, H., France, M. P., Korner, H., Sedgwick, J. D., and Britton, W. J. (1999). Structural deficiencies in granuloma formation in TNF gene-targeted mice underlie the heightened susceptibility to aerosol *Mycobacterium tuberculosis* infection, which is not compensated for by lymphotoxin. *J. Immunol.* 162, 3504–3511.

Beharka, A. A., Gaynor, C. D., Kang, B. K., Voelker, D. R., McCormack, F. X., and Schlesinger, L. S. (2002). Pulmonary surfactant protein A up-regulates activity of the mannose receptor, a pattern recognition receptor expressed on human macrophages. *J. Immunol.* 169, 3565–3573.

Behr, M. A. (2004). Tuberculosis due to multiple strains: a concern for the patient? A concern for tuberculosis control? *Am. J. Respir. Crit. Care Med.* 169, 554–555.

Berrebi, D., Bruscoli, S., Cohen, N., Foussat, A., Migliorati, G., Bouchet-Delbos, L., Maillot, M. C., Portier, A., Couderc, J., Galanaud, P., Peuchmaur, M., Riccardi, C., and Emilie, D. (2003). Synthesis of glucocorticoid-induced leucine zipper (GILZ) by macrophages: an anti-inflammatory and immunosuppressive mechanism shared by glucocorticoids and IL-10. *Blood* 101, 729–738.

Boehm, U., Klamp, T., Groot, M., and Howard, J. C. (1997). Cellular responses to interferon-gamma. *Annu. Rev. Immunol.* 15, 749–795.

Bogdan, C., Vodovotz, Y., and Nathan, C. (1991). Macrophage deactivation by interleukin 10. *J. Exp. Med.* 174, 1549–1555.

Bouchonnet, F., Boechat, N., Bonay, M., and Hance, A. J. (2002). Alpha/beta interferon impairs the ability of human macrophages to control growth of *Mycobacterium bovis* BCG. *Infect. Immun.* 70, 3020–3025.

Boussiotis, V. A., Tsai, E. Y., Yunis, E. J., Thim, S., Delgado, J. C., Dascher, C. C., Berezovskaya, A., Rousset, D., Reynes, J. M., and Goldfeld, A. E. (2000). IL-10-producing T cells suppress immune responses in anergic tuberculosis patients. *J. Clin. Invest.* 105, 1317–1325.

Bright, J. J., and Sriram, S. (1998). TGF-beta inhibits IL-12-induced activation of Jak-STAT pathway in T lymphocytes. *J. Immunol.* 161, 1772–1777.

Brooks, M. N., Rajaram, M. V., Azad, A. K., Amer, A. O., Valdivia-Arenas, M. A., Park, J. H., Nuñez, G., and Schlesinger, L. S. (2010). NOD2 controls the nature of the inflammatory response and subsequent fate of *Mycobacterium tuberculosis* and *M. bovis* BCG in human macrophages. *Cell. Microbiol.* doi: 10.1111/j.1462-5822.2010.01544.x. [Epub ahead of print].

Bulut, Y., Michelsen, K. S., Hayrapetian, L., Naiki, Y., Spallek, R., Singh, M., and Arditi, M. (2005). *Mycobacterium tuberculosis* heat shock proteins use diverse Toll-like receptor pathways to activate pro-inflammatory signals. *J. Biol. Chem.* 280, 20961–20967.

Byrd, T. F. (1997). Tumor necrosis factor alpha (TNFalpha) promotes growth of virulent *Mycobacterium tuberculosis* in human monocytes: iron-mediated growth suppression is correlated with decreased release of TNF-alpha from iron-treated infected monocytes. *J. Clin. Invest.* 99, 2518–2529.

Capuano, S. V. III, Croix, D. A., Pawar, S., Zinovik, A., Myers, A., Lin, P. L., Bissel, S., Fuhrman, C., Klein, E., and Flynn, J. L. (2003). Experimental *Mycobacterium tuberculosis* infection of cynomolgus macaques closely resembles the various manifestations of human *M. tuberculosis* infection. *Infect. Immun.* 71, 5831–5844.

Carlson, T. K., Torrelles, J. B., Smith, K., Horlacher, T., Castelli, R., Seeberger, P. H., Crouch, E. C., and Schlesinger, L. S. (2009). Critical role of amino acid position 343 of surfactant protein-D in the selective binding of glycolipids from *Mycobacterium tuberculosis*. *Glycobiology* 19, 1473–1484.

Casarini, M., Ameglio, F., Alemanno, L., Zangrilli, P., Mattia, P., Paone, G., Bisetti, A., and Giosue, S. (1999). Cytokine levels correlate with a radiologic score in active pulmonary tuberculosis. *Am. J. Respir. Crit. Care Med.* 159, 143–148.

Cassatella, M. A., Gasperini, S., Bovolenta, C., Calzetti, F., Vollebregt, M., Scapini, P., Marchi, M., Suzuki, R., Suzuki, A., and Yoshimura, A. (1999). Interleukin-10 (IL-10) selectively enhances CIS3/SOCS3 mRNA expression in human neutrophils: evidence for an IL-10-induced pathway that is independent of STAT protein activation. *Blood* 94, 2880–2889.

Cenci, E., Romani, L., Mencacci, A., Spaccapelo, R., Schiaffella, E., Puccetti, P., and Bistoni, F. (1993). Interleukin-4 and interleukin-10 inhibit nitric oxide-dependent macrophage killing of *Candida albicans*. *Eur. J. Immunol.* 23, 1034–1038.

Chackerian, A., Alt, J., Perera, V., and Behar, S. M. (2002). Activation of NKT cells protects mice from tuberculosis. *Infect. Immun.* 70, 6302–6309.

Chan, E. D., Chan, J., and Schluger, N. W. (2001). What is the role of nitric oxide in murine and human host defense against tuberculosis? Current knowledge. *Am. J. Respir. Cell Mol. Biol.* 25, 606–612.

Chen, Z. M., O'Shaughnessy, M. J., Gramaglia, I., Panoskaltsis-Mortari, A., Murphy, W. J., Narula, S., Roncarolo, M. G., and Blazar, B. R. (2003). IL-10 and TGF-beta induce alloreactive CD4+ CD25− T cells to acquire regulatory cell function. *Blood* 101, 5076–5083.

Chieppa, M., Bianchi, G., Doni, A., Del Prete, A., Sironi, M., Laskarin, G., Monti, P., Piemonti, L., Biondi, A., Mantovani, A., Introna, M., and Allavena, P. (2003). Cross-linking of the mannose receptor on monocyte-derived dendritic cells activates an anti-inflammatory immunosup-

pressive program. *J. Immunol.* 171, 4552–4560.

Cho, H. J., Hayashi, T., Datta, S. K., Takabayashi, K., Van Uden, J. H., Horner, A., Corr, M., and Raz, E. (2002). IFN-alpha beta promote priming of antigen-specific CD8+ and CD4+ T lymphocytes by immunostimulatory DNA-based vaccines. *J. Immunol.* 168, 4907–4913.

Clegg, S., and Hughes, K. T. (2002). FimZ is a molecular link between sticking and swimming in *Salmonella enterica* serovar Typhimurium. *J. Bacteriol.* 184, 1209–1213.

Co, D. O., Hogan, L. H., Kim, S. I., and Sandor, M. (2004). Mycobacterial granulomas: keys to a long-lasting host–pathogen relationship. *Clin. Immunol.* 113, 130–136.

Collison, L. W., Workman, C. J., Kuo, T. T., Boyd, K., Wang, Y., Vignali, K. M., Cross, R., Sehy, D., Blumberg, R. S., and Vignali, D. A. (2007). The inhibitory cytokine IL-35 contributes to regulatory T-cell function. *Nature* 450, 566–569.

Cooper, A. M. (2009). Cell-mediated immune responses in tuberculosis. *Annu. Rev. Immunol.* 27, 393–422.

Cooper, A. M., Dalton, D. K., Stewart, T. A., Griffin, J. P., Russell, D. G., and Orme, I. M. (1993). Disseminated tuberculosis in interferon gamma gene-disrupted mice. *J. Exp. Med.* 178, 2243–2247.

Cooper, A. M., Magram, J., Ferrante, J., and Orme, I. M. (1997). Interleukin 12 (IL-12) is crucial to the development of protective immunity in mice intravenously infected with *Mycobacterium tuberculosis. J. Exp. Med.* 186, 39–45.

Cooper, A. M., Roberts, A. D., Rhoades, E. R., Callahan, J. E., Getzy, D. M., and Orme, I. M. (1995). The role of interleukin-12 in acquired immunity to *Mycobacterium tuberculosis* infection. *Immunology* 84, 423–432.

Cooper, A. M., Solache, A., and Khader, S. A. (2007). Interleukin-12 and tuberculosis: an old story revisited. *Curr. Opin. Immunol.* 19, 441–447.

Cunha, F. Q., Moncada, S., and Liew, F. Y. (1992). Interleukin 10 (IL-10) inhibits the induction of nitric oxide synthase by interferon-gamma in murine macrophages. *Biochem. Biophys. Res. Commun.* 182, 1155–1159.

D'Andrea, A., Aste-Amezaga, M., Valiante, N. M., Ma, X., Kubin, M., and Trinchieri, G. (1993). Interleukin 10 (IL-10) inhibits human lymphocyte interferon gamma-production by suppressing natural killer cell stimulatory factor/IL-12 synthesis in accessory cells. *J. Exp. Med.* 178, 1041–1048.

Daley, C. L. (2010). "Tuberculosis latency in humans," in *Tuberculosis*, eds W. N. Rom and S. M. Garay (Philadelphia:

Lippincott Williams & Wilkins), 85–99.

de Jong, R., Altare, F., Haagen, I. A., Elferink, D. G., Boer, T., van Breda, V., Kabel, P. J., Draaisma, J. M., Van Dissel, J. T., Kroon, F. P., Casanova, J. L., and Ottenhoff, T. H. (1998). Severe mycobacterial and *Salmonella* infections in interleukin-12 receptor-deficient patients. *Science* 280, 1435–1438.

de la Barrera, S., Aleman, M., Musella, R., Schierloh, P., Pasquinelli, V., Garcia, V., Abbate, E., and Sasiain, M. C. (2004). IL-10 down-regulates costimulatory molecules on *Mycobacterium tuberculosis*-pulsed macrophages and impairs the lytic activity of CD4 and CD8 CTL in tuberculosis patients. *Clin. Exp. Immunol.* 138, 128–138.

de Waal Malefyt, R., Abrams, J., Bennett, B., Figdor, C. G., and De Vries, J. E. (1991). Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. *J. Exp. Med.* 174, 1209–1220.

de Waal Malefyt, R., Figdor, C. G., Huijbens, R., Mohan-Peterson, S., Bennett, B., Culpepper, J., Dang, W., Zurawski, G., and De Vries, J. E. (1993). Effects of IL-13 on phenotype, cytokine production, and cytotoxic function of human monocytes: comparison with IL-4 and modulation by IFN-gamma or IL-10. *J. Immunol.* 151, 6370–6381.

Dheda, K., Booth, H., Huggett, J. F., Johnson, M. A., Zumla, A., and Rook, G. A. (2005). Lung remodeling in pulmonary tuberculosis. *J. Infect. Dis.* 192, 1201–1209.

Di, L. D., Caccamo, N., Meraviglia, S., Guggino, G., La Manna, M. P., Sireci, G., Salerno, A., and Dieli, F. (2009). Tuning inflammation in tuberculosis: the role of decoy receptors. *Microbes Infect.* 11, 821–827.

Di, L. D., Locati, M., Caccamo, N., Vecchi, A., Meraviglia, S., Salerno, A., Sireci, G., Nebuloni, M., Caceres, N., Cardona, P. J., Dieli, F., and Mantovani, A. (2008). Role of the chemokine decoy receptor D6 in balancing inflammation, immune activation, and antimicrobial resistance in *Mycobacterium tuberculosis* infection. *J. Exp. Med.* 205, 2075–2084.

Dinarello, C. A. (2009). Immunological and inflammatory functions of the interleukin-1 family. *Annu. Rev. Immunol.* 27, 519–550.

Divangahi, M., Desjardins, D., Nunes-Alves, C., Remold, H. G., and Behar, S. M. (2010). Eicosanoid pathways regulate adaptive immunity to *Mycobacterium tuberculosis. Nat. Immunol.* 11, 751–758.

Divangahi, M., Mostowy, S., Coulombe, F., Kozak, R., Guillot, L., Veyrier, F., Kobayashi, K. S., Flavell, R. A., Gros, P., and Behr, M. A. (2008). NOD2-

deficient mice have impaired resistance to *Mycobacterium tuberculosis* infection through defective innate and adaptive immunity. *J. Immunol.* 181, 7157–7165.

Dommett, R. M., Klein, N., and Turner, M. W. (2006). Mannose-binding lectin in innate immunity: past, present and future. *Tissue Antigens* 68, 193–209.

Drennan, M. B., Nicolle, D., Quesniaux, V. J., Jacobs, M., Allie, N., Mpagi, J., Fremond, C., Wagner, H., Kirschning, C., and Ryffel, B. (2004). Toll-like receptor 2-deficient mice succumb to *Mycobacterium tuberculosis* infection. *Am. J. Pathol.* 164, 49–57.

Ehlers, S. (2009). DC-SIGN and mannosylated surface structures of *Mycobacterium tuberculosis*: a deceptive liaison. *Eur. J. Cell Biol.* 89, 95–101.

Feng, C. G., Kullberg, M. C., Jankovic, D., Cheever, A. W., Caspar, P., Coffman, R. L., and Sher, A. (2002). Transgenic mice expressing human interleukin-10 in the antigen-presenting cell compartment show increased susceptibility to infection with *Mycobacterium avium* associated with decreased macrophage effector function and apoptosis. *Infect. Immun.* 70, 6672–6679.

Fenton, M. J., Riley, L. W., and Schlesinger, L. S. (2005). "Receptor-mediated recognition of *Mycobacterium tuberculosis* by host cells," in *Tuberculosis and the Tubercle Bacillus*, eds S. T. Cole, K. D. Eisenach, D. N. McMurray, and W. R. Jacobs Jr. (New York: ASM Press), 405–426.

Fenton, M. J., Vermeulen, M. W., Kim, S., Burdick, M., Strieter, R. M., and Kornfeld, H. (1997). Induction of gamma interferon production in human alveolar macrophages by *Mycobacterium tuberculosis. Infect. Immun.* 65, 5149–5156.

Ferguson, J. S., Martin, J. L., Azad, A. K., McCarthy, T. R., Kang, P. B., Voelker, D. R., Crouch, E. C., and Schlesinger, L. S. (2006). Surfactant protein D increases fusion of *Mycobacterium tuberculosis*-containing phagosomes with lysosomes in human macrophages. *Infect. Immun.* 74, 7005–7009.

Ferguson, J. S., Voelker, D. R., McCormack, F. X., and Schlesinger, L. S. (1999). Surfactant protein D binds to *Mycobacterium tuberculosis* bacilli and lipoarabinomannan via carbohydrate-lectin interactions resulting in reduced phagocytosis of the bacteria by macrophages. *J. Immunol.* 163, 312–321.

Ferguson, J. S., Weis, J. J., Martin, J. L., and Schlesinger, L. S. (2004). Complement protein C3 binding to *Mycobacterium tuberculosis* is initiated by the classical pathway in human bronchoalveolar lavage fluid. *Infect. Immun.* 72, 2564–2573.

Ferwerda, G., Girardin, S. E., Kullberg, B. J., Le Bourhis, L., de Jong, D. J., Langenberg, D. M., van Crevel, R., Adema, G. J., Ottenhoff, T. H., Van der Meer, J. W., and Netea, M. G. (2005). NOD2 and toll-like receptors are nonredundant recognition systems of *Mycobacterium tuberculosis. PLoS Pathog.* 1, 279–285. doi: 10.1371/journal.ppat.0010034

Fieschi, C., and Casanova, J. L. (2003). The role of interleukin-12 in human infectious diseases: only a faint signature. *Eur. J. Immunol.* 33, 1461–1464.

Fiorentino, D. F., Zlotnik, A., Mosmann, T. R., Howard, M., and O'Garra, A. (1991). IL-10 inhibits cytokine production by activated macrophages. *J. Immunol.* 147, 3815–3822.

Flores-Villanueva, P. O., Ruiz-Morales, J. A., Song, C. H., Flores, L. M., Jo, E. K., Montano, M., Barnes, P. F., Selman, M., and Granados, J. (2005). A functional promoter polymorphism in monocyte chemoattractant protein-1 is associated with increased susceptibility to pulmonary tuberculosis. *J. Exp. Med.* 202, 1649–1658.

Flynn, J. L., and Chan, J. (2001). Immunology of tuberculosis. *Annu. Rev. Immunol.* 19, 93–129.

Flynn, J. L., and Chan, J. (2005). What's good for the host is good for the bug. *Trends Microbiol.* 13, 98–102.

Flynn, J. L., Chan, J., Triebold, K. J., Dalton, D. K., Stewart, T. A., and Bloom, B. R. (1993). An essential role for interferon gamma in resistance to *Mycobacterium tuberculosis* infection. *J. Exp. Med.* 178, 2249–2254.

Flynn, J. L., Goldstein, M. M., Chan, J., Triebold, K. J., Pfeffer, K., Lowenstein, C. J., Schreiber, R., Mak, T. W., and Bloom, B. R. (1995a). Tumor necrosis factor-alpha is required in the protective immune response against *Mycobacterium tuberculosis* in mice. *Immunity* 2, 561–572.

Flynn, J. L., Goldstein, M. M., Triebold, K. J., Sypek, J., Wolf, S., and Bloom, B. R. (1995b). IL-12 increases resistance of BALB/c mice to *Mycobacterium tuberculosis* infection. *J. Immunol.* 155, 2515–2524.

Franchi, L., Park, J. H., Shaw, M. H., Marina-Garcia, N., Chen, G., Kim, Y. G., and Nunez, G. (2008). Intracellular NOD-like receptors in innate immunity, infection and disease. *Cell. Microbiol.* 10, 1–8.

Frank, G. M., Divito, S. J., Maker, D. M., Xu, M., and Hendricks, R. L. (2010). A novel p40-independent function of IL-12p35 is required for progression and maintenance of herpes stromal keratitis. *Invest. Ophthalmol. Vis. Sci.* 51, 3591–3598.

Fremond, C. M., Nicolle, D. M., Torres, D. S., and Quesniaux, V. F. (2003).

Control of *Mycobacterium bovis* BCG infection with increased inflammation in TLR4-deficient mice. *Microbes Infect.* 5, 1070–1081.

Fremond, C. M., Togbe, D., Doz, E., Rose, S., Vasseur, V., Maillet, I., Jacobs, M., Ryffel, B., and Quesniaux, V. F. (2007). IL-1 receptor-mediated signal is an essential component of MyD88-dependent innate response to *Mycobacterium tuberculosis* infection. *J. Immunol.* 179, 1178–1189.

Gandotra, S., Jang, S., Murray, P. J., Salgame, P., and Ehrt, S. (2007). Nucleotide-binding oligomerization domain protein 2-deficient mice control infection with *Mycobacterium tuberculosis*. *Infect. Immun.* 75, 5127–5134.

Geijtenbeek, T. B., Torensma, R., Van Vliet, S. J., van Duijnhoven, G. C., Adema, G. J., Van Kooyk, Y., and Figdor, C. G. (2000). Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses. *Cell* 100, 575–585.

Geijtenbeek, T. B., Van Vliet, S. J., Koppel, E. A., Sanchez-Hernandez, M., Vandenbroucke-Grauls, C. M., Appelmelk, B., and Van Kooyk, Y. (2003). Mycobacteria target DC-SIGN to suppress dendritic cell function. *J. Exp. Med.* 197, 7–17.

Geldmacher, C., Ngwenyama, N., Schuetz, A., Petrovas, C., Reither, K., Heeregrave, E. J., Casazza, J. P., Ambrozak, D. R., Louder, M., Ampofo, W., Pollakis, G., Hill, B., Sanga, E., Saathoff, E., Maboko, L., Roederer, M., Paxton, W. A., Hoelscher, M., and Koup, R. A. (2010). Preferential infection and depletion of *Mycobacterium tuberculosis*-specific CD4 T cells after HIV-1 infection. *J. Exp. Med.* 207, 2869–2881.

Geurtsen, J., Chedammi, S., Mesters, J., Cot, M., Driessen, N. N., Sambou, T., Kakutani, R., Ummels, R., Maaskant, J., Takata, H., Baba, O., Terashima, T., Bovin, N., Vandenbroucke-Grauls, C. M., Nigou, J., Puzo, G., Lemassu, A., Daffe, M., and Appelmelk, B. J. (2009). Identification of mycobacterial alpha-glucan as a novel ligand for DC-SIGN: involvement of mycobacterial capsular polysaccharides in host immune modulation. *J. Immunol.* 183, 5221–5231.

Gomez, L. M., Camargo, J. F., Castiblanco, J., Ruiz-Narvaez, E. A., Cadena, J., and Anaya, J. M. (2006). Analysis of IL1B, TAP1, TAP2 and IKBL polymorphisms on susceptibility to tuberculosis. *Tissue Antigens* 67, 290–296.

Gorham, J. D., Guler, M. L., Fenoglio, D., Gubler, U., and Murphy, K. M. (1998). Low dose TGF-beta attenuates IL-12 responsiveness in murine Th cells. *J. Immunol.* 161, 1664–1670.

Gruber, M. F., Williams, C. C., and Gerrard, T. L. (1994). Macrophage-colony-stimulating factor expression by anti-CD45 stimulated human monocytes is transcriptionally up-regulated by IL-1β and inhibited by IL-4 and IL-10. *J. Immunol.* 152, 1354–1361.

Gutierrez, O., Pipaon, C., Inohara, N., Fontalba, A., Ogura, Y., Prosper, F., Nunez, G., and Fernandez-Luna, J. L. (2002). Induction of Nod2 in myelomonocytic and intestinal epithelial cells via nuclear factor-kappa B activation. *J. Biol. Chem.* 277, 41701–41705.

Happel, K. I., Lockhart, E. A., Mason, C. M., Porretta, E., Keoshkerian, E., Odden, A. R., Nelson, S., and Ramsay, A. J. (2005). Pulmonary interleukin-23 gene delivery increases local T-cell immunity and controls growth of *Mycobacterium tuberculosis* in the lungs. *Infect. Immun.* 73, 5782–5788.

Harding, C. V., and Boom, W. H. (2010). Regulation of antigen presentation by *Mycobacterium tuberculosis*: a role for Toll-like receptors. *Nat. Rev. Microbiol.* 8, 296–307.

Hawn, T. R., Dunstan, S. J., Thwaites, G. E., Simmons, C. P., Thuong, N. T., Lan, N. T., Quy, H. T., Chau, T. T., Hieu, N. T., Rodrigues, S., Janer, M., Zhao, L. P., Hien, T. T., Farrar, J. J., and Aderem, A. (2006). A polymorphism in Toll-interleukin 1 receptor domain containing adaptor protein is associated with susceptibility to meningeal tuberculosis. *J. Infect. Dis.* 194, 1127–1134.

Henning, L. N., Azad, A. K., Parsa, K. V., Crowther, J. E., Tridandapani, S., and Schlesinger, L. S. (2008). Pulmonary surfactant protein A regulates TLR expression and activity in human macrophages. *J. Immunol.* 180, 7847–7858.

Hernandez-Pando, R., Orozco-Esteves, H., Maldonado, H. A., Guilar-Leon, D., Vilchis-Landeros, M. M., Mata-Espinosa, D. A., Mendoza, V., and Lopez-Casillas, F. (2006). A combination of a transforming growth factor-beta antagonist and an inhibitor of cyclooxygenase is an effective treatment for murine pulmonary tuberculosis. *Clin. Exp. Immunol.* 144, 264–272.

Hildebrand, A., Romaris, M., Rasmussen, L. M., Heinegard, D., Twardzik, D. R., Border, W. A. and Ruoslahti, E. (1994). Interaction of the small interstitial proteoglycans biglycan, decorin and fibromodulin with transforming growth factor beta. *Biochem. J.* 302(Pt 2), 527–534.

Hirsch, C. S., Ellner, J. J., Russell, D. G., and Rich, E. A. (1994). Complement receptor-mediated uptake and tumor necrosis factor-alpha-mediated growth inhibition of *Mycobacterium tuberculosis* by human alveolar macrophages. *J. Immunol.* 152, 743–753.

Holscher, C., Holscher, A., Ruckerl, D., Yoshimoto, T., Yoshida, H., Mak, T., Saris, C., and Ehlers, S. (2005). The IL-27 receptor chain WSX-1 differentially regulates antibacterial immunity and survival during experimental tuberculosis. *J. Immunol.* 174, 3534–3544.

Horsburgh, C. R. Jr. (1991). *Mycobacterium avium* complex infection in the acquired immunodeficiency syndrome. *N. Engl. J. Med.* 324, 1332–1338.

Hu, C., Mayadas-Norton, T., Tanaka, K., Chan, J., and Salgame, P. (2000). *Mycobacterium tuberculosis* infection in complement receptor 3-deficient mice. *J. Immunol.* 165, 2596–2602.

Hunter, C. A. (2005). New IL-12-family members: IL-23 and IL-27, cytokines with divergent functions. *Nat. Rev. Immunol.* 5, 521–531.

Ilonidis, G., Parapanisiou, E., Anogeianaki, A., Giavazis, I., Theofilogiannakos, E. K., Tsekoura, P., Kidonopoulou, K., Trakatelli, C., Polimenidis, Z., Conti, P., and Anogianakis, G. (2006). Interleukin-1beta (IL-1 beta), interleukin 6 (IL-6) and tumor necrosis factor (TNF) in plasma and pleural fluid of pneumonia, lung cancer and tuberculous pleuritis. *J. Biol. Regul. Homeost. Agents* 20, 41–46.

Inohara, N., and Nunez, G. (2003). NODs: intracellular proteins involved in inflammation and apoptosis. *Nat. Rev. Immunol.* 3, 371–382.

Ishikawa, E., Ishikawa, T., Morita, Y. S., Toyonaga, K., Yamada, H., Takeuchi, O., Kinoshita, T., Akira, S., Yoshikai, Y., and Yamasaki, S. (2009). Direct recognition of the mycobacterial glycolipid, trehalose dimycolate, by C-type lectin Mincle. *J. Exp. Med.* 206, 2879–2888.

Jacobs, M., Brown, N., Allie, N., and Ryffel, B. (2000). Fatal *Mycobacterium bovis* BCG infection in TNF-LT-alpha-deficient mice. *Clin. Immunol.* 94, 192–199.

Jo, E. K., Yang, C. S., Choi, C. H., and Harding, C. V. (2007). Intracellular signalling cascades regulating innate immune responses to mycobacteria: branching out from Toll-like receptors. *Cell. Microbiol.* 9, 1087–1098.

Jones, B. W., Heldwein, K. A., Means, T. K., Saukkonen, J. J., and Fenton, M. J. (2001). Differential roles of Toll-like receptors in the elicitation of proinflammatory responses by macrophages. *Ann. Rheum. Dis.* 60(Suppl. 3), iii6–iii12.

Juffermans, N. P., Florquin, S., Camoglio, L., Verbon, A., Kolk, A. H., Speelman, P., van Deventer, S. J., and Van Der, P. T. (2000). Interleukin-1 signaling is essential for host defense during murine pulmonary tuberculosis. *J. Infect. Dis.* 182, 902–908.

Juffermans, N. P., Verbon, A., van Deventer, S. J., van, D. H., Belisle, J. T., Ellis, M. E., Speelman, P., and Van Der, P. T. (1999). Elevated chemokine concentrations in sera of human immunodeficiency virus (HIV)-seropositive and HIV-seronegative patients with tuberculosis: a possible role for mycobacterial lipoarabinomannan. *Infect. Immun.* 67, 4295–4297.

Kaneko, H., Yamada, H., Mizuno, S., Udagawa, T., Kazumi, Y., Sekikawa, K., and Sugawara, I. (1999). Role of tumor necrosis factor-alpha in *Mycobacterium*-induced granuloma formation in tumor necrosis factor-alpha-deficient mice. *Lab. Invest.* 79, 379–386.

Kang, P. B., Azad, A. K., Torrelles, J. B., Kaufman, T. M., Beharka, A., Tibesar, E., Desjardin, L. E., and Schlesinger, L. S. (2005). The human macrophage mannose receptor directs *Mycobacterium tuberculosis* ipoarabinomannan-mediated phagosome biogenesis. *J. Exp. Med.* 202, 987–999.

Kaplan, G., Post, F. A., Moreira, A. L., Wainwright, H., Kreiswirth, B. N., Tanverdi, M., Mathema, B., Ramaswamy, S. V., Walther, G., Steyn, L. M., Barry, C. E., III, and Bekker, L. G. (2003). *Mycobacterium tuberculosis* growth at the cavity surface: a microenvironment with failed immunity. *Infect. Immun.* 71, 7099–7108.

Kawai, T., and Akira, S. (2010). The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nat. Immunol.* 11, 373–384.

Keane, J., Gershon, S., Wise, R. P., Mirabile-Levens, E., Kasznica, J., Schwieterman, W. D., Siegel, J. N., and Braun, M. M. (2001). Tuberculosis associated with infliximab, a tumor necrosis factor alpha-neutralizing agent. *N. Engl. J. Med.* 345, 1098–1104.

Khader, S. A., Bell, G. K., Pearl, J. E., Fountain, J. J., Rangel-Moreno, J., Cilley, G. E., Shen, F., Eaton, S. M., Gaffen, S. L., Swain, S. L., Locksley, R. M., Haynes, L., Randall, T. D., and Cooper, A. M. (2007). IL-23 and IL-17 in the establishment of protective pulmonary CD4+ T cell responses after vaccination and during *Mycobacterium tuberculosis* challenge. *Nat. Immunol.* 8, 369–377.

Khader, S. A., and Cooper, A. M. (2008). IL-23 and IL-17 in tuberculosis. *Cytokine* 41, 79–83.

Khader, S. A., Pearl, J. E., Sakamoto, K., Gilmartin, L., Bell, G. K., Jelley-Gibbs, D. M., Ghilardi, N., deSauvage, F., and Cooper, A. M. (2005). IL-23 compensates for the absence of IL-12p70 and is essential for the IL-17 response during tuberculosis but is dispensable for protection and antigen-specific IFN-gamma responses if IL-12p70 is available. *J. Immunol.* 175, 788–795.

Khanna, K. V., Choi, C. S., Gekker, G., Peterson, P. K., and Molitor, T. W. (1996). Differential infection of porcine alveolar macrophage subpopulations by nonopsonized *Mycobacterium bovis* involves CD14 receptors. *J. Leukoc. Biol.* 60, 214–220.

Klug, K., Ehlers, S., Uhlig, S., and Reiling, N. (2010). Mitogen-activated protein kinases p38 and ERK1/2 regulated control of *Mycobacterium avium* replication in primary murine macrophages is independent of tumor necrosis factor-alpha and interleukin-10. *Innate Immun.* doi: 10.1177/1753425910377799 [Epub ahead of print].

Kuga, S., Otsuka, T., Niiro, H., Nunoi, H., Nemoto, Y., Nakano, T., Ogo, T., Umei, T., and Niho, Y (1996). Suppression of superoxide anion production by interleukin-10 is accompanied by a down-regulation of the genes for subunit proteins of NADPH oxidase. *Exp. Hematol.* 24, 151–157.

Kurashima, K., Mukaida, N., Fujimura, M., Yasui, M., Nakazumi, Y., Matsuda, T., and Matsushima, K. (1997). Elevated chemokine levels in bronchoalveolar lavage fluid of tuberculosis patients. *Am. J. Respir. Crit. Care Med.* 155, 1474–1477.

Ladel, C. H., Blum, C., Dreher, A., Reifenberg, K., Kopf, M., and Kaufmann, S. H. (1997). Lethal tuberculosis in interleukin-6-deficient mutant mice. *Infect. Immun.* 65, 4843–4849.

Latchumanan, V. K., Balkhi, M. Y., Sinha, A., Singh, B., Sharma, P., and Natarajan, K. (2005). Regulation of immune responses to *Mycobacterium tuberculosis* secretory antigens by dendritic cells. *Tuberculosis (Edinb.)* 85, 377–383.

Lee, Y. J., Han, Y., Lu, H. T., Nguyen, V., Qin, H., Howe, P. H., Hocevar, B. A., Boss, J. M., Ransohoff, R. M., and Benveniste, E. N. (1997). TGF-beta suppresses IFN-gamma induction of class II MHC gene expression by inhibiting class II transactivator messenger RNA expression. *J. Immunol.* 158, 2065–2075.

Li, X., McKinstry, K. K., Swain, S. L., and Dalton, D. K. (2007). IFN-gamma acts directly on activated CD4+ T cells during mycobacterial infection to promote apoptosis by inducing components of the intracellular apoptosis machinery and by inducing extracellular proapoptotic signals. *J. Immunol.* 179, 939–949.

Lin, P. L., Myers, A., Smith, L., Bigbee, C., Bigbee, M., Fuhrman, C., Grieser, H., Chiosea, I., Voitenek, N. N., Capuano, S. V., Klein, E., and Flynn, J. L. (2010). Tumor necrosis factor neutralization results in disseminated disease in acute and latent *Mycobacterium tuberculosis* infection with normal granuloma structure in a cynomolgus macaque model. *Arthritis Rheum.* 62, 340–350.

Lin, Y. G., Zhang, M., Hofman, F. M., Gong, J. H., and Barnes, P. F. (1996). Absence of a prominent Th2 cytokine response in human tuberculosis. *Infect. Immun.* 64, 1351–1356.

Lockhart, E., Green, A. M., and Flynn, J. L. (2006). IL-17 production is dominated by gammadelta T cells rather than CD4 T cells during *Mycobacterium tuberculosis* infection. *J. Immunol.* 177, 4662–4669.

Manca, C., Tsenova, L., Bergtold, A., Freeman, S., Tovey, M., Musser, J. M., Barry, C. E., III, Freedman, V. H., and Kaplan, G. (2001). Virulence of a *Mycobacterium tuberculosis* clinical isolate in mice is determined by failure to induce Th1 type immunity and is associated with induction of IFN-α/B. *Proc. Natl. Acad. Sci. U.S.A.* 98, 5752–5757.

Martinez de la, T. Y., Locati, M., Buracchi, C., Dupor, J., Cook, D. N., Bonecchi, R., Nebuloni, M., Rukavina, D., Vago, L., Vecchi, A., Lira, S. A., and Mantovani, A. (2005). Increased inflammation in mice deficient for the chemokine decoy receptor D6. *Eur. J. Immunol.* 35, 1342–1346.

Martinez-Pomares, L., Linehan, S. A., Taylor, P. R., and Gordon, S. (2001). Binding properties of the mannose receptor. *Immunobiology* 204, 527–535.

McElvania, T. E., Allen, I. C., Hulseberg, P. D., Sullivan, J. T., McCann, J. R., Sandor, M., Braunstein, M., and Ting, J. P. (2010). Granuloma formation and host defense in chronic *Mycobacterium tuberculosis* infection requires PYCARD/ASC but not NLRP3 or caspase-1. *PLoS ONE* 5, e12320. doi: 10.1371/journal.pone.0012320

McGreal, E. P., Miller, J. L., and Gordon, S. (2005). Ligand recognition by antigen-presenting cell C-type lectin receptors. *Curr. Opin. Immunol.* 17, 18–24.

Mendez-Samperio, P. (2008). Expression and regulation of chemokines in mycobacterial infection. *J. Infect.* 57, 374–384.

Mendez-Samperio, P. (2010). Role of interleukin-12 family cytokines in the cellular response to mycobacterial disease. *Int. J. Infect. Dis.* 14, e366–e371.

Mendez-Samperio, P., Vazquez, A., and Ayala, H. (2003). Infection of human monocytes with *Mycobacterium bovis* BCG induces production of CC-chemokines. *J. Infect.* 47, 139–147.

Moore, K. W., de Waal, M. R., Coffman, R. L., and O'Garra, A. (2001). Interleukin-10 and the interleukin-10 receptor. *Annu. Rev. Immunol.* 19, 683–765.

Murray, P. J., Wang, L., Onufryk, C., Tepper, R. I., and Young, R. A. (1997). T cell-derived IL-10 antagonizes macrophage function in mycobacterial infection. *J. Immunol.* 158, 315–321.

Nakao, A., Afrakhte, M., Moren, A., Nakayama, T., Christian, J. L., Heuchel, R., Itoh, S., Kawabata, M., Heldin, N. E., Heldin, C. H., and ten, D. P. (1997). Identification of Smad7, a TGF-beta-inducible antagonist of TGF-beta signalling. *Nature* 389, 631–635.

Netea, M. G., Azam, T., Lewis, E. C., Joosten, L. A., Wang, M., Langenberg, D., Meng, X., Chan, E. D., Yoon, D. Y., Ottenhoff, T., Kim, S. H., and Dinarello, C. A. (2006). *Mycobacterium tuberculosis* induces interleukin-32 production through a caspase-1/IL-18/interferon-gamma-dependent mechanism. *PLoS Med.* 3, e277. doi: 10.1371/journal.pmed.0030277

Nibbering, P. H., Pos, O., Stevenhagen, A., and Van Furth, R. (1993). Interleukin-8 enhances nonoxidative intracellular killing of *Mycobacterium fortuitum* by human granulocytes. *Infect. Immun.* 61, 3111–3116.

Nigou, J., Zelle-Rieser, C., Gilleron, M., Thurnher, M., and Puzo, G. (2001). Mannosylated lipoarabinomannans inhibit IL-12 production b human dendritic cells: evidence for a negative signal delivered through the mannose receptor. *J. Immunol.* 166, 7477–7485.

Niiro, H., Otsuka, T., Abe, M., Satoh, H., Ogo, T., Nakano, T., Furukawa, Y., and Niho, Y. (1992). Epstein–Barr virus BCRF1 gene product (viral interleukin 10) inhibits superoxide anion production by human monocytes. *Lymphokine Cytokine Res.* 11, 209–214.

North, R. J. (1998). Mice incapable of making IL-4 or IL-10 display normal resistance to infection with *Mycobacterium tuberculosis*. *Clin. Exp. Immunol.* 113, 55–58.

Ogura, Y., Inohara, N., Benito, A., Chen, F. F., Yamaoka, S., and Nunez, G. (2001). Nod2, a Nod1/Apaf-1 family member that is restricted to monocytes and activates NF-kappaB. *J. Biol. Chem.* 276, 4812–4818.

O'Leary, S., O'Sullivan, M. P., and Keane, J. (2010). IL-10 blocks phagosome maturation in *Mycobacterium tuberculosis*-infected human macrophages. *Am. J. Respir. Cell Mol. Biol.* doi: 10.1165/rcmb.2010-0319OC. [Epub ahead of print].

Oppmann, B., Lesley, R., Blom, B., Timans, J. C., Xu, Y., Hunte, B., Vega, F., Yu, N., Wang, J., Singh, K., Zonin, F., Vaisberg, E., Churakova, T., Liu, M., Gorman, D., Wagner, J., Zurawski, S., Liu, Y., Abrams, J. S., Moore, K. W., Rennick, D., Waal-Malefyt, R., Hannum, C., Bazan, J. F., and Kastelein, R. A. (2000). Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. *Immunity* 13, 715–725.

Oswald, I. P., Gazzinelli, R. T., Sher, A., and James, S. L. (1992). IL-10 synergizes with IL-4 and transforming growth factor-beta to inhibit macrophage cytotoxic activity. *J. Immunol.* 148, 3578–3582.

Owaki, T., Asakawa, M., Morishima, N., Hata, K., Fukai, F., Matsui, M., Mizuguchi, J., and Yoshimoto, T. (2005). A role for IL-27 in early regulation of Th1 differentiation. *J. Immunol.* 175, 2191–2200.

Pace, E., Gjomarkaj, M., Melis, M., Profita, M., Spatafora, M., Vignola, A. M., Bonsignore, G., and Mody, C. H. (1999). Interleukin-8 induces lymphocyte chemotaxis into the pleural space. Role of pleural macrophages. *Am. J. Respir. Crit. Care Med.* 159, 1592–1599.

Park, C. G., Takahara, K., Umemoto, E., Yashima, Y., Matsubara, K., Matsuda, Y., Clausen, B. E., Inaba, K., and Steinman, R. M. (2001). Five mouse homologues of the human dendritic cell C-type lectin, DC-SIGN. *Int. Immunol.* 13, 1283–1290.

Park, Y., Kim, H. S., Ahn, J. Y., Yun, D., Cho, M. L., Hong, S., Kim, H. Y., and Chung, D. H. (2010). IL-12p35 promotes antibody-induced joint inflammation by activating NKT cells and suppressing TGF-beta. *J. Immunol.* 185, 1476–1484.

Pearl, J. E., Saunders, B., Ehlers, S., Orme, I. M., and Cooper, A. M. (2001). Inflammation and lymphocyte activation during mycobacterial infection in the interferon-gamma-deficient mouse. *Cell. Immunol.* 211, 43–50.

Polentarutti, N., Rol, G. P., Muzio, M., Bosisio, D., Camnasio, M., Riva, F., Zoja, C., Benigni, A., Tomasoni, S., Vecchi, A., Garlanda, C., and Mantovani, A. (2003). Unique pattern of expression and inhibition of IL-1 signaling by the IL-1 receptor family member TIR8/SIGIRR. *Eur. Cytokine Netw.* 14, 211–218.

Powlesland, A. S., Ward, E. M., Sadhu, S. K., Guo, Y., Taylor, M. E., and Drickamer, K. (2006). Widely divergent biochemical properties of the complete set of mouse DC-SIGN-related proteins. *J. Biol. Chem.* 281, 20440–20449.

Quesniaux, V. J., Nicolle, D. M., Torres, D., Kremer, L., Guerardel, Y., Nigou, J., Puzo, G., Erard, F., and Ryffel, B. (2004). Toll-like receptor 2 (TLR2)-dependent-positive and TLR2-independent-negative regulation of proinflammatory cytokines by myco-

bacterial lipomannans. *J. Immunol.* 172, 4425–4434.

Rajaram, M. V., Brooks, M. N., Morris, J. D., Torrelles, J. B., Azad, A. K., and Schlesinger, L. S. (2010). *Mycobacterium tuberculosis* activates human macrophage peroxisome proliferator-activated receptor gamma linking mannose receptor recognition to regulation of immune responses. *J. Immunol.* 185, 929–942.

Redford, P. S., Boonstra, A., Read, S., Pitt, J., Graham, C., Stavropoulos, E., Bancroft, G. J., and O'Garra, A. (2010). Enhanced protection to *Mycobacterium tuberculosis* infection in IL-10-deficient mice is accompanied by early and enhanced Th1 responses in the lung. *Eur. J. Immunol.* 40, 2200–2210.

Remoli, M. E., Giacomini, E., Lutfalla, G., Dondi, E., Orefici, G., Battistini, A., Uzé, G., Pellegrini, S., and Coccia, E. M. (2002). Selective expression of type I IFN genes in human dendritic cells infected with *Mycobacterium tuberculosis*. *J. Immunol.* 169, 366–374.

Remus, N., Reichenbach, J., Picard, C., Rietschel, C., Wood, P., Lammas, D., Kumararatne, D. S., and Casanova, J. L. (2001). Impaired interferon gamma-mediated immunity and susceptibility to mycobacterial infection in childhood. *Pediatr. Res.* 50, 8–13.

Roach, D. R., Bean, A. G., Demangel, C., France, M. P., Briscoe, H., and Britton, W. J. (2002). TNF regulates chemokine induction essential for cell recruitment, granuloma formation, and clearance of mycobacterial infection. *J. Immunol.* 168, 4620–4627.

Roach, S. K., and Schorey, J. S. (2002). Differential regulation of the mitogen-activated protein kinases by pathogenic and nonpathogenic mycobacteria. *Infect. Immun.* 70, 3040–3052.

Roilides, E., Dimitriadou, A., Kadiltsoglou, I., Sein, T., Karpouzas, J., Pizzo, P. A., and Walsh, T. J. (1997). IL-10 exerts suppressive and enhancing effects on antifungal activity of mononuclear phagocytes against *Aspergillus fumigatus*. *J. Immunol.* 158, 322–329.

Romano, M. F., Lamberti, A., Petrella, A., Bisogni, R., Tassone, P. F., Formisano, S., Venuta, S., and Turco, M. C. (1996). IL-10 inhibits nuclear factor-kappa B/Rel nuclear activity in CD3-stimulated human peripheral T lymphocytes. *J. Immunol.* 156, 2119–2123.

Russell, D. G. (2007). Who puts the tubercle in tuberculosis? *Nat. Rev. Microbiol.* 5, 39–47.

Sabat, R. (2010). IL-10 family of cytokines. *Cytokine Growth Factor Rev.* 21, 315–324.

Saharinen, J., and Keski-Oja, J. (2000). Specific sequence motif of 8-Cys repeats of TGF-beta binding proteins, LTBPs, creates a hydrophobic interaction surface for binding of small latent TGF-beta. *Mol. Biol. Cell* 11, 2691–2704.

Samten, B., Townsend, J. C., Sever-Chroneos, Z., Pasquinelli, V., Barnes, P. F., and Chroneos, Z. C. (2008). An antibody against the surfactant protein A (SP-A)-binding domain of the SP-A receptor inhibits T cell-mediated immune responses to *Mycobacterium tuberculosis*. *J. Leukoc. Biol.* 84, 115–123.

Saunders, B. M., and Britton, W. J. (2007). Life and death in the granuloma: immunopathology of tuberculosis. *Immunol. Cell Biol.* 85, 103–111.

Saunders, B. M., and Cooper, A. M. (2002). Restraining mycobacteria: Role of granulomas in mycobacterial infections. *Immunol. Cell Biol.* 78, 334–341.

Saunders, B. M., Frank, A. A., and Orme, I. M. (1999). Granuloma formation is required to contain bacillus growth and delay mortality in mice chronically infected with *Mycobacterium tuberculosis*. *Immunology* 98, 324–328.

Schaefer, M., Reiling, N., Fessler, C., Stephani, J., Taniuchi, I., Hatam, F., Yildirim, A. O., Fehrenbach, H., Walter, K., Ruland, J., Wagner, H., Ehlers, S., and Sparwasser, T. (2008). Decreased pathology and prolonged survival of human DC-SIGN transgenic mice during mycobacterial infection. *J. Immunol.* 180, 6836–6845.

Schlesinger, L. S., Azad, A. K., Torrelles, J. B., Roberts, E., Vergne, I., and Deretic, V. (2008). "Determinants of phagocytosis, phagosome biogenesis and autophagy for *Mycobacterium tuberculosis*," in *Handbook of Tuberculosis. Immunology and Cell Biology*, eds S. H. E. Kaufmann and W. J. Britton (Weinheim: Wiley-VCH Verlag GmbH & Co. KGaA), 1–22.

Schlesinger, L. S., Bellinger-Kawahara, C. G., Payne, N. R., and Horwitz, M. A. (1990). Phagocytosis of *Mycobacterium tuberculosis* is mediated by human monocyte complement receptors and complement component C3. *J. Immunol.* 144, 2771–2780.

Schlesinger, L. S., Hull, S. R., and Kaufman, T. M. (1994). Binding of the terminal mannosyl units of lipoarabinomannan from a virulent strain of *Mycobacterium tuberculosis* to human macrophages. *J. Immunol.* 152, 4070–4079.

Schneider, B. E., Korbel, D., Hagens, K., Koch, M., Raupach, B., Enders, J., Kaufmann, S. H., Mittrucker, H. W., and Schaible, U. E. (2010). A role for IL-18 in protective immunity against *Mycobacterium tuberculosis*. *Eur. J. Immunol.* 40, 396–405.

Schoenen, H., Bodendorfer, B., Hitchens, K., Manzanero, S., Werninghaus, K., Nimmerjahn, F., Agger, E. M., Stenger, S., Andersen, P., Ruland, J., Brown, G. D., Wells, C., and Lang, R. (2010). Cutting edge: Mincle is essential for recognition and adjuvanticity of the mycobacterial cord factor and its synthetic analog trehalose-dibehenate. *J. Immunol.* 184, 2756–2760.

Schottelius, A. J., Mayo, M. W., Sartor, R. B., and Baldwin, A. S. Jr. (1999). Interleukin-10 signaling blocks inhibitor of kappaB kinase activity and nuclear factor kappaB DNA binding. *J. Biol. Chem.* 274, 31868–31874.

Seder, R. A., Gazzinelli, R., Sher, A., and Paul, W. E. (1993). Interleukin 12 acts directly on CD4+ T cells to enhance priming for interferon gamma production and diminishes interleukin 4 inhibition of such priming. *Proc. Natl. Acad. Sci. U.S.A.* 90, 10188–10192.

Settas, L. D., Tsimirikas, G., Vosvotekas, G., Triantafyllidou, E., and Nicolaides, P. (2007). Reactivation of pulmonary tuberculosis in a patient with rheumatoid arthritis during treatment with IL-1 receptor antagonists (anakinra). *J. Clin. Rheumatol.* 13, 219–220.

Sher, A., and Coffman, R. L. (1992). Regulation of immunity to parasites by T cells and T cell-derived cytokines. *Annu. Rev. Immunol.* 10, 385–409.

Simmons, D. P., Canaday, D. H., Liu, Y., Li, Q., Huang, A., Boom, W. H., and Harding, C. V. (2010). *Mycobacterium tuberculosis* and TLR2 agonists inhibit induction of type I IFN and class I MHC antigen cross processing by TLR9. *J. Immunol.* 185, 2405–2415.

Sonnenberg, P., Murray, J., Glynn, J. R., Shearer, S., Kambashi, B., and Godfrey-Faussett, P. (2001). HIV-1 and recurrence, relapse, and reinfection of tuberculosis after cure: a cohort study in South African mineworkers. *Lancet* 358, 1687–1693.

Strobl, H., and Knapp, W. (1999). TGF-beta1 regulation of dendritic cells. *Microbes Infect.* 1, 1283–1290.

Sugawara, I., Yamada, H., Hua, S., and Mizuno, S. (2001). Role of interleukin (IL)-1 type 1 receptor in mycobacterial infection. *Microbiol. Immunol.* 45, 743–750.

Sugawara, I., Yamada, H., Kaneko, H., Mizuno, S., Takeda, K., and Akira, S. (1999). Role of interleukin-18 (IL-18) in mycobacterial infection in IL-18-gene-disrupted mice. *Infect. Immun.* 67, 2585–2589.

Tanne, A., Ma, B., Boudou, F., Tailleux, L., Botella, H., Badell, E., Levillain, F., Taylor, M. E., Drickamer, K., Nigou, J., Dobos, K. M., Puzo, G., Vestweber, D., Wild, M. K., Marcinko, M., Sobieszczuk, P., Stewart, L., Lebus, D., Gicquel, B., and Neyrolles, O. (2009). A murine DC-SIGN homologue contributes to early host defense against *Mycobacterium tuberculosis*. *J. Exp. Med.* 206, 2205–2220.

Thoma-Uszynski, S., Stenger, S., Takeuchi, O., Ochoa, M., Engele, M., Sieling, P., Barnes, P., Rollinghoff, M., Bolcskei, P., Wagner, M., Akira, S., Norgard, M., Belisle, J., Godowski, P., Bloom, B., and Modlin, R. (2001). Induction of direct antimicrobial activity through mammalian Toll-like receptors. *Science* 291, 1544–1547.

Toossi, Z., Gogate, P., Shiratsuchi, H., Young, T., and Ellner, J. J. (1995). Enhanced production of TGF-β by blood monocytes from patients with active tuberculosis and presence of TGF-β in tuberculous granulomatous lung lesions. *J. Immunol.* 154, 465–473.

Torrelles, J. B., Azad, A. K., Henning, L. N., Carlson, T. K., and Schlesinger, L. S. (2008a). Role of C-type lectins in mycobacterial infections. *Curr. Drug Targets* 9, 102–112.

Torrelles, J. B., Azad, A. K., and Schlesinger, L. S. (2006). Fine discrimination in the recognition of individual species of phosphatidyl-myo-inositol mannosides from *Mycobacterium tuberculosis* by C-type lectin pattern recognition receptors. *J. Immunol.* 177, 1805–1816.

Torrelles, J. B., Knaup, R., Kolareth, A., Slepushkina, T., Kaufman, T. M., Kang, P. B., Hill, P., Brennan, P. J., Chatterjee, D., Belisle, J. T., Musser, J. M., and Schlesinger, L. S. (2008b). Identification of *Mycobacterium tuberculosis* clinical isolates with altered phagocytosis by human macrophages due to a truncated lipoarabinomannan. *J. Biol. Chem.* 283, 31417–31428.

Torrelles, J. B., and Schlesinger, L. S. (2010). Diversity in *Mycobacterium tuberculosis* mannosylated cell wall determinants impacts adaptation to the host. *Tuberculosis* 90, 84–93.

Trinchieri, G., Pflanz, S., and Kastelein, R. A. (2003). The IL-12 family of heterodimeric cytokines: new players in the regulation of T cell responses. *Immunity* 19, 641–644.

Tsai, M. C., Chakravarty, S., Zhu, G., Xu, J., Tanaka, K., Koch, C., Tufariello, J., Flynn, J., and Chan, J. (2006). Characterization of the tuberculous granuloma in murine and human lungs: cellular composition and relative tissue oxygen tension. *Cell Microbiol.* 8, 218–232.

Tsao, T. C., Hong, J., Huang, C., Yang, P., Liao, S. K., and Chang, K. S. (1999). Increased TNF-alpha, IL-1 beta and IL-6 levels in the bronchoalveolar lavage fluid with the upregulation of their mRNA in macrophages lavaged from patients with active pulmonary

tuberculosis. *Tuber. Lung Dis.* 79, 279–285.

Turner, J. (2011). "Growing old and immunity to bacteria," in *The Immune Response to Infection*, eds S. H. E. Kaufmann, B. T. Rouse, and D. L. Sacks (Washington, DC: ASM Press), 413–423.

Turner, J., Frank, A. A., Brooks, J. V., Gonzalez-Juarrero, M., and Orme, I. M. (2001). The progression of chronic tuberculosis in the mouse does not require the participation of B lymphocytes or interleukin-4. *Exp. Gerontol.* 36, 537–545.

Turner, J., Gonzalez-Juarrero, M., Ellis, D. L., Basaraba, R. J., Kipnis, A., Orme, I. M., and Cooper, A. M. (2002). In vivo IL-10 production reactivates chronic pulmonary tuberculosis in C57BL/6 mice. *J. Immunol.* 169, 6343–6351.

Ulrichs, T., and Kaufmann, S. H. (2006). New insights into the function of granulomas in human tuberculosis. *J. Pathol.* 208, 261–269.

Ulrichs, T., Kosmiadi, G. A., Jorg, S., Pradl, L., Titukhina, M., Mishenko, V., Gushina, N., and Kaufmann, S. H. (2005). Differential organization of the local immune response in patients with active cavitary tuberculosis or with nonprogressive tuberculoma. *J. Infect. Dis.* 192, 89–97.

Ulrichs, T., Kosmiadi, G. A., Trusov, V., Jorg, S., Pradl, L., Titukhina, M., Mishenko, V., Gushina, N., and Kaufmann, S. H. (2004). Human tuberculous granulomas induce peripheral lymphoid follicle-like structures to orchestrate local host defence in the lung. *J. Pathol.* 204, 217–228.

Umemura, M., Yahagi, A., Hamada, S., Begum, M. D., Watanabe, H., Kawakami, K., Suda, T., Sudo, K., Nakae, S., Iwakura, Y., and Matsuzaki, G. (2007). IL-17-mediated regulation of innate and acquired immune response against pulmonary *Mycobacterium bovis* bacille Calmette-Guerin infection. *J. Immunol.* 178, 3786–3796.

Underhill, D. M., Ozinsky, A., Smith, K. D., and Aderem, A. (1999). Toll-like receptor-2 mediates mycobacteria-induced proinflammatory signaling in macrophages. *Proc. Natl. Acad. Sci. U.S.A.* 96, 14459–14463.

Vesosky, B., Rottinghaus, E. K., Stromberg, P., Turner, J., and Beamer, G. (2010). CCL5 participates in early protection against *Mycobacterium tuberculosis. J. Leukoc. Biol.* 87, 1153–1165.

Vesosky, B., Turner, O. C., Turner, J., and Orme, I. M. (2004). Gamma interferon production by bovine gamma delta T cells following stimulation with mycobacterial mycolyl-arabinogalactan-peptidoglycan. *Infect. Immun.* 72, 4612–4618.

Villeneuve, C., Gilleron, M., Maridonneau-Parini, I., Daffe, M., Astarie-Dequeker, C., and Etienne, G. (2005). Mycobacteria use their surface-exposed glycolipids to infect human macrophages through a receptor-dependent process. *J. Lipid Res.* 46, 475–483.

Wang, J., Wakeham, J., Harkness, R., and Xing, Z. (1999). Macrophages are a significant source of type 1 cytokines during mycobacterial infection. *J. Clin. Invest.* 103, 1023–1029.

Wang, P., Wu, P., Siegel, M. I., Egan, R. W., and Billah, M. M. (1995). Interleukin (IL)-10 inhibits nuclear factor kappaB (NFkappaB) activation in human monocytes. IL-10 and IL-4 suppress cytokine synthesis by different mechanisms. *J. Biol. Chem.* 270, 9558–9563.

Wieland, C. W., Koppel, E. A., den Dunnen, J., Florquin, S., McKenzie, A. N., van Kooyk, Y., Van Der, P. T., and Geijtenbeek, T. B. (2007). Mice lacking SIGNR1 have stronger T helper 1 responses to *Mycobacterium tuberculosis. Microbes Infect.* 9, 134–141.

Wozniak, T. M., Ryan, A. A., and Britton, W. J. (2006). Interleukin-23 restores immunity to *Mycobacterium tuberculosis* infection in IL-12p40-deficient mice

and is not required for the development of IL-17-secreting T cell responses. *J. Immunol.* 177, 8684–8692.

Wright, J. R. (2005). Immunoregulatory functions of surfactant proteins. *Nat. Rev. Immunol.* 5, 58–68.

Yadav, M., and Schorey, J. S. (2006). The β-glucan receptor Dectin-1 functions together with TLR2 to mediate macrophage activation by mycobacteria. *Blood* 108, 3168–3175.

Yamada, H., Mizumo, S., Horai, R., Iwakura, Y., and Sugawara, I. (2000). Protective role of interleukin-1 in mycobacterial infection in IL-1 alpha/beta double-knockout mice. *Lab. Invest.* 80, 759–767.

Yamasaki, S., Ishikawa, E., Sakuma, M., Hara, H., Ogata, K., and Saito, T. (2008). Mincle is an ITAM-coupled activating receptor that senses damaged cells. *Nat. Immunol.* 9, 1179–1188.

Yang, C. S., Yuk, J. M., Shin, D. M., Kang, J., Lee, S. J., and Jo, E. K. (2009). Secretory phospholipase A2 plays an essential role in microglial inflammatory responses to *Mycobacterium tuberculosis. Glia* 57, 1091–1103.

Zaffran, Y., Zhang, L., and Ellner, J. J. (1998). Role of CR4 in *Mycobacterium tuberculosis*-human macrophages binding and signal transduction in the absence of serum. *Infect. Immun.* 66, 4541–4544.

Zeller, J. C., Panoskaltsis-Mortari, A., Murphy, W. J., Ruscetti, F. W., Narula, S., Roncarolo, M. G., and Blazar, B. R. (1999). Induction of CD4+ T cell alloantigen-specific hyporesponsiveness by IL-10 and TGF-beta. *J. Immunol.* 163, 3684–3691.

Zhang, F. R., Huang, W., Chen, S. M., Sun, L. D., Liu, H., Li, Y., Cui, Y., Yan, X. X., Yang, H. T., Yang, R. D., Chu, T. S., Zhang, C., Zhang, L., Han, J. W., Yu, G. Q., Quan, C., Yu, Y. X., Zhang, Z., Shi, B. Q., Zhang, L. H., Cheng, H., Wang, C. Y., Lin, Y., Zheng, H. F., Fu, X. A., Zuo, X. B., Wang, Q., Long, H., Sun, Y. P., Cheng, Y. L., Tian, H. Q., Zhou, F. S., Liu, H. X., Lu,

W. S., He, S. M., Du, W. L., Shen, M., Jin, Q. Y., Wang, Y., Low, H. Q., Erwin, T., Yang, N. H., Li, J. Y., Zhao, X., Jiao, Y. L., Mao, L. G., Yin, G., Jiang, Z. X., Wang, X. D., Yu, J. P., Hu, Z. H., Gong, C. H., Liu, Y. Q., Liu, R. Y., Wang, D. M., Wei, D., Liu, J. X., Cao, W. K., Cao, H. Z., Li, Y. P., Yan, W. G., Wei, S. Y., Wang, K. J., Hibberd, M. L., Yang, S., Zhang, X. J., and Liu, J. J. (2009). Genomewide association study of leprosy. *N. Engl. J. Med.* 361, 2609–2618.

Zhang, M., Lin, Y., Iyer, D. V., Gong, J., Abrams, J. S., and Barnes, P. F. (1995). T-cell cytokine responses in human infection with *Mycobacterium tuberculosis. Infect. Immun.* 63, 3231–3234.

Zimmerli, S., Edwards, S., and Ernst, J. D. (1996). Selective receptor blockade during phagocytosis does not alter the survival and growth of *Mycobacterium tuberculosis* in human macrophages. *Am. J. Respir. Cell Mol. Biol.* 15, 760–770.

Francisella recognition by inflammasomes: differences between mice and men

Mikhail A. Gavrilin* and Mark D. Wewers*

Division of Pulmonary, Allergy, Critical Care and Sleep Medicine, Davis Heart and Lung Research Institute, The Center for Microbial Interface Biology, The Ohio State University, Columbus, OH, USA

Pathogen recognition by intracellular sensors involves the assembly of a caspase-1 activation machine termed the inflammasome. Intracellular pathogens like *Francisella* that gain access to the cytosolic detection systems are useful tools to uncover the details of caspase-1 activation events. This review overviews *Francisella* function in the mononuclear phagocyte with particular attention to inflammasome versus pyroptosome roles and outlines differences between mouse and human caspase-1 activation pathways. Specific attention is placed on functional differences between human and murine pyrin as an intracellular recognition molecule for *Francisella*.

Keywords: AIM2, *Francisella*, IL-1β, inflammasome, M-CSF, monocytes, pyrin, pyroptosome

OVERVIEW

Understanding the innate host response to infectious challenges is a process greatly accelerated by the discovery of intracellular pathogen sensors and the inflammasome concept, reviewed extensively in this special edition. Much can be learned by focusing on the details of individual pathogen types, which can refine our understanding of innate host functions. In this mini-review, we will focus on the detection of *Francisella* and how this particular organism has allowed a clearer understanding of intracellular pathogen recognition. We will also briefly review *Francisella* infection and the peculiarities regarding detection in mouse and man.

Francisella tularensis is a zoonotic agent circulating in the Northern Hemisphere. It is transmitted from host to host by several pathways where arthropod vectors predominate (Ellis et al., 2002; Sjostedt, 2007). *F. tularensis* represents four subspecies differing in geographical location, infectivity, and virulence to the various hosts (Oyston, 2008). The most virulent species is *F. tularensis* subsp. *tularensis* (*F. tularensis*) with median lethal dose <10 CFU for humans, mice, and rabbits. However, *F. tularensis* subsp. *holarctica* (*F. holarctica*) has more broad virulence with calculated LD50 ranging from ~1 CFU for mice, <10^3 CFU for humans, and >10^6 CFU for rabbits. *F. tularensis* subsp. *novicida* (*F. novicida*) while still highly virulent in mice (LD50 < 10^3 CFU), shows low virulence for humans and rabbits (reviewed in Ellis et al., 2002).

The disease caused by *F. tularensis* is called tularemia, otherwise known as rabbit fever (Ellis et al., 2002). The characteristics of tularemia include fever, aches, and signs of toxicity lasting for several days (Parmely et al., 2009). If left untreated, tularemia may result in high mortality, reaching up to 60% with the pneumonic form of the disease (reviewed in Parmely et al., 2009).

Because *Francisella* has a low dose requirement, high virulence, and high morbidity, it is an attractive organism for bacteriological weapons (Sjostedt, 2007). Since *F. tularensis* was included in the list of select agents as a potential bioterrorist weapon (Ellis et al., 2002; Rotz et al., 2002), research into the pathogenicity of this bacteria in host organisms has been greatly accelerated. Large data sets have been generated regarding the specific details of bacteria–host interactions, albeit with some controversy. The controversy may be explained by differences in models used, including *Francisella* subspecies, host organisms, or cell types.

Francisella belongs to a select group of bacteria, including *Listeria, Shigella, Burkholderia,* and *Rickettsia* spp., which proliferate within the host cell cytosol (Ray et al., 2009). *Francisella* evolved to replicate in macrophages, which function to provide the first line of defense against pathogens. To survive in this biological niche, *Francisella* developed a strategy to avoid recognition and destruction by the immune system in order to utilize the host resources and multiply. The employment of this strategy by different *F. tularensis* subspecies dictates the differences in bacterial pathogenicity and evolutionary success.

Accordingly, a delay in recognition and immune system activation should be beneficial. *F. tularensis* (Schu4, type A) is the most virulent subspecies for both mice and humans, suppressing all pro-inflammatory responses for at least 72 h following infection (Kirimanjeswara et al., 2008). Since the Schu4 strain requires a BSL3 facility, more work has been done with the less virulent

strains, *F. holarctica* (LVS, type B) and *F. novicida*. However, these two subspecies differ in their relative virulence for mice versus human – i.e., virulent in mice and attenuated in humans.

Could this difference in virulence be related to distinctions in recognition? Based on differences in virulence between *Francisella* subspecies and host organisms, it is reasonable to suggest that differences in *Francisella* recognition are based upon host response differences. Notably, virulent and attenuated *Francisella* subspecies have escape capacities that drive release from the phagosome in both humans and mice (Golovliov et al., 2003; Mariathasan et al., 2005; Gavrilin et al., 2006). Upon phagosomal escape *Francisella* may be also recognized by intracellular pathogen sensors, many of which belong to the NLR family of proteins. The focus of current review is to highlight *Francisella* recognition by different hosts.

INFLAMMASOME VERSUS PYROPTOSOME

INFLAMMASOME

Francisella recognition induces fever and cell death and is characterized by a wave of pro-inflammatory cytokines, where IL-1β plays a major role. This cytokine is induced upon mononuclear cell stimulation and synthesized as a biologically inactive proIL-1β (Dinarello, 1998). Its conversion to the biologically active 17 kDa form, which includes cleavage and release from cells, is tightly regulated by caspase-1 which, in turn, also requires an activation event (Yamin et al., 1996). Caspase-1 activation depends upon assembly of a multiprotein complex called the inflammasome (Martinon et al., 2002). All protein partners in the inflammasome possess either CARD (caspase recruitment domain) or PYD (pyrin domain) and assemble via CARD–CARD and PYD–PYD interactions (Martinon and Tschopp, 2006; Martinon et al., 2009). The prototypical inflammasome consists of CARD containing caspase-1, CARD, and PYD containing adaptor molecule ASC, and an NLR sensor of pathogen- or danger-associated molecule patterns (PAMPs or DAMPs). Depending on the presence of CARD or PYD domains, these sensors are subdivided as NLRC or NLRP family members, respectively (Ting et al., 2008). Because caspase-1 is the central protein/component of every inflammasome, while ASC is present in the majority of them, inflammasomes are named based on the participating pattern recognition receptor – PRR (NLR or other CARD or PYD containing protein). As such, several inflammasome structures have been described. These include the NLRP1 inflammasome (NLRP1, ASC, caspase-1, and caspase-5; Martinon et al., 2002), the NLRP3 inflammasome (NLRP3, CARD8, ASC, caspase-1; Agostini et al., 2004), the NLRC4 (IPAF) inflammasome (NLRC4, ASC, caspase-1; Mariathasan et al., 2004), and the NLRC5 inflammasome (NLRC5, ASC, caspase-1; Davis et al., 2010). However, it was proposed that several non-NLR proteins may also initiate the assembly of inflammasomes. For example, the RIG-I inflammasome (RIG-I, ASC, caspase-1; Poeck et al., 2010), the AIM2 inflammasome (AIM2, ASC, caspase-1; Burckstummer et al., 2009; Fernandes-Alnemri et al., 2009; Hornung et al., 2009; Roberts et al., 2009), and the pyrin inflammasome (pyrin, ASC, caspase-1; Yu et al., 2006; Gavrilin et al., 2009).

PYROPTOSOME

In addition to the widely accepted inflammasome concept, there is another caspase-1 activation platform, which may be distinct from the inflammasome, called a pyroptosome (Fernandes-Alnemri

et al., 2007). The pyroptosome is a single (one per cell) 1–2 μm supra-molecular speck which contains ASC and caspase-1. This speck is different from the *in vitro* reconstituted inflammasome, which forms a ring-like structure with an outer diameter of ~13 nm and an inner diameter of ~4 nm (Faustin et al., 2007). Pyroptosome assembly is associated with host cell death that occurs within minutes of the ASC speck complex formation (Fernandes-Alnemri et al., 2007). This type of cell death is uniquely dependent on caspase-1 activation and, because of its ability to induce IL-1β dependent fever, named pyroptosis (Fink and Cookson, 2005). Host cell pyroptosis results in ASC pyroptosome aggregation, followed by cell rupture and the accompanied release of IL-1β (Fernandes-Alnemri et al., 2007). However, IL-1β and caspase-1 accumulation in cell culture media is often seen in the absence of cell death following LPS or bacterial infection of mononuclear phagocytes which suggests that inflammasomes may be distinct from pyroptosomes (Mangan et al., 1991; Gavrilin et al., 2009; Santic et al., 2010). In this regard, at least some of the IL-1β and caspase-1 (as well as ASC and some other PRR) are released from live monocytes in the form of microvesicles (MacKenzie et al., 2001; Sarkar et al., 2009). Since the size of exosomal microvesicles is 80–100 nm, these inflammasomes are likely distinct from the micrometer sized pyroptosome. Thus, pyroptosome formation accompanying pyroptosis may be a radically different way to eliminate a cytosolic bacterial burden compared to microvesicular IL-1β and the inflammasome release pathway. How host cell death is regulated by caspase-1 and how it may differ with respect to caspase-1 activation pathways is an area of particular interest for future studies. The summary of the inflammasome versus pyroptosome is shown in **Table 1**.

MURINE RECOGNITION OF INTRACELLULAR *FRANCISELLA*

INTERFERON SIGNALING

It is clear in mice that type I interferon signaling is necessary for inflammasome activation, IL-1β/IL-18 release, and the extensive cell death that follows *Francisella* infection (Henry et al., 2007). Murine macrophages showed 10^3-fold increase of *Ifnb* mRNA 8 h after infection with *F. novicida* (Henry et al., 2007). The 100-fold induction of the *Ifnb* gene was also observed after mouse macrophage infection with the LVS *Francisella* strain, but in contrast to *Il1b* gene expression which peaks at 4 h post-infection, induction of the *Ifnb* gene

Table 1 | Pyroptosome versus inflammasome.

	Pyroptosome	Inflammasome
Localization	Intracellular	Intra and extra-cellular
Size	1000–2000 nm	13 nm
Cell death	+++	+
Inflammation	+	+++
ASC	+	+
PRR	+	+
Caspase-1	+	+
ProIL-1β processing	+	+

Reference Cheng et al. (2010), Cookson and Brennan (2001), Fernandes-Alnemri and Alnemri (2008), Fernandes-Alnemri et al. (2007), Jones et al. (2010)
Faustin et al. (2007), Gavrilin et al. (2009), Kahlenberg and Dubyak (2004), Martinon et al. (2002), Sarkar et al. (2009), Zhou et al. (2010)

was observed much later (starting at 12 h post-infection; Cole et al., 2008). In response to intracellular *Francisella*, mouse macrophages secrete IFN-β, which in an autocrine fashion activates caspase-1 and induces extensive cell death (Henry et al., 2007). In a paracrine fashion, IFN-β negatively regulates the number of IL-17A(+) gamma delta T cells and resistance to infection (Henry et al., 2010). However, while many researchers agree that IFN-β activates the inflammasome, there is a controversy surrounding its effect on the expression of some potential *Francisella*-sensing PRR. In particular, it was reported that Aim2 expression is either induced (Jones et al., 2010) or not affected (Alnemri, 2010; Fernandes-Alnemri et al., 2010) in murine macrophages by IFN-β treatment. Thus, IFN-β's role in inflammasome function requires additional study.

AIM2 AS *FRANCISELLA*-SENSING PRR

Knockout animals provide an extremely powerful tool to evaluate the effects of specific innate molecules on biological processes. In experiments with knockout mice, the importance of the caspase-1/ASC axis activation following *Francisella* escape into the cytosol was clearly shown (Mariathasan et al., 2005). It was also shown that in mice Nlrp1, Nlrp3, and Nlrc4 do not recognize *Francisella* (Weiss et al., 2007; Huang et al., 2010). Recently, several groups showed that absent in melanoma 2 (Aim2), an interferon-inducible protein, is responsible for intracellular *Francisella* detection in murine macrophages (Fernandes-Alnemri et al., 2010; Jones et al., 2010; Rathinam et al., 2010). Aim2 belongs to the HIN-200 (PYHIN) protein family (Ludlow et al., 2005). Mice and humans share 55% amino acid identity of Aim2 (Choubey et al., 2010). Structurally, Aim2 has a HIN-200 amino acid repeat, which can bind double strand DNA, and a PYD domain, which is able to homo-dimerize with other PYD domains (Burckstummer et al., 2009; Choubey et al., 2010). The PYD domain of Aim2 may recruit the PYD of the inflammasome adaptor ASC, as shown by co-localization after dual co-expression (Burckstummer et al., 2009). This co-localization is functional, as over-expression of mouse Aim2 induces ASC-dependent activation of an *NF-κB* reporter gene (Rathinam et al., 2010) and promotes caspase-1 activation (Fernandes-Alnemri et al., 2009). When Aim2 is expressed in the cell, ASC oligomerization is induced by variety of factors, such as double stranded DNA (Burckstummer et al., 2009; Fernandes-Alnemri et al., 2009; Roberts et al., 2009). Thus, Aim2-ASC-caspase-1 may form an Aim2 inflammasome or pyroptosome, whereas bacterial DNA serves as the DAMP which induces this assembly. In support of this conclusion, it was recently shown that *F. novicida* and LVS strains activate murine caspase-1 in an Aim2 dependent manner (Fernandes-Alnemri et al., 2010; Jones et al., 2010; Rathinam et al., 2010). Caspase-1 activation, IL-1β secretion, and cell death were absent in *Aim2*−/− macrophages in response to *F. novicida* infection (Fernandes-Alnemri et al., 2010), while bacterial burden was increased (Jones et al., 2010). It is proposed that bacterial DNA could leak into the cytoplasm during escape from the phagosome as a result of *Francisella* lysis and phagosome acidification (Fernandes-Alnemri et al., 2010). Thus far, there is no consensus as to whether the Aim2-ASC speck represents the inflammasome or the pyroptosome. To avoid confusion, some researchers refer to the speck as an "ASC focus," with the belief that assembly of this focus leads to secretion of mature IL-1β and cell death (Jones et al., 2010).

INTRACELLULAR *FRANCISELLA* RECOGNITION IN HUMAN PHAGOCYTES

INTERFERON SIGNALING AND AIM2 EXPRESSION

Although Aim2 is clearly important in murine responses to *Francisella*, the process of *Francisella* recognition in human mononuclear cells is less clear. In sharp contrast to the mouse macrophages, infection of human monocytes with attenuated *F. novicida* does not lead to the up-regulation of type I interferon signaling (Butchar et al., 2008). However, virulent *F. tularensis* Schu4 induce some *IFNB* gene expression (Butchar et al., 2008). At the same time, *F. novicida* induces significantly higher IL-1β release in human monocytes compared to Schu4 or LVS (Gavrilin et al., 2006; Butchar et al., 2008). Of note, we are unable to find detectable expression of AIM2 in primary human CD14+ monocytes by Western blot (unpublished results). However, it has been shown that human PBMC express detectable amounts of AIM2 (Meixenberger et al., 2010). Human *AIM2* gene is expressed in the spleen, small intestine, and peripheral leukocytes (DeYoung et al., 1997). Because PBMC contain lymphocytes as well as CD14+ monocytes, lymphocyte contributions may explain this difference in AIM2 detection. Also, in agreement with our data are reports that human monocytes do not express AIM2 except after interferon induction (Hornung et al., 2009; Rathinam et al., 2010). However, the inability of *F. novicida* to induce IFN-β in human monocytes (Butchar et al., 2008), may explain the failure to detect human monocyte AIM2. Concomitantly, human monocytes release large amounts of mature IL-1β following infection with *F. novicida,* suggesting that human monocytes may utilize a PRR other than AIM2 for intracellular *Francisella* recognition (Gavrilin et al., 2009). Interestingly, *Francisella*-infected human CD14+ monocytes show high induction of interferon gamma gene (*IFNG*), i.e., type II interferon signaling (Butchar et al., 2008). This induction may represent a defense mechanism by which IFN-γ restricts cytosolic growth of *Francisella* without affecting its phagosomal escape (Edwards et al., 2010). Of note, it was shown that IFN-γ also may induce AIM2 expression in human monocytic cell line THP-1 (Fernandes-Alnemri et al., 2009) as well as pyrin in human CD14+ monocytes (Centola et al., 2000).

PECULIARITIES OF PYRIN FUNCTION IN HUMAN VERSUS MURINE CELLS

Pyrin, the familial Mediterranean fever (FMF) protein, belongs to the tripartite motif (TRIM) family of proteins, all of which share structural homology (Nisole et al., 2005). Although pyrin has an amino terminal PYD, it is not a member of NLR family of intracellular sensors of pathogens. Nonetheless, pyrin has been shown to interact via its PYD with the adaptor protein ASC (Richards et al., 2001). Importantly, the CARD of the ASC molecule can interact directly with caspase-1 via the amino terminal CARD. As a result, these proteins could assemble into an inflammasome. We observed that pyrin, ASC, and caspase-1, tagged to various fluorescent proteins and expressed in HEK 293 cells, co-localize into one speck (unpublished data), which is similar to the AIM2 pyroptosome discussed earlier.

The hypothetical model of pyrin inflammasome with its C-terminal B30.2 domain as a pathogen sensor was first proposed by Alnemri and colleagues (Yu et al., 2006). Importantly, this lab showed that the *Mefv* knockout animals still respond to *F. novicida,* whereas *Aim2*−/− animals do not (Fernandes-Alnemri

et al., 2010). This implies that Aim2, but not pyrin, is responsible for *Francisella*-induced caspase-1 activation and IL-1β release in murine macrophages.

Our work suggests that the human response to *Francisella* may be quite distinct from the murine response. For example, pyrin is an important component of caspase-1 activation and IL-1β release after challenging human mononuclear cells with *F. novicida* (Gavrilin et al., 2009). At first glance, this result is difficult to reconcile with the *Mefv* murine knockout results (Fernandes-Alnemri et al., 2010). However, it is important to highlight an important difference between pyrin protein coded by *MEFV* in human and mice (**Figure 1**). Although these two species share 47.6% identity and 65.5% similarity in pyrin amino acid sequence, murine pyrin lacks the complete B30.2 domain, a potential pathogen sensing region (Chae et al., 2000). Importantly, mutations responsible for FMF in humans are mostly located in the B30.2 domain (Ting and Davis, 2005). Thus, it is possible that mice have lost their ability to detect *Francisella* via pyrin due to genetic changes in murine pyrin gene.

Pyrin's role in caspase-1 function has also been a subject of controversy. Discovered as a gene mutation that leads to bouts of inflammation, several groups immediately suggested an anti-inflammatory role for pyrin by showing that pyrin can inhibit IL-1β activation (Chae et al., 2003, 2006; Papin et al., 2007). In contrast, other groups provide evidence that pyrin is a pro-inflammatory protein, activating caspase-1 dependent IL-1β cleavage (Yu et al., 2006, 2007; Seshadri et al., 2007; Gavrilin et al., 2009). Working with human primary CD14+ monocytes and their derived macrophages, we observed a correlation between pyrin levels and the ability of the cell to process and release IL-1β in response to *Francisella* infection. Fresh human monocytes show high levels of pyrin and readily release large quantities of IL-1β, whereas macrophages are relatively deficient in pyrin and defective in processing, but not in the synthesis of proIL-1β. Alveolar macrophages also follow this pattern and do not readily process and release IL-1β (Wewers and Herzyk, 1989). In addition, the modulation of intracellular pyrin levels in mononuclear phagocytes in various stages of differentiation correlates with the ability of pyrin to affect IL-1β release in response to *Francisella* infection (Gavrilin et al., 2006, 2009; Seshadri et al., 2007).

FIGURE 1 | Differences between pyrin in human and mice.
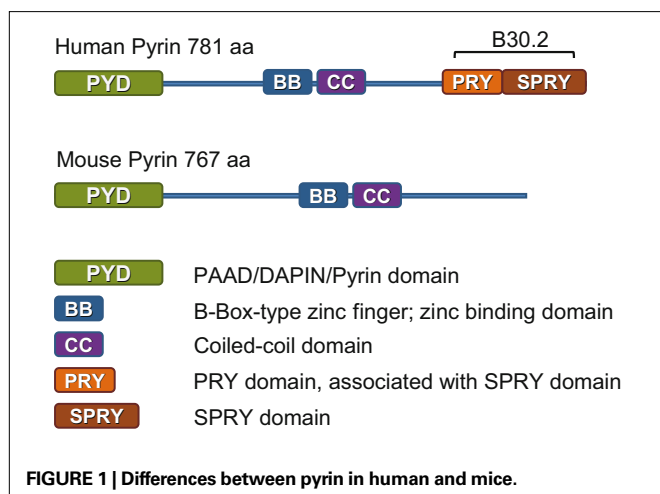
M-CSF

Particularly noticeable is the striking effect of M-CSF on pyrin levels in human cells. Macrophages matured in the presence of M-CSF maintain pyrin levels and readily release IL-1β followed by infection with *Francisella*, similar to freshly harvested CD14+ human monocytes. Moreover, macrophages matured in the absence of growth factors, while defective in proIL-1β processing and release, re-induce pyrin and the ability to process IL-1β with M-CSF treatments (Gavrilin et al., 2009). Thus, the local microenvironment may modulate a broad-range of macrophage phenotypes that extend well beyond M1/M2 classification models.

THP-1 CELL LINE AS A MODEL OF HOST–PATHOGEN INTERACTION

In discussing the differences in AIM2 and pyrin expression and function between mice and primary human mononuclear cells, it is important to mention the human pre-monocytic cell line THP-1 because a plethora of experiments have been performed with this cell line. THP-1 cells are phagocytic, can be infected with *Francisella*, and show activation of caspase-1 (Mohapatra et al., 2007; Zakharova et al., 2010). It was shown that stimulation of THP-1 cells with PMA, Sendai virus, or INF-γ induces AIM2 expression (Burckstummer et al., 2009; Fernandes-Alnemri et al., 2009; Hornung et al., 2009). PMA treated THP-1 cells also release IL-1β after infection with *F. novicida*. During THP-1 differentiation to THP-derived macrophages (TDM), pyrin disappears as is also observed with CD14+ monocyte differentiation to monocyte-derived macrophages (MDM). Consistent with a role for pyrin in caspase-1 activation after *F. novicida*, TDMs transduced to express pyrin release about sixfold more IL-1β upon infection with *F. novicida* as compared to TDM controls (Gavrilin et al., 2009). Nevertheless, even in the absence of pyrin, TDM release more IL-1β than do fresh THP-1 cells, suggesting the existence of a non-pyrin activation mechanism as well. Thus, it is interesting to speculate that pyrin and AIM2 may both be sensors of *Francisella* and that these two sensors may work in concert with each other. This speculation is more important in human cells with fully intact pyrin B30.2 domains.

In keeping with the pyrin/AIM2 dual detection hypothesis, it is notable that each PRR may recognize certain DAMP or PAMP covering discreet molecular patterns, such that overlapping regions of recognition may lead to more precise detection of pathogen types. The example of such composite detection is shown for *Listeria*, where mouse Nlrc4, Nlrp3, and Aim2 work in cooperation (Tsuchiya et al., 2010; Warren et al., 2010; Wu et al., 2010) while human PBMC appear to detect *Listeria* only via NLRP3 and not in cooperation with AIM2, NLRC4, and other tested NLRs (Meixenberger et al., 2010). The newly described NLRC5 inflammasome also demonstrated cooperativity with NLRP3 when THP-1 cells were stimulated with panel of PAMPs and DAMPs, typically activating NLRP3 inflammasome (Davis et al., 2010).

Regarding *Francisella* detection, we cannot rule out the possibility that AIM2 expression in human monocytes exists below the limits of confident detection, but still in amounts sufficient to trigger ASC oligomerization and caspase-1 activation upon DNA recognition. Apparently, NLRs may be able to act at low concentrations relative to ASC and caspase-1, triggering an initial node of activation. For example, NLRP3 initiates ASC pyroptosome oligomerization at the molar ratio of 1:500 (Fernandes-Alnemri et al., 2007). Following

initial dimerization, ASC oligomerization may become independent of NLR requirements, having reached a critical threshold concentration (Cheng et al., 2010). Yet, maximum caspase-1 activity using an *in vitro* reconstructed inflammasome was shown at molar ratio of caspase-1: ASC-NALP1 as 2:1:1 (Faustin et al., 2007). Thus, much remains to be learned about the mechanisms that drive inflammasome and pyroptosome formation and function.

CONCLUSION

In summary, the study of specific pathogens continues to uncover particulars of inflammasome regulation. In the case of *Francisella*, human mononuclear cells recognize *Francisella* before its internalization resulting in activation of multiple signaling pathways, including proIL-1β synthesis. Thus intracellular *Francisella*, after escape from the phagosome, may be detected by several pattern recognition receptors, where AIM2 and pyrin may trigger formation of the pyroptosome or the inflammasome platform for caspase-1 activation. This event results in the initiation of cell death and/or IL-1β processing and release. However, comparisons between mice and humans reveal that there are important evolutionary changes in pathogen sensing that are likely to have been driven by environmental pressures. Understanding the peculiarities of pathogen host sensing is dependent upon powerful animal models but must continue to be interpreted along side of human studies.

REFERENCES

Agostini, L., Martinon, F., Burns, K., McDermott, M. F., Hawkins, P. N., and Tschopp, J. (2004). NALP3 forms an IL-1beta-processing inflammasome with increased activity in Muckle-Wells autoinflammatory disorder. *Immunity* 20, 319–325.

Alnemri, E. S. (2010). Sensing cytoplasmic danger signals by the inflammasome. *J. Clin. Immunol.* 30, 512–519.

Burckstummer, T., Baumann, C., Bluml, S., Dixit, E., Durnberger, G., Jahn, H., Planyavsky, M., Bilban, M., Colinge, J., Bennett, K. L., and Superti-Furga, G. (2009). An orthogonal proteomic-genomic screen identifies AIM2 as a cytoplasmic DNA sensor for the inflammasome. *Nat. Immunol.* 10, 266–272.

Butchar, J. P., Cremer, T. J., Clay, C. D., Gavrilin, M. A., Wewers, M. D., Marsh, C. B., Schlesinger, L. S., and Tridandapani, S. (2008). Microarray analysis of human monocytes infected with *Francisella tularensis* identifies new targets of host response subversion. *PLoS ONE* 3, e2924. doi: 10.1371/journal.pone.0002924

Centola, M., Wood, G., Frucht, D. M., Galon, J., Aringer, M., Farrell, C., Kingma, D. W., Horwitz, M. E., Mansfield, E., Holland, S. M., O'Shea, J. J., Rosenberg, H. F., Malech, H. L., and Kastner, D. L. (2000). The gene for familial Mediterranean fever, MEFV, is expressed in early leukocyte development and is regulated in response to inflammatory mediators. *Blood* 95, 3223–3231.

Chae, J. J., Centola, M., Aksentijevich, I., Dutra, A., Tran, M., Wood, G., Nagaraju, K., Kingma, D. W., Liu, P. P., and Kastner, D. L. (2000). Isolation, genomic organization, and expression analysis of the mouse and rat homologs of MEFV, the gene for familial mediterranean fever. *Mamm. Genome* 11, 428–435.

Chae, J. J., Komarow, H. D., Cheng, J., Wood, G., Raben, N., Liu, P. P., and Kastner, D. L. (2003). Targeted disruption of pyrin, the FMF protein, causes heightened sensitivity to endotoxin and a defect in macrophage apoptosis. *Mol. Cell* 11, 591–604.

Chae, J. J., Wood, G., Masters, S. L., Richard, K., Park, G., Smith, B. J., and Kastner, D. L. (2006). The B30.2 domain of pyrin, the familial Mediterranean fever protein, interacts directly with caspase-1 to modulate IL-1beta production. *Proc. Natl. Acad. Sci. U.S.A.* 103, 9982–9987.

Cheng, J., Waite, A. L., Tkaczyk, E. R., Ke, K., Richards, N., Hunt, A. J., and Gumucio, D. L. (2010). Kinetic properties of ASC protein aggregation in epithelial cells. *J. Cell. Physiol.* 222, 738–747.

Choubey, D., Duan, X., Dickerson, E., Ponomareva, L., Panchanathan, R., Shen, H., and Srivastava, R. (2010). Interferon-inducible p200-family proteins as novel sensors of cytoplasmic DNA: role in inflammation and autoimmunity. *J. Interferon Cytokine Res.* 30, 371–380.

Cole, L. E., Santiago, A., Barry, E., Kang, T. J., Shirey, K. A., Roberts, Z. J., Elkins, K. L., Cross, A. S., and Vogel, S. N. (2008). Macrophage proinflammatory response to *Francisella tularensis* live vaccine strain requires coordination of multiple signaling pathways. *J. Immunol.* 180, 6885–6891.

Cookson, B. T., and Brennan, M. A. (2001). Pro-inflammatory programmed cell death. *Trends Microbiol.* 9, 113–114.

Davis, B. K., Roberts, R. A., Huang, M. T., Willingham, S. B., Conti, B. J., Brickey, W. J., Barker, B. R., Kwan, M., Taxman, D. J., Accavitti-Loper, M. A., Duncan, J. A., and Ting, J. P. (2010). Cutting edge: NLRC5-dependent activation of the inflammasome. *J. Immunol.* 186, 1333–1337.

DeYoung, K. L., Ray, M. E., Su, Y. A., Anzick, S. L., Johnstone, R. W., Trapani, J. A., Meltzer, P. S., and Trent, J. M. (1997). Cloning a novel member of the human interferon-inducible gene family associated with control of tumorigenicity in a model of human melanoma. *Oncogene* 15, 453–457.

Dinarello, C. A. (1998). Interleukin-1 beta, interleukin-18, and the interleukin-1 beta converting enzyme. *Ann. N. Y. Acad. Sci.* 856, 1–11.

Edwards, J. A., Rockx-Brouwer, D., Nair, V., and Celli, J. (2010). Restricted cytosolic growth of *Francisella tularensis* subsp. *tularensis* by IFN-gamma activation of macrophages. *Microbiology* 156, 327–339.

Ellis, J., Oyston, P. C., Green, M., and Titball, R. W. (2002). Tularemia. *Clin. Microbiol. Rev.* 15, 631–646.

Faustin, B., Lartigue, L., Bruey, J. M., Luciano, F., Sergienko, E., Bailly-Maitre, B., Volkmann, N., Hanein, D., Rouiller, I., and Reed, J. C. (2007). Reconstituted NALP1 inflammasome reveals two-step mechanism of caspase-1 activation. *Mol. Cell* 25, 713–724.

Fernandes-Alnemri, T., and Alnemri, E. S. (2008). Assembly, purification, and assay of the activity of the ASC pyroptosome. *Meth. Enzymol.* 442, 251–270.

Fernandes-Alnemri, T., Wu, J., Yu, J. W., Datta, P., Miller, B., Jankowski, W., Rosenberg, S., Zhang, J., and Alnemri, E. S. (2007). The pyroptosome: a supramolecular assembly of ASC dimers mediating inflammatory cell death via caspase-1 activation. *Cell Death Differ.* 14, 1590–1604.

Fernandes-Alnemri, T., Yu, J. W., Datta, P., Wu, J., and Alnemri, E. S. (2009). AIM2 activates the inflammasome and cell death in response to cytoplasmic DNA. *Nature* 458, 509–513.

Fernandes-Alnemri, T., Yu, J. W., Juliana, C., Solorzano, L., Kang, S., Wu, J., Datta, P., McCormick, M., Huang, L.,

McDermott, E., Eisenlohr, L., Landel, C. P., and Alnemri, E. S. (2010). The AIM2 inflammasome is critical for innate immunity to *Francisella tularensis*. *Nat. Immunol.* 11, 385–393.

Fink, S. L., and Cookson, B. T. (2005). Apoptosis, pyroptosis, and necrosis: mechanistic description of dead and dying eukaryotic cells. *Infect. Immun.* 73, 1907–1916.

Gavrilin, M. A., Bouakl, I. J., Knatz, N. L., Duncan, M. D., Hall, M. W., Gunn, J. S., and Wewers, M. D. (2006). Internalization and phagosome escape required for *Francisella* to induce human monocyte IL-1beta processing and release. *Proc. Natl. Acad. Sci. U.S.A.* 103, 141–146.

Gavrilin, M. A., Mitra, S., Seshadri, S., Nateri, J., Berhe, F., Hall, M. W., and Wewers, M. D. (2009). Pyrin critical to macrophage IL-1beta response to *Francisella* challenge. *J. Immunol.* 182, 7982–7989.

Golovliov, I., Baranov, V., Krocova, Z., Kovarova, H., and Sjostedt, A. (2003). An attenuated strain of the facultative intracellular bacterium *Francisella tularensis* can escape the phagosome of monocytic cells. *Infect. Immun.* 71, 5940–5950.

Henry, T., Brotcke, A., Weiss, D. S., Thompson, L. J., and Monack, D. M. (2007). Type I interferon signaling is required for activation of the inflammasome during *Francisella* infection. *J. Exp. Med.* 204, 987–994.

Henry, T., Kirimanjeswara, G. S., Ruby, T., Jones, J. W., Peng, K., Perret, M., Ho, L., Sauer, J. D., Iwakura, Y., Metzger, D. W., and Monack, D. M. (2010). Type I IFN signaling constrains IL-17A/F secretion by gammadelta T cells during bacterial infections. *J. Immunol.* 184, 3755–3767.

Hornung, V., Ablasser, A., Charrel-Dennis, M., Bauernfeind, F., Horvath, G., Caffrey, D. R., Latz, E., and Fitzgerald, K. A. (2009). AIM2 recognizes cytosolic dsDNA and forms a

caspase-1-activating inflammasome with ASC. *Nature* 458, 514–518.

Huang, M. T., Mortensen, B. L., Taxman, D. J., Craven, R. R., Taft-Benz, S., Kijek, T. M., Fuller, J. R., Davis, B. K., Allen, I. C., Brickey, W. J., Gris, D., Wen, H., Kawula, T. H., and Ting, J. P. (2010). Deletion of ripA alleviates suppression of the inflammasome and MAPK by *Francisella tularensis*. *J. Immunol.* 185, 5476–5485.

Jones, J. W., Kayagaki, N., Broz, P., Henry, T., Newton, K., O'Rourke, K., Chan, S., Dong, J., Qu, Y., Roose-Girma, M., Dixit, V. M., and Monack, D. M. (2010). Absent in melanoma 2 is required for innate immune recognition of *Francisella tularensis*. *Proc. Natl. Acad. Sci. U.S.A.* 107, 9771–9776.

Kahlenberg, J. M., and Dubyak, G. R. (2004). Mechanisms of caspase-1 activation by P2X7 receptor-mediated K+ release. *Am. J. Physiol. Cell Physiol.* 286, C1100–C1108.

Kirimanjeswara, G. S., Olmos, S., Bakshi, C. S., and Metzger, D. W. (2008). Humoral and cell-mediated immunity to the intracellular pathogen *Francisella tularensis*. *Immunol. Rev.* 225, 244–255.

Ludlow, L. E., Johnstone, R. W., and Clarke, C. J. (2005). The HIN-200 family: more than interferon-inducible genes? *Exp. Cell Res.* 308, 1–17.

MacKenzie, A., Wilson, H. L., Kiss-Toth, E., Dower, S. K., North, R. A., and Surprenant, A. (2001). Rapid secretion of interleukin-1beta by microvesicle shedding. *Immunity* 15, 825–835.

Mangan, D. F., Welch, G. R., and Wahl, S. M. (1991). Lipopolysaccharide, tumor necrosis factor-alpha, and IL-1 beta prevent programmed cell death (apoptosis) in human peripheral blood monocytes. *J. Immunol.* 146, 1541–1546.

Mariathasan, S., Newton, K., Monack, D. M., Vucic, D., French, D. M., Lee, W. P., Roose-Girma, M., Erickson, S., and Dixit, V. M. (2004). Differential activation of the inflammasome by caspase-1 adaptors ASC and Ipaf. *Nature* 430, 213–218.

Mariathasan, S., Weiss, D. S., Dixit, V. M., and Monack, D. M. (2005). Innate immunity against *Francisella tularensis* is dependent on the ASC/caspase-1 axis. *J. Exp. Med.* 202, 1043–1049.

Martinon, F., Burns, K., and Tschopp, J. (2002). The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. *Mol. Cell* 10, 417–426.

Martinon, F., Mayor, A., and Tschopp, J. (2009). The inflammasomes: guardians of the body. *Annu. Rev. Immunol.* 27, 229–265.

Martinon, F., and Tschopp, J. (2006). Inflammatory caspases and inflammasomes: master switches of inflammation. *Cell Death Differ.* 14, 10–22.

Meixenberger, K., Pache, F., Eitel, J., Schmeck, B., Hippenstiel, S., Slevogt, H., N'Guessan, P., Witzenrath, M., Netea, M. G., Chakraborty, T., Suttorp, N., and Opitz, B. (2010). Listeria monocytogenes-infected human peripheral blood mononuclear cells produce IL-1beta, depending on listeriolysin O and NLRP3. *J. Immunol.* 184, 922–930.

Mohapatra, N. P., Balagopal, A., Soni, S., Schlesinger, L. S., and Gunn, J. S. (2007). AcpA is a *Francisella* acid phosphatase that affects intramacrophage survival and virulence. *Infect. Immun.* 75, 390–396.

Nisole, S., Stoye, J. P., and Saib, A. (2005). TRIM family proteins: retroviral restriction and antiviral defence. *Nat. Rev. Microbiol.* 3, 799–808.

Oyston, P. C. (2008). *Francisella tularensis*: unravelling the secrets of an intracellular pathogen. *J. Med. Microbiol.* 57, 921–930.

Papin, S., Cuenin, S., Agostini, L., Martinon, F., Werner, S., Beer, H. D., Grutter, C., Grutter, M., and Tschopp, J. (2007). The SPRY domain of pyrin, mutated in familial Mediterranean fever patients, interacts with inflammasome components and inhibits proIL-1beta processing. *Cell Death Differ.* 14, 1457–1466.

Parmely, M. J., Fischer, J. L., and Pinson, D. M. (2009). Programmed cell death and the pathogenesis of tissue injury induced by type A *Francisella tularensis*. *FEMS Microbiol. Lett.* 301, 1–11.

Poeck, H., Bscheider, M., Gross, O., Finger, K., Roth, S., Rebsamen, M., Hannesschlager, N., Schlee, M., Rothenfusser, S., Barchet, W., Kato, H., Akira, S., Inoue, S., Endres, S., Peschel, C., Hartmann, G., Hornung, V., and Ruland, J. (2010). Recognition of RNA virus by RIG-I results in activation of CARD9 and inflammasome signaling for interleukin 1 beta production. *Nat. Immunol.* 11, 63–69.

Rathinam, V. A., Jiang, Z., Waggoner, S. N., Sharma, S., Cole, L. E., Waggoner, L., Vanaja, S. K., Monks, B. G., Ganesan, S., Latz, E., Hornung, V., Vogel, S. N., Szomolanyi-Tsuda, E., and Fitzgerald, K. A. (2010). The AIM2 inflammasome is essential for host defense against cytosolic bacteria and DNA viruses. *Nat. Immunol.* 11, 395–402.

Ray, K., Marteyn, B., Sansonetti, P. J., and Tang, C. M. (2009). Life on the inside: the intracellular lifestyle of cytosolic bacteria. *Nat. Rev. Microbiol.* 7, 333–340.

Richards, N., Schaner, P., Diaz, A., Stuckey, J., Shelden, E., Wadhwa, A., and Gumucio, D. L. (2001). Interaction between pyrin and the apoptotic speck protein (ASC) modulates ASC-induced apoptosis. *J. Biol. Chem.* 276, 39320–39329.

Roberts, T. L., Idris, A., Dunn, J. A., Kelly, G. M., Burnton, C. M., Hodgson, S., Hardy, L. L., Garceau, V., Sweet, M. J., Ross, I. L., Hume, D. A., and Stacey, K. J. (2009). HIN-200 proteins regulate caspase activation in response to foreign cytoplasmic DNA. *Science* 323, 1057–1060.

Rotz, L. D., Khan, A. S., Lillibridge, S. R., Ostroff, S. M., and Hughes, J. M. (2002). Public health assessment of potential biological terrorism agents. *Emerging Infect. Dis.* 8, 225–230.

Santic, M., Pavokovic, G., Jones, S., Asare, R., and Kwaik, Y. A. (2010). Regulation of apoptosis and anti-apoptosis signalling by *Francisella tularensis*. *Microbes Infect.* 12, 126–134.

Sarkar, A., Mitra, S., Mehta, S., Raices, R., and Wewers, M. D. (2009). Monocyte derived microvesicles deliver a cell death message via encapsulated caspase-1. *PLoS ONE* 4, e7140. doi: 10.1371/journal.pone.0007140

Seshadri, S., Duncan, M. D., Hart, J. M., Gavrilin, M. A., and Wewers, M. D. (2007). Pyrin levels in human monocytes and monocyte-derived macrophages regulate IL-1beta processing and release. *J. Immunol.* 179, 1274–1281.

Sjostedt, A. (2007). Tularemia: history, epidemiology, pathogen physiology, and clinical manifestations. *Ann. N. Y. Acad. Sci.* 1105, 1–29.

Ting, J. P., and Davis, B. K. (2005). CATERPILLER: a novel gene family important in immunity, cell death, and diseases. *Annu. Rev. Immunol.* 23, 387–414.

Ting, J. P., Lovering, R. C., Alnemri, E. S., Bertin, J., Boss, J. M., Davis, B. K., Flavell, R. A., Girardin, S. E., Godzik, A., Harton, J. A., Hoffman, H. M., Hugot, J. P., Inohara, N., Mackenzie, A., Maltais, L. J., Nunez, G., Ogura, Y., Otten, L. A., Philpott, D., Reed, J. C., Reith, W., Schreiber, S., Steimle, V., and Ward, P. A. (2008). The NLR gene family: a standard nomenclature. *Immunity* 28, 285–287.

Tsuchiya, K., Hara, H., Kawamura, I., Nomura, T., Yamamoto, T., Daim, S., Dewamitta, S. R., Shen, Y., Fang, R., and Mitsuyama, M. (2010). Involvement of absent in melanoma 2 in inflammasome activation in macrophages infected with *Listeria monocytogenes*. *J. Immunol.* 185, 1186–1195.

Warren, S. E., Armstrong, A., Hamilton, M. K., Mao, D. P., Leaf., I. A., Miao, E. A., and Aderem, A. (2010). Cutting edge: cytosolic bacterial DNA activates the inflammasome via Aim2. *J. Immunol.* 185, 818–821.

Weiss, D. S., Henry, T., and Monack, D. M. (2007). *Francisella tularensis*: activation of the inflammasome. *Ann. N. Y. Acad. Sci.* 1105, 219–237.

Wewers, M. D., and Herzyk, D. J. (1989). Alveolar macrophages differ from blood monocytes in human IL-1 beta release. Quantitation by enzyme-linked immunoassay. *J. Immunol.* 143, 1635–1641.

Wu, J., Fernandes-Alnemri, T., and Alnemri, E. S. (2010). Involvement of the AIM2, NLRC4, and NLRP3 inflammasomes in caspase-1 activation by Listeria monocytogenes. *J. Clin. Immunol.* 30, 693–702.

Yamin, T. T., Ayala, J. M., and Miller, D. K. (1996). Activation of the native 45-kDa precursor form of interleukin-1-converting enzyme. *J. Biol. Chem.* 271, 13273–13282.

Yu, J. W., Fernandes-Alnemri, T., Datta, P., Wu, J., Juliana, C., Solorzano, L., McCormick, M., Zhang, Z., and Alnemri, E. S. (2007). Pyrin activates the ASC pyroptosome in response to engagement by autoinflammatory PSTPIP1 mutants. *Mol. Cell* 28, 214–227.

Yu, J. W., Wu, J., Zhang, Z., Datta, P., Ibrahimi, I., Taniguchi, S., Sagara, J., Fernandes-Alnemri, T., and Alnemri, E. S. (2006). Cryopyrin and pyrin activate caspase-1, but not NF-kappaB, via ASC oligomerization. *Cell Death Differ.* 13, 236–249.

Zakharova, E., Grandhi, J., Wewers, M. D., and Gavrilin, M. A. (2010). Mycoplasma suppression of THP-1 cell TLR responses is corrected with antibiotics. *PLoS ONE* 5, e9900. doi: 10.1371/journal.pone.0009900

Zhou, R., Yazdi, A. S., Menu, P., and Tschopp, J. (2010). A role for mitochondria in NLRP3 inflammasome activation. *Nature* 469, 221–225.

Replication of *Legionella pneumophila* in human cells: why are we susceptible?

Arwa Abu Khweek and Amal Amer*

Division of Pulmonary, Allergy, Critical Care and Sleep Medicine, Center for Microbial Interface Biology and the Department of Internal Medicine, Ohio State University, Columbus, OH, USA

Legionella pneumophila is the causative agent of Legionnaires' disease, a serious and often fatal form of pneumonia. The susceptibility to *L. pneumophila* arises from the ability of this intracellular pathogen to multiply in human alveolar macrophages and monocytes. *L. pneumophila* also replicates in several professional and non-professional phagocytic human-derived cell lines. With the exception of the A/J mouse strain, most mice strains are restrictive, thus they do not support *L. pneumophila* replication. Mice lacking the NOD-like receptor Nlrc4 or caspase-1 are also susceptible to *L. pneumophila*. On the other hand, in the susceptible human hosts, *L. pneumophila* utilizes several strategies to ensure intracellular replication and protect itself against the host immune system. Most of these strategies converge to prevent the fusion of the *L. pneumophila* phagosome with the lysosome, inhibiting host cell apoptosis, activating survival pathways, and sequestering essential nutrients for replication and pathogenesis. In this review, we summarize survival mechanisms employed by *L. pneumophila* to maintain its replication in human cells. In addition, we highlight different human-derived cell lines that support the multiplication of this intracellular bacterium. Therefore, these *in vitro* models can be applicable and are reproducible when investigating *L. pneumophila*/phagocyte interactions at the molecular and cellular levels in the human host.

Keywords: NOD-like receptors, Toll-like receptors, pathogen-associated molecular patterns, neuronal apoptosis-inhibitory proteins

LEGIONELLA PNEUMOPHILA

Legionella pneumophila is a facultative intracellular lung pathogen (Horwitz and Silverstein, 1980) and the causative agent of Legionnaires' disease (LD), a serious and often fatal, life-threatening bacterial pneumonia. LD is also a relatively common cause of community-acquired and nosocomial pneumonia in adults. *L. pneumophila* was recognized in 1976 after an outbreak of pneumonia at an American Legion convention in Philadelphia. Soon after, the etiologic agent was identified as a fastidious Gram-negative bacillus and named *L. pneumophila*. Although several other species of the genus *Legionella* were subsequently identified, the great majority of LD is caused by *L. pneumophila* (Marston et al., 1997; Yu et al., 2002). Most nosocomial infections and hospital outbreaks have been linked to contaminated air or water supplies. *L. pneumophila* has been recovered from different aquatic habitats including showers, streams, whirlpools, air conditioners, cooling towers, fountains, and spa baths (Fraser et al., 1979; Fliermans et al., 1981; Sethi and Brandis, 1983; Spitalny et al., 1984; Lettinga et al., 2002). Within water systems, *L. pneumophila* colonizes into biofilm; which are complex bacterial communities attached to a substratum by means of exopolysaccharides (EPS; Rogers et al., 1994). Biofilm develop into mushroom-like structures with water channels that allow access to nutrients and oxygen within these bacterial communities. *L. pneumophila* is able to obtain nutrients such as amino acids and organic carbon sources from the microbial consortium located in the biofilm (Watnick and Kolter, 2000). Humans are accidental dead-end hosts for *L. pneumophila*, thus, there is no person-to-person transmission whereas, protozoa are considered the environmental hosts for this intracellular pathogen, and are required for replication of biofilm-associated *L. pneumophila* (Rowbotham, 1980, 1986; Nash et al., 1984; Kuiper et al., 2004). While providing a niche for *L. pneumophila* replication, amoebae also protect *L. pneumophila* from harsh environmental conditions. Replication within different protozoa enhances bacterial multiplication in human alveolar macrophages. Growth within the protozoa also induces resistance to chemical disinfectant, biocides and antibiotics and induces phenotypic changes in *L. pneumophila* (Barker et al., 1992, 1995; Abu Kwaik et al., 1993; Cirillo et al., 1994).

RISK FACTORS

The majority of people exposed to *L. pneumophila* remains asymptomatic or suffer only mild self-limiting infection. Susceptible patients to LD disease are likely to exhibit a defect in cell-mediated immunity rendering them less capable of limiting the early multiplication of *L. pneumophila*. Cigaret smoking, chronic lung disease, and immunosuppression (especially that caused by corticosteroid therapy) have been consistently implicated as risk factors (Carratala et al., 1994). Surgery is a major predisposing factor in nosocomial infection, with transplant recipients at highest risk. Other factors associated with the development of LD include, old age, cancer, and alcohol intake (Marston et al., 1994; Den Boer et al., 2002).

L. PNEUMOPHILA MULTIPLIES IN HUMAN MONOCYTES, ALVEOLAR MACROPHAGES, AND HUMAN-DERIVED CELL LINES

Legionella pneumophila multiplies in human monocytes (Horwitz and Silverstein, 1980; Horwitz, 1983b). The intracellular fate of *L. pneumophila* in both human and protozoa is similar, where the

bacteria evade fusion of the phagosome to lysosome, although the mechanism maybe different in some aspects (Horwitz, 1983a,b; Horwitz and Maxfield, 1984; Bozue and Johnson, 1996). Attachments and binding of *L. pneumophila* to the complement receptor 1 (CR1), and complement receptor 3 (CR3) on human phagocytic cells is followed by entry into these cells. In some cases, the entry of the pathogen is mediated by "coiling phagocytosis," in which a single long phagocyte pseudopod coils around the bacterium as it is internalized (Horwitz, 1984; Payne and Horwitz, 1987; **Figure 1**). Following phagocytosis, *L. pneumophila* avoids interaction with endosomes, early and late lysosomes, and instead, fuses transiently with mitochondria and intercepts the endoplasmic reticulum (ER) exit vesicles (Horwitz, 1983b; Swanson and Isberg, 1995; Kagan and Roy, 2002; Liang et al., 2006; Isberg et al., 2009). The bacteria maintain interactions with ER-derived vesicles and replicate in a vacuole surrounded by a membrane that resembles rough ER (Horwitz and Silverstein, 1980; Horwitz, 1983b; **Figure 1**). The *L. pneumophila*-containing

vacuole (LCV) remains studded with ribosomes until hundreds of the bacteria fill the vacuole and the monocyte ruptures (**Figure 1**; Nash et al., 1984). In contrast, formalin-killed *L. pneumophila* does not inhibit fusion or induce the formation of specialized phagosome and the dead bacteria are degraded within the phagolysosome (Horwitz and Silverstein, 1980; Horwitz, 1983b).

Alveolar macrophages are the most abundant potential effector cells in the lung and play a role in host defense against *L. pneumophila* (Nash et al., 1984). *L. pneumophila* that reaches the alveoli encounters and multiplies in human alveolar macrophages at the site of infection, which is crucial to the pathogenesis of LD (Green, 1970; Goldstein et al., 1974). Lung tissue specimens from LD patients frequently contain large numbers of intracellular *L. pneumophila* (Winn Jr. and Myerowitz, 1981; Nash et al., 1984). Furthermore, alveolar macrophages are a major feature of the alveolar secretions in lung biopsies from patients with LD (Winn Jr. and Myerowitz, 1981; Nash et al., 1984).
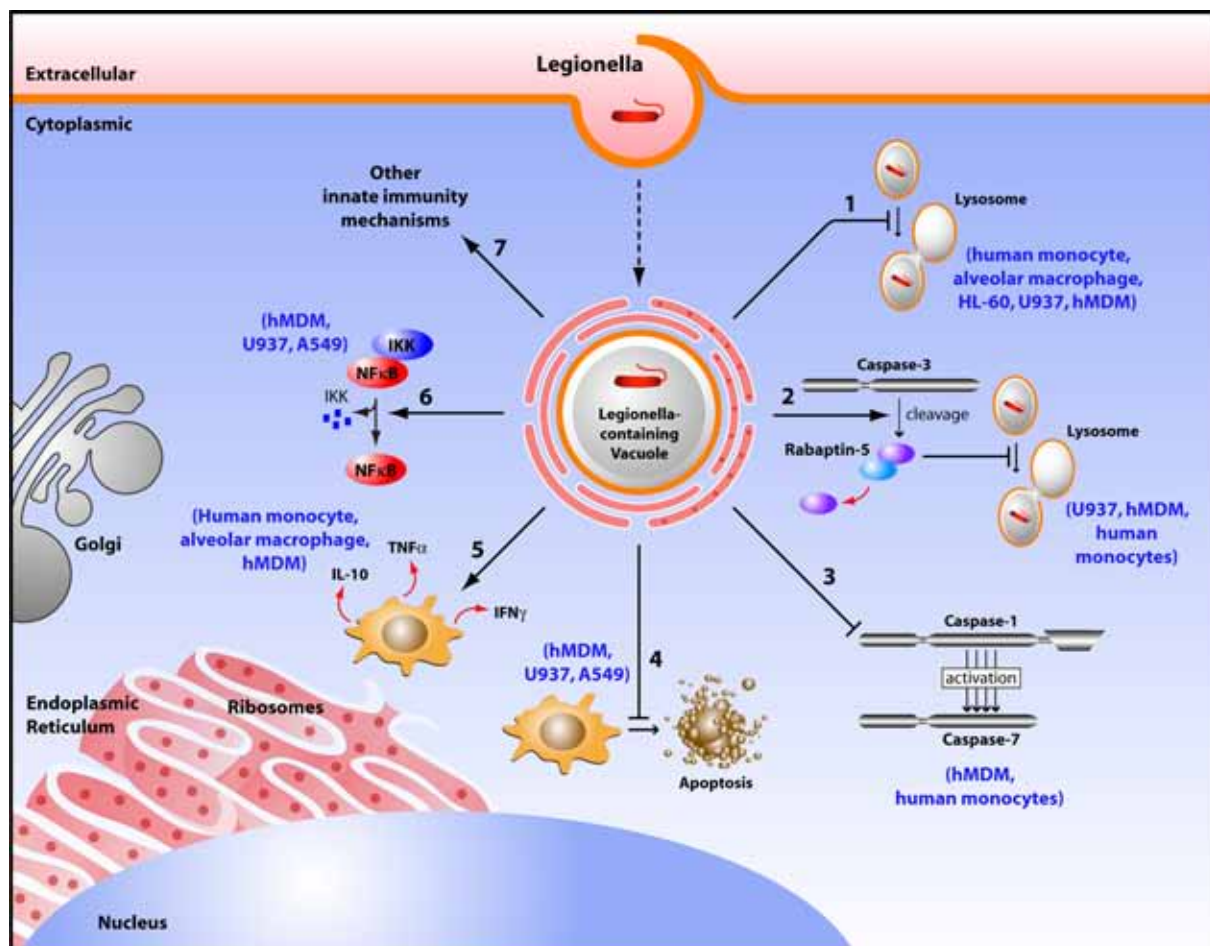
FIGURE 1 | Diagram depicting survival mechanisms employed by *L. pneumophila* in human phagocytes. Upon entry into a human phagocyte, *L. pneumophila* becomes contained into a vacuole called *Legionella*-containing phagosome that avoids the typical fusion with the lysosome, and instead remodels into an ER-like vesicle allowing bacterial replication (1). *L. pneumophila* promotes the cleavage of Rabaptin-5 by caspase-3, thus preventing the default phagosome–lysosome fusion (2). *L. pneumophila* does not activate caspase-1

and -7 in human monocytes, consequently aborting the phagosome–lysosome fusion (3). This pathogen inhibits host cell apoptosis by up regulating anti-apoptotic genes (4). *L. pneumophila* controls the local balance of activating cytokines (INF-γ, TNF-α) that inhibit its replication, and inhibiting cytokines (IL-10) that allow its survival (5). *L. pneumophila* activates the NF-κB pathway to maintain host cell survival (6). *L. pneumophila* modulates other innate immune responses to establish a replicative niche (7).

Beside human monocytes and alveolar macrophages, investigators have employed a variety of cell lines that not only have similar characteristics to human phagocytes, but are also capable of supporting growth of this intracellular pathogen. For example, *L. pneumophila* multiplies within and kills HL-60 cells, a macrophage-like cell line that is derived from promyelocyte (Marra et al., 1990). In HL-60 cells, *L. pneumophila* enters by adhering to the CR3 and is taken by coiling phagocytosis. Similar to human phagocytes, the LCV is surrounded by ER-derived vesicles studded with ribosomes and therefore, HL-60 cells are utilized as a model to study the interaction of human macrophages with the LD bacterium (Marra et al., 1990). Similarly, *L. pneumophila* grows within U937 cells; a human monocytes lymphoma cell line that displays many monocytic features and can be maintained continuously in culture (Pearlman et al., 1988). These cells can be differentiated to adherent, non-replicative cells with characteristics of tissue macrophages. Additionally, they express surface markers specific for cells of monocyte lineage and are able to differentiate between virulent and avirulent strains of *L. pneumophila*. Their activation state is controlled experimentally and consequently can be standardized (Rovera et al., 1979). THP-1 is another human monocytic cell line that matures into macrophage-like adherent cells following stimulation with phorbol esters or 1,25-dihydroxyvitamin D3. Cirillo et al. (1994) reported that *L. pneumophila* could invade and multiply within THP-1 cells in a manner similar to that in human monocytes and macrophages. Mono Mac 6 (MM6) is an additional example of human monocytic cell line. MM6 was generated from peripheral blood of patient with monoblastic leukemia. Phenotypically and functionally, MM6 represents a more mature macrophage-like cell line. Unlike the other monoblastic cell lines U937 and THP-1, MM6 does not require stimulation by phorbol esters or 1,25-dihydroxyvitamin D3 and expresses several markers that are lacking in U937 and THP-1 (Ziegler-Heitbrock et al., 1988; Neumeister et al., 1997). MM6 has been successfully utilized to study the molecular pathogenesis of *L. pneumophila* and other intracellular pathogens that can replicate within human monocytes (Neumeister, 2004). Furthermore, non-professional phagocytic cell lines have been utilized to study *L. pneumophila* replication such as A549, a human epithelial cell line derived from a human lung cancer. They are non-professional phagocytes and have characteristics of type II alveolar epithelial cells (Maruta et al., 1998). It has been shown that *L. pneumophila* is internalized and replicate effectively in A549 cells (Maruta et al., 1998; Vinzing et al., 2008; Bartfeld et al., 2009). Therefore, many human-derived cell lines have been used to characterize the molecular mechanisms associated with *L. pneumophila* pathogenesis. While some features are unique to the interactions between *L. pneumophila* and specific host cells, the primary mechanisms of infection appears to be the same.

THE INNATE IMMUNE RESPONSE OF PRIMARY HUMAN CELLS AND HUMAN-DERIVED CELL LINES TO *L. PNEUMOPHILA*

Host cells express a range of receptors that act as microbial sensors. These receptors sense microorganisms and transduce signals that activate immune responses. Pathogen-associated molecular patterns (PAMPs) such as peptidoglycan, bacterial flagellin, and nucleic acid can be recognized by means of cell surface pattern recognition receptors (PRRs) named Toll-like receptors (TLRs) (Underhill et al., 1999; Janeway Jr. and Medzhitov, 2002; Takeda et al., 2003). TLRs recognize PAMPs via an extracellular domain and initiate inflammatory signaling pathways through an intracellular domain (Underhill et al., 1999; Janeway Jr. and Medzhitov, 2002; Takeda et al., 2003). Humans express functional TLRs 1–10 whereas mice express TLRs 1–7 and 9–13 (**Table 1**; Vinzing et al., 2008).

Other means of sensing the presence of microbes or their factors in the host cytosol is mediated by NOD-like receptors (NLRs), which in turn, initiate signaling cascades that mediate the production of inflammatory cytokines, recruitment of phagocytic cells and direction of the innate and the acquired immune responses. There are 23 NLRs in human and 34 in mice (Abdelaziz et al., 2010; Amer, 2010). Typically, NLRs are composed of a leucine-rich repeat (LRR) domain, a central nucleotide oligomerization domain (NOD) and N-terminal effector binding domain, which consists of caspase recruitment domain (CARD) and the pyrin domain.

One of the major NLRs contributing to the restriction of *L. pneumophila* infection in human and in mice is NLRC4 (IPAF) (Damiano et al., 2001; Poyet et al., 2001; Amer et al., 2006; Vinzing et al., 2008; Akhter et al., 2009). Human NLRC4 is highly homologous to the mouse Nlrc4 and is expressed in human macrophages but not in human lung epithelial cells (Vinzing et al., 2008). Like most NLRs, mouse Nlrc4 assembles in a large multiprotein complex called the inflammasome leading to the activation of caspase-1. Caspases are family of cysteine proteases that play a distinct role in apoptosis and inflammation (Fink and Cookson, 2005). Caspases can be divided into two groups; one category of caspases is involved in apoptosis such as caspase-3 and -7 (Martinon and Tschopp, 2004). Another category is involved in inflammation and is required for cytokine processing (Creagh et al., 2003). As an inflammatory caspase, caspase-1 is required for the activation of cytokines such as IL-1β, IL-18, and IL-33 and induces a form of cell death named pyroptosis (Fink and Cookson, 2005). Wild-type

Table 1 | Differences between human and mice cells that affects *Legionella* permissiveness.

	Human	Mice	References
TLRs	1–10	1–7 and 9–13	Akira et al. (2006 #137)
NLRs	23 members	34 members	Abdelaziz et al. (2010 #149)
NAIPs	1	7 paralogs	Vinzing et al. (2008 #7)
Caspases	11 caspases	10 caspases	
Caspase-1	Not activated in response to *Legionella* infection	Activated in response to *Legionella* infection	Akhter et al. (2009 #5)
Caspase-7	Not activated in response to *Legionella* infection	Activated in response to *Legionella* infection	Akhter et al. (2009 #5)
Caspase-3	activated in response to *Legionella* infection	Not activated in response to *Legionella* infection	Santic et al. (2007 #8)

mouse macrophages restrict *L. pneumophila* replication via caspase-1-mediated caspase-7 activation resulting in fusion of the LCV with the lysosome leading to bacterial degradation and growth restriction (Akhter et al., 2009). However, human monocytes do not activate caspase-1 and -7 upon *L. pneumophila* infection, allowing bacterial growth (**Table 1**; Santic et al., 2007; Akhter et al., 2009). Although THP-1 cells were not shown to activate caspase-1 in response to *L. pneumophila*, the depletion of human NLRC4 allows more *L. pneumophila* replication (Vinzing et al., 2008). This data implies the existence of uncharacterized caspase-1-independent restriction mechanism of *L. pneumophila* replication in human phagocytes.

A subset of NLRs contains baculovirus inhibitor of apoptosis repeat domains (BIR) instead of CARD or pyrin domains. These proteins are called the neuronal apoptosis-inhibitory proteins (NAIPs). The LRR domain recognizes microbial products, while the BIR domains are essential for their interactions with caspase-3 and -7 (Liston et al., 1996; Takahashi et al., 1998; Diez et al., 2003). The importance of Naip5 during *L. pneumophila* infection stemmed from studies showing that the A/J mice are permissive to *L. pneumophila* in contrast to most other inbred mouse strains (Diez et al., 2003; Wright et al., 2003). Gros and his group identified 14 amino acid substitutions in the A/J derived Naip5 when compared to wild-type Naip5 and they proposed that several of these mutations are responsible for permissiveness to *L. pneumophila* (Beckers et al., 1995; Diez et al., 2003). Furthermore, in contrast to the seven mouse Naips, only one NAIP has been identified in human and exhibits 68% homology to the mouse C57BL/6 and A/J Naip5. Human NAIP is expressed in THP-1, primary macrophages, and A549 epithelial cells. Furthermore, depletion of the human orthologs of Naip5 results in increased replication of *L. pneumophila* in human THP-1 macrophages and A549 lung epithelial cells (Vinzing et al., 2008). However, the mechanism by which human NAIP restricts *L. pneumophila* replication is yet to be elucidated.

THE ACTIVATION OF SURVIVAL PATHWAYS IN *L. PNEUMOPHILA*-INFECTED HUMAN CELLS

Studies with the human macrophage-like U937 and human epithelial cells A549 showed that *L. pneumophila* induces the activation of the transcriptional regulator nuclear factor κB (NF-κB) upon infection (Losick and Isberg, 2006; Abu-Zant et al., 2007; Schmeck et al., 2007; Bartfeld et al., 2009). *L. pneumophila* induces biphasic activation of the NF-κB pathway. The early phase is strong, but transient and is flagellin, TLR5 and MYD88-dependent. It is terminated quickly to prevent the autotoxic overproduction of inflammatory mediators. *L. pneumophila* activates the NF-κB pathway in human epithelial cells leading to secretion of several cytokines and chemokines (Schmeck et al., 2007). This activation is beneficial for the host since it triggers the innate immune response. The second phase of NF-κB activation is characterized by long-term activation for up to 30 h. However, this phase is flagellin, TLR5, MYD88-independent (Bartfeld et al., 2009). The long-term activation of NF-κB is linked to bacterial replication and upregulation of anti-apoptotic genes (Bartfeld et al., 2009). Therefore, this phase seems to be beneficial for the pathogen. The mechanism for this late stage NF-κB activation is still unclear.

THE ROLE OF CASPASE-3 IN *L. PNEUMOPHILA* INFECTION OF HUMAN CELLS

The host can employ several mechanisms to overcome intracellular infection. Among these, is the elimination of infected cells by caspase-mediated apoptosis. Arising reports demonstrate new distinct roles for executioner caspases independent of cell death (Li and Yuan, 2008; Walsh et al., 2008; Amer, 2010). *L. pneumophila* induces caspase-3 activation in human macrophages and U937 (Gao and Abu Kwaik, 1999a,b; Zink et al., 2002; Molmeret et al., 2004; Santic et al., 2007). The earlier studies with macrophages and epithelial cells demonstrated that high numbers of *L. pneumophila* induces apoptosis through activation of caspase-3 (Gao and Abu Kwaik, 1999a; Neumeister et al., 2001). Remarkably, other studies demonstrated that the early caspase-3 activation by *L. pneumophila* is independent of the intrinsic and extrinsic pathways of apoptosis (Molmeret et al., 2004). Notably, at physiological levels of infection, *L. pneumophila* utilizes the early mild caspase-3 activation in human cells to evade the phagosome–lysosome fusion. Activation of caspase-3 by low numbers of *L. pneumophila* is accompanied with cleavage of rabaptin-5, a downstream effector of rab-5 (Molmeret et al., 2004). Rab-5 GTPase is involved in the maturation of the early endosome. Therefore, according to the number of *L. pneumophila* invading a human cell, caspase-3 activation can lead either to apoptosis or to delayed phagosome maturation.

MODULATION OF THE UBIQUITINATION MACHINERY BY *L. PNEUMOPHILA*

Several studies showed that in human cells, the LCV is decorated with polyubiquitinated proteins upon *L. pneumophila* infection (Ivanov and Roy, 2009). Ubiquitination is a reversible post-translational modification that controls the abundance of many critical regulatory proteins (Craig and Tyers, 1999; Lomma et al., 2010). Degradation of intracellular proteins is mediated via means of the ubiquitin system and its specificity is determined at the level of substrate recognition by the E3 ubiquitin ligases, that catalyzes binding of the activated ubiquitin to the target protein for degradation (Craig and Tyers, 1999). Additionally, the *L. pneumophila* genome encodes for proteins with F-box and U-box domains (Cazalet et al., 2004; Price et al., 2009; Lomma et al., 2010) that are similar to eukaryotic SCF ubiquitin ligases. F-box proteins are known components of E3 ubiquitin ligases named SCF complexes. The F-box motif within the F-box protein interacts with the components of the ubiquitin machinery while the protein–protein interaction domain selectively binds to specific substrate. The U-box is a domain of approximately 70 amino acids that is present in proteins from yeast to humans. Mammalian U-box proteins in conjugation with E1 and E2 mediate protein ubiquitination, and thus have been classified as E3 ligases (Hatakeyama et al., 2001). Three F-box-containing proteins have been shown to be required for intracellular replication of *L. pneumophila* in THP-1 macrophages and A549 cells, and single, double, or triple mutations in the F-box proteins impair infection within these cells (Lomma et al., 2010). Al-Khodor et al. (2008) showed that the F-box-containing protein AnkB is required for intracellular replication of *L. pneumophila* within human monocytes-derived macrophages (hMDM). Furthermore, AnkB translocated to the host cytosol via the type IV secretion system mediates *L. pneumophila* replication via decorating the LCV with polyubiquitinated

proteins (Price et al., 2009). The focal adhesion protein ParvB, is the target for AnkB (Lomma et al., 2010), and AnkB interferes with the ubiquitination of the ParvB, likely by competing with the eukaryotic E3 ligase for the protein–protein interaction domain of ParvB (Lomma et al., 2010). Therefore, *L. pneumophila* hijacks the host cell ubiquitination system to mediate intracellular replication in human cells.

THE ROLE OF CYTOKINES DURING *L. PNEUMOPHILA* INFECTION IN HUMAN CELLS

Other innate immune mechanisms might modulate *L. pneumophila* infection in human cells, including cytokine production such as Interferon gamma-γ (INF-γ) and Tumor necrosis-α (TNF-α) (Coers et al., 2007). Induction of innate immunity includes macrophage activation by the antimicrobial cytokine IFN-γ, protection by TNF-α, and production of IL-6 and IL-1, as well as chemokines from inflammatory cells (Friedman et al., 2002). *In vitro* studies with primary human macrophages or cell lines derived from human macrophages that are activated with INF-γ and TNF-α restricted *L. pneumophila* replication (Bhardwaj et al., 1986; Nash et al., 1988; Matsiota-Bernard et al., 1993). Furthermore, pretreatment of hMDM with INF-γ inhibited intracellular replication of *L. pneumophila* and restricted the multiplication of the pathogen (Santic et al., 2005). The authors speculate that these processes are distinct between human and mouse macrophages and ultimately determine the permissiveness to *L. pneumophila*. TNF-α has been detected in the broncho-alveolar lavage fluid during *L. pneumophila* infection (Blanchard et al., 1987). It is produced readily by human peripheral blood lymphocytes in response to *L. pneumophila* antigens (Friedman et al., 2002). TNF-α is a potent cytokine that enhances the bactericidal activity of the macrophages. Furthermore, inhibition of TNF-α production is associated with increased bacterial replication (Nash et al., 1984; Matsiota-Bernard et al., 1993).

In contrast to the macrophage activating cytokines INF-γ and TNF-α, Interleukin-10 (IL-10) is an inhibitory cytokine. IL-10 treatment significantly enhances *L. pneumophila* growth in monocytes, and completely reverses the protective effect of INF-γ on *L. pneumophila* replication (Park and Skerrett, 1996). IL-10 has similar, but less potent, effects on alveolar macrophages and INF-γ-activated alveolar macrophages are less sensitive to deactivation by IL-10. Park and Skerrett (1996) identified important differences in alveolar macrophages and monocytes response to *L. pneumophila* infection and to IL-10. They also suggested that the induction of IL-10 during *L. pneumophila* infection may be a virulence mechanism that promotes intracellular bacterial replication. Therefore, the local balance between the INF-γ and IL-10 production by *L. pneumophila* might determine if monocytes that are recruited to the site of infection will become effector cells or develop into susceptible targets available for bacterial growth.

Cell-mediated immunity seems to play a critical role in restricting *L. pneumophila* replication, and the activated human monocytes have the capacity to inhibit the multiplication of virulent *L. pneumophila* both via decreasing the phagocytosis and the rate of intracellular multiplication (Horwitz and Silverstein, 1981b). Consistent with that, patients with LD produce less INF-γ than non-LD patients, suggesting that impairment in the INF-γ response may increase the susceptibility to the disease (Lettinga et al., 2003).

However, patients with acute LD are able to restrict *L. pneumophila* replication as a result elevated level of the Th1-cytokines such as INF-γ and IL-12 (Takeda et al., 2003). Sera from patients with *L. pneumophila* contain antibodies to *L. pneumophila* protein antigens (Friedman et al., 2002). However, humoral immunity seems to play a limited role in host defense against LD. Horwitz and Silverstein (1981a) demonstrated that complement and antibody, which promote entry of *L. pneumophila* into monocytes, do not inhibit the rate of *L. pneumophila* multiplication in monocytes.

OTHER IMMUNE RESPONSES TO *L. PNEUMOPHILA* IN HUMAN CELLS

Another major strategy of innate resistance to intracellular infection is to sequester key nutrients, such as iron, from invading bacteria. Within host cells, iron is predominantly bound to proteins and unavailable in free soluble form. Under iron-limited conditions, *L. pneumophila* secretes a high affinity iron chelator named siderophore, which sequesters iron from the host and the surrounding environment (Liles et al., 2000; Cianciotto, 2007). *L. pneumophila* utilizes iron to maintain its replication in human monocytes and macrophages. Consistently, treatment of human monocytes and macrophages with iron chelator aborts *L. pneumophila* replication (Gebran et al., 1994; Viswanathan et al., 2000). Accordingly, restrictive monocytes seem to exhibit low level of expression of transferrin receptor similar to the low level of expression of transferrin receptor in INF-γ activated monocytes (Byrd and Horwitz, 2000). Consequently, supplementation of iron abolishes the effect of INF-γ-mediated growth restriction of *L. pneumophila* in hMDM (Santic et al., 2005).

Bacterial infection stimulates the production of reactive nitrogen intermediate by the inducible nitric oxide synthase (iNOS). Transcription of NOS2 is mediated by type I and type II interferons; suggesting that the production of nitric oxide (NO) is not an immediate response for the infection. NO mediates its action on bacteria in the vacuole or the cytosol. However, stimulation with 1,25-dihydroxyvitamin D(3) to enhance NO production or the inhibition of NO production by treatment with *N*(G)-methyl-L-arginine did not modify the intracellular multiplication of *L. pneumophila* within MM6. This study suggested that NO radicals do not play a role in restricting different *L. pneumophila* species in human monocytic cell lines (Neumeister et al., 2001). So far, the role of NO radicals in human macrophages seems to be controversial (Neumeister et al., 2001; Radtke and O'Riordan, 2006).

GENETIC SUSCEPTIBILITY TO LEGIONNAIRES' DISEASE

While the genetic factors that predispose individuals for LD are not well known, it has been shown that TLR5 influences human susceptibility to *L. pneumophila* infection. TLR5 recognizes bacterial flagellin (Hayashi et al., 2001) and is the predominant mediator of Interleukin-8 (IL-8) in lung epithelial cells A549. A common stop codon polymorphism in the ligand-binding domain of TLR5 (TLR5[329STOP]) occurs in 10% of the population resulting in the inability to mediate flagellin signaling and is associated with increased risk to LD (Hawn et al., 2003). TLR5[329STOP] has a dominant negative effect and peripheral blood monocytes (PBMCs) from heterozygous individuals stimulated with flagellin had less IL-6 and IL-8 secretion. Likely, TLR5[329STOP] mutation increases susceptibility to

LD by decreasing the cytokine secretion. However, the role of the mouse TLR5 in *L. pneumophila* infection is controversial (Hawn et al., 2007; Archer et al., 2009). On the other hand, polymorphism in the TLR4 receptor, SNP A896G, is associated with resistance to LD in heterozygous individuals (Hawn et al., 2005).

Another factor associated with resistance to *L. pneumophila* infection is the mannose-binding lectin (MBL), a PRR of the innate immune system that activates complement via the lectin pathway (Turner, 1996). MBL deficiency compromises opsonization by complement leading to an increased risk of infection. In infection with intracellular pathogens, the role of MBL deficiency is more ambiguous, as more intracellular pathogens use opsonization by MBL to enter their host cell. Herpers et al. (2009) showed that Legionellosis is not associated with MBL deficiency. However, a large number of patients with Legionellosis displayed deficient MBL-mediated complement activation even though they carried MBL-sufficient genotypes. Furthermore, patients with MBL-sufficient genotypes showed functional deficiency at the acute phase of disease, yet the lectin pathway functionality

was restored at convalescence. However, the deficiency of lectin pathway seems to be an effect rather than a risk factor for LD development (Herpers et al., 2009).

CONCLUSIONS

Legionella pneumophila exploits several strategies to overcome the innate immune defense in human phagocytes (**Figure 1**). However, most of these converge toward preventing the fusion of its phagosome with the lysosome, inhibiting host cell apoptosis, maintaining host cell survival, modulating the local balance between activating and inhibiting cytokines, sequestering nutrients from the host and overcoming the killing mechanisms available within phagocytes.

ACKNOWLEDGMENTS

REFERENCES

Abdelaziz, D. H., Amr, K., and Amer, A. O. (2010). Nlrc4/Ipaf/CLAN/CARD12: more than a flagellin sensor. *Int. J. Biochem. Cell Biol.* 42, 789–791.

Abu Kwaik, Y., Eisenstein, B. I., and Engleberg, N. C. (1993). Phenotypic modulation by *Legionella pneumophila* upon infection of macrophages. *Infect. Immun.* 61, 1320–1329.

Abu-Zant, A., Jones, S., Asare, R., Suttles, J., Price, C., Graham, J., and Kwaik, Y. A. (2007). Anti-apoptotic signalling by the Dot/Icm secretion system of *L. pneumophila. Cell. Microbiol.* 9, 246–264.

Akhter, A., Gavrilin, M. A., Frantz, L., Washington, S., Ditty, C., Limoli, D., Day, C., Sarkar, A., Newland, C., Butchar, J., Marsh, C. B., Wewers, M. D., Tridandapani, S., Kanneganti, T.-D., and Amer, A. O. (2009). Caspase-7 activation by the Nlrc4/Ipaf inflammasome restricts *Legionella pneumophila* infection. *PLoS Pathog.* 5, e1000361. doi: 10.1371/journal.ppat.1000361.

Akira, S., Uematsu, S., and Takeuchi, O. (2006). Pathogen recognition and innate immunity. *Cell* 124, 783–801.

Al-Khodor, S., Price, C. T., Habyarimana, F., Kalia, A., and Abu Kwaik, Y. (2008). A Dot/Icm-translocated ankyrin protein of *Legionella pneumophila* is required for intracellular proliferation within human macrophages and protozoa. *Mol. Microbiol.* 70, 908–923.

Amer, A., Franchi, L., Kanneganti, T. D., Body-Malapel, M., Ozören, N., Brady, G., Meshinchi, S., Jagirdar, R., Gewirtz, A., Akira, S., and Núñez, G. (2006). Regulation of *Legionella* phagosome maturation and infection through flagellin and host Ipaf. *J. Biol. Chem.* 281, 35217–35223.

Amer, A. O. (2010). Modulation of caspases and their non-apoptotic functions by *Legionella pneumophila. Cell. Microbiol.* 12, 140–147.

Archer, K. A., Alexopoulou, L., Flavell, R. A., and Roy, C. R. (2009). Multiple MyD88-dependent responses contribute to pulmonary clearance of *Legionella pneumophila. Cell. Microbiol.* 11, 21–36.

Barker, J., Brown, M. R., Collier, P. J., Farrell, I., and Gilbert, P. (1992). Relationship between *Legionella pneumophila* and *Acanthamoeba polyphaga*: physiological status and susceptibility to chemical inactivation. *Appl. Environ. Microbiol.* 58, 2420–2425.

Barker, J., Scaife, H., and Brown, M. R. (1995). Intraphagocytic growth induces an antibiotic-resistant phenotype of *Legionella pneumophila. Antimicrob. Agents Chemother.* 39, 2684–2688.

Bartfeld, S., Engels, C., Bauer, B., Aurass, P., Flieger, A., Bruggemann, H., and Meyer, T. F. (2009). Temporal resolution of two-tracked NF-kappaB activation by *Legionella pneumophila. Cell. Microbiol.* 11, 1638–1651.

Beckers, M. C., Yoshida, S., Morgan, K., Skamene, E., and Gros, P. (1995). Natural resistance to infection with *Legionella pneumophila*: chromosomal localization of the Lgn1 susceptibility gene. *Mamm. Genome* 6, 540–545.

Bhardwaj, N., Nash, T. W., and Horwitz, M. A. (1986). Interferon-gamma-activated human monocytes inhibit the intracellular multiplication of *Legionella pneumophila. J. Immunol.* 137, 2662–2669.

Blanchard, D. K., Djeu, J. Y., Klein, T. W., Friedman, H., and Stewart, W. E. (1987). Induction of tumor necrosis

factor by *Legionella pneumophila. Infect. Immun.* 55, 433–437.

Bozue, J. A., and Johnson, W. (1996). Interaction of *Legionella pneumophila* with *Acanthamoeba castellanii*: uptake by coiling phagocytosis and inhibition of phagosome–lysosome fusion. *Infect. Immun.* 64, 668–673.

Byrd, T. F., and Horwitz, M. A. (2000). Aberrantly low transferrin receptor expression on human monocytes is associated with nonpermissiveness for *Legionella pneumophila* growth. *J. Infect. Dis.* 181, 1394–1400.

Carratala, J., Gudiol, F., Pallares, R., Dorca, J., Verdaguer, R., Ariza, J., and Manresa, F. (1994). Risk factors for nosocomial *Legionella pneumophila* pneumonia. *Am. J. Respir. Crit. Care Med.* 149(Pt 1), 625–629.

Cazalet, C., Rusniok, C., Brüggemann, H., Zidane, N., Magnier, A., Ma, L., Tichit, M., Jarraud, S., Bouchier, C., Vandenesch, F., Kunst, F., Etienne, J., Glaser, P., and Buchrieser, C. (2004). Evidence in the *Legionella pneumophila* genome for exploitation of host cell functions and high genome plasticity. *Nat. Genet.* 36, 1165–1173.

Cianciotto, N. P. (2007). Iron acquisition by *Legionella pneumophila. Biometals* 20, 323–331.

Cirillo, J. D., Falkow, S., and Tompkins, L. S. (1994). Growth of *Legionella pneumophila* in *Acanthamoeba castellanii* enhances invasion. *Infect. Immun.* 62, 3254–3261.

Coers, J., Vance, R. E., Fontana, M. F., and Dietrich, W. F. (2007). Restriction of *Legionella pneumophila* growth in macrophages requires the concerted action of cytokine and Naip5/Ipaf signalling pathways. *Cell. Microbiol.* 9, 2344–2357.

Craig, K. L., and Tyers, M. (1999). The F-box: a new motif for ubiquitin dependent proteolysis in cell cycle regulation and signal transduction. *Prog. Biophys. Mol. Biol.* 72, 299–328.

Creagh, E. M., Conroy, H., and Martin, S. J. (2003). Caspase-activation pathways in apoptosis and immunity. *Immunol. Rev.* 193, 10–21.

Damiano, J. S., Stehlik, C., Pio, F., Godzik, A., and Reed, J. C. (2001). CLAN, a novel human CED-4-like gene. *Genomics* 75, 77–83.

Den Boer, J. W., Yzerman, E. P., Schellekens, J., Lettinga, K. D., Boshuizen, H. C., Van Steenbergen, J. E., Bosman, A., Van den Hof, S., Van Vliet, H. A., Peeters, M. F., Van Ketel, R. J., Speelman, P., Kool, J. L., and Conyn-Van Spaendonck, M. A. (2002). A large outbreak of Legionnaires' disease at a flower show, the Netherlands, 1999. *Emerging Infect. Dis.* 8, 37–43.

Diez, E., Lee, S. H., Gauthier, S., Yaraghi, Z., Tremblay, M., Vidal, S., and Gros, P. (2003). Birc1e is the gene within the Lgn1 locus associated with resistance to *Legionella pneumophila. Nat. Genet.* 33, 55–60.

Fink, S. L., and Cookson, B. T. (2005). Apoptosis, pyroptosis, and necrosis: mechanistic description of dead and dying eukaryotic cells. *Infect. Immun.* 73, 1907–1916.

Fliermans, C. B., Cherry, W. B., Orrison, L. H., Smith, S. J., Tison, D. L., and Pope, D. H. (1981). Ecological distribution of *Legionella pneumophila. Appl. Environ. Microbiol.* 41, 9–16.

Fraser, D. W., Deubner, D. C., Hill, D. L., and Gilliam, D. K. (1979). Nonpneumonic, short-incubation-period Legionellosis (Pontiac fever) in men who cleaned a

steam turbine condenser. *Science* 205, 690–691.

Friedman, H., Yamamoto, Y., and Klein, T. W. (2002). *Legionella pneumophila* pathogenesis and immunity. *Semin. Pediatr. Infect. Dis.* 13, 273–279.

Gao, L. Y., and Abu Kwaik, Y. (1999a). Activation of caspase 3 during *Legionella pneumophila*-induced apoptosis. *Infect. Immun.* 67, 4886–4894.

Gao, L. Y., and Abu Kwaik, Y. (1999b). Apoptosis in macrophages and alveolar epithelial cells during early stages of infection by *Legionella pneumophila* and its role in cytopathogenicity. *Infect. Immun.* 67, 862–870.

Gebran, S. J., Newton, C., Yamamoto, Y., Widen, R., Klein, T. W., and Friedman, H. (1994). Macrophage permissiveness for *Legionella pneumophila* growth modulated by iron. *Infect. Immun.* 62, 564–568.

Goldstein, E., Lippert, W., and Warshauer, D. (1974). Pulmonary alveolar macrophage. Defender against bacterial infection of the lung. *J. Clin. Invest.* 54, 519–528.

Green, G. M. (1970). The J. Burns Amberson Lecture – in defense of the lung. *Am. Rev. Respir. Dis.* 102, 691–703.

Hatakeyama, S., Yada, M., Matsumoto, M., Ishida, N., and Nakayama, K. I. (2001). U box proteins as a new family of ubiquitin-protein ligases. *J. Biol. Chem.* 276, 33111–33120.

Hawn, T. R., Berrington, W. R., Smith, I. A., Uematsu, S., Akira, S., Aderem, A., Smith, K. D., and Skerrett, S. J. (2007). Altered inflammatory responses in TLR5-deficient mice infected with *Legionella pneumophila*. *J. Immunol.* 179, 6981–6987.

Hawn, T. R., Verbon, A., Lettinga, K. D., Zhao, L. P., Li, S. S., Laws, R. J. Skerrett, S. J., Beutler, B., Schroeder, L., Nachman, A., Ozinsky, A., Smith, K. D., and Aderem, A. (2003). A common dominant TLR5 stop codon polymorphism abolishes flagellin signaling and is associated with susceptibility to legionnaires' disease. *J. Exp. Med.* 198, 1563–1572.

Hawn, T. R., Verbon, A., Janer, M., Zhao, L. P., Beutler, B., and Aderem, A. (2005). Toll-like receptor 4 polymorphisms are associated with resistance to Legionnaires' disease. *Proc. Natl. Acad. Sci. U.S.A.* 102, 2487–2489.

Hayashi, F., Smith, K. D., Ozinsky, A., Hawn, T. R., Yi, E. C., Goodlett, D. R., Eng, J. K., Akira, S., Underhill, D. M., and Aderem, A. (2001). The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. *Nature* 410, 1099–1103.

Herpers, B. L., Yzerman, E. P., de Jong, B. A., Bruin, J. P., Lettinga, K. D., Kuipers, S., Den Boer, J. W., van Hannen, E. J., Rijkers, G. T., van Velzen-Blad, H., and de Jongh, B. M. (2009). Deficient mannose-binding lectin-mediated complement activation despite mannose-binding lectin-sufficient genotypes in an outbreak of *Legionella pneumophila* pneumonia. *Hum. Immunol.* 70, 125–129.

Horwitz, M. A. (1983a). Formation of a novel phagosome by the Legionnaires' disease bacterium (*Legionella pneumophila*) in human monocytes. *J. Exp. Med.* 158, 1319–1331.

Horwitz, M. A. (1983b). The Legionnaires' disease bacterium (*Legionella pneumophila*) inhibits phagosome–lysosome fusion in human monocytes. *J. Exp. Med.* 158, 2108–2126.

Horwitz, M. A. (1984). Phagocytosis of the Legionnaires' disease bacterium (*Legionella pneumophila*) occurs by a novel mechanism: engulfment within a pseudopod coil. *Cell* 36, 27–33.

Horwitz, M. A., and Maxfield, F. R. (1984). *Legionella pneumophila* inhibits acidification of its phagosome in human monocytes. *J. Cell Biol.* 99, 1936–1943.

Horwitz, M. A., and Silverstein, S. C. (1980). Legionnaires' disease bacterium (*Legionella pneumophila*) multiples intracellularly in human monocytes. *J. Clin. Invest.* 66, 441–450.

Horwitz, M. A., and Silverstein, S. C. (1981a). Activated human monocytes inhibit the intracellular multiplication of Legionnaires' disease bacteria. *J. Exp. Med.* 154, 1618–1635.

Horwitz, M. A., and Silverstein, S. C. (1981b). Interaction of the Legionnaires' disease bacterium (*Legionella pneumophila*) with human phagocytes. I. *L. pneumophila* resists killing by polymorphonuclear leukocytes, antibody, and complement. *J. Exp. Med.* 153, 386–397.

Isberg, R. R., O'Connor, T. J., and Heidtman, M. (2009). The *Legionella pneumophila* replication vacuole: making a cosy niche inside host cells. *Nat. Rev. Microbiol.* 7, 13–24.

Ivanov, S. S., and Roy, C. R. (2009). Modulation of ubiquitin dynamics and suppression of DALIS formation by the *Legionella pneumophila* Dot/Icm system. *Cell. Microbiol.* 11, 261–278.

Janeway, C. A. Jr., and Medzhitov, R. (2002). Innate immune recognition. *Annu. Rev. Immunol.* 20, 197–216.

Kagan, J. C., and Roy, C. R. (2002). *Legionella* phagosomes intercept vesicular traffic from endoplasmic reticulum exit sites. *Nat. Cell Biol.* 4, 945–954.

Kuiper, M. W., Wullings, B. A., Akkermans, A. D., Beumer, R. R., and van der Kooij, D. (2004). Intracellular proliferation of *Legionella pneumophila* in *Hartmannella vermiformis* in aquatic biofilms grown on plasticized polyvinyl chloride. *Appl. Environ. Microbiol.* 70, 6826–6833.

Lettinga, K. D., Verbon, A., Weverling, G. J., Schellekens, J. F., Den Boer, J. W., Yzerman, E. P., Prins, J., Boersma, W. G., van Ketel, R. J., Prins, J. M., and Speelman, P. (2002). Legionnaires' disease at a Dutch flower show: prognostic factors and impact of therapy. *Emerging Infect. Dis.* 8, 1448–1454.

Lettinga, K. D., Weijer, S., Speelman, P., Prins, J. M., Van Der Poll, T., and Verbon, A. (2003). Reduced interferon-gamma release in patients recovered from Legionnaires' disease. *Thorax* 58, 63–67.

Li, J., and Yuan, J. (2008). Caspases in apoptosis and beyond. *Oncogene* 27, 6194–6206.

Liang, J. L., Dziuban, E. J., Craun, G. F., Hill, V., Moore, M. R., Gelting, R. J., Calderon, R. L., Beach, M. J., Roy, S. L., and Centers for Disease Control and Prevention (CDC). (2006). Surveillance for waterborne disease and outbreaks associated with drinking water and water not intended for drinking – United States, 2003–2004. *MMWR Surveill. Summ.* 55, 31–65.

Liles, M. R., Scheel, T. A., and Cianciotto, N. P. (2000). Discovery of a nonclassical siderophore, legiobactin, produced by strains of *Legionella pneumophila*. *J. Bacteriol.* 182, 749–757.

Liston, P., Roy, N., Tamai, K., Lefebvre, C., Baird, S., Cherton-Horvat, G., Farahani, R., McLean, M., Ikeda, J. E., MacKenzie, A., and Korneluk, R. G. (1996). Suppression of apoptosis in mammalian cells by NAIP and a related family of IAP genes. *Nature* 379, 349–353.

Lomma, M., Dervins-Ravault, D., Rolando, M., Nora, T., Newton, H. J., Samson, F. M., Sahr, T., Gomez-Valero, L., Jules, M., Hartland, E. L., and Buchrieser, C. (2010). The *Legionella pneumophila* F-box protein Lpp2082 (AnkB) modulates ubiquitination of the host protein parvin B and promotes intracellular replication. *Cell. Microbiol.* 12, 1272–1291.

Losick, V. P., and Isberg, R. R. (2006). NF-kappaB translocation prevents host cell death after low-dose challenge by *Legionella pneumophila*. *J. Exp. Med.* 203, 2177–2189.

Marra, A., Horwitz, M. A., and Shuman, H. A. (1990). The HL-60 model for the interaction of human macrophages with the Legionnaires' disease bacterium. *J. Immunol.* 144, 2738–2744.

Marston, B. J., Lipman, H. B., and Breiman, R. F. (1994). Surveillance for Legionnaires' disease. Risk factors for morbidity and mortality. *Arch. Intern. Med.* 154, 2417–2422.

Marston, B. J., Plouffe, J. F., File, T. M., Jr., Hackman, B. A., Salstrom, S. J., Lipman, H. B., Kolczak, M. S., and Breiman, R. F. (1997). Incidence of community-acquired pneumonia requiring hospitalization. Results of a population-based active surveillance Study in Ohio. The Community-Based Pneumonia Incidence Study Group. *Arch. Intern. Med.* 157, 1709–1718.

Martinon, F., and Tschopp, J. (2004). Inflammatory caspases: linking an intracellular innate immune system to autoinflammatory diseases. *Cell* 117, 561–574.

Maruta, K., Miyamoto, H., Hamada, T., Ogawa, M., Taniguchi, H., and Yoshida, S. (1998). Entry and intracellular growth of *Legionella dumoffii* in alveolar epithelial cells. *Am. J. Respir. Crit. Care Med.* 157(Pt 1), 1967–1974.

Matsiota-Bernard, P., Lefebre, C., Sedqui, M., Cornillet, P., and Guenounou, M. (1993). Involvement of tumor necrosis factor alpha in intracellular multiplication of *Legionella pneumophila* in human monocytes. *Infect. Immun.* 61, 4980–4983.

Molmeret, M., Zink, S. D., Han, L., Abu-Zant, A., Asari, R., Bitar, D. M., and Abu Kwaik, Y. (2004). Activation of caspase-3 by the Dot/Icm virulence system is essential for arrested biogenesis of the *Legionella*-containing phagosome. *Cell. Microbiol.* 6, 33–48.

Nash, T. W., Libby, D. M., and Horwitz, M. A. (1984). Interaction between the legionnaires' disease bacterium (*Legionella pneumophila*) and human alveolar macrophages. Influence of antibody, lymphokines, and hydrocortisone. *J. Clin. Invest.* 74, 771–782.

Nash, T. W., Libby, D. M., and Horwitz, M. A. (1988). IFN-gamma-activated human alveolar macrophages inhibit the intracellular multiplication of *Legionella pneumophila*. *J. Immunol.* 140, 3978–3981.

Neumeister, B. (2004). Intracellular multiplication of *Legionella* species and the influence of amoebae on their intracellular growth in human monocytes: mono mac 6 cells and *Acanthamoeba castellanii* as suitable in vitro models. *Methods Mol. Biol.* 268, 141–151.

Neumeister, B., Bach, V., Faigle, M., and Northoff, H. (2001). Induction of iNOS in human monocytes infected with different *Legionella* species. *FEMS Microbiol. Lett.* 202, 31–38.

Neumeister, B., Schoniger, S., Faigle, M., Eichner, M., and Dietz, K. (1997). Multiplication of different *Legionella* species in Mono Mac 6 cells and in *Acanthamoeba castellanii*. *Appl. Environ. Microbiol.* 63, 1219–1224.

Park, D. R., and Skerrett, S. J. (1996). IL-10 enhances the growth of

Legionella pneumophila in human mononuclear phagocytes and reverses the protective effect of IFN-gamma: differential responses of blood monocytes and alveolar macrophages. *J. Immunol.* 157, 2528–2538.

Payne, N. R., and Horwitz, M. A. (1987). Phagocytosis of *Legionella pneumophila* is mediated by human monocyte complement receptors. *J. Exp. Med.* 166, 1377–1389.

Pearlman, E., Jiwa, A. H., Engleberg, N. C., and Eisenstein, B. I. (1988). Growth of *Legionella pneumophila* in a human macrophage-like (U937) cell line. *Microb. Pathog.* 5, 87–95.

Poyet, J.L., Srinivasula, S. M., Tnani, M., Razmara, M., Fernandes-Alnemri, T., and Alnemri, E. S. (2001). Identification of Ipaf, a human caspase-1-activating protein related to Apaf-1. *J. Biol. Chem.* 276, 28309–28313.

Price, C. T., Al-Khodor, S., Al-Quadan, T., Santic, M., Habyarimana, F., Kalia, A., and Kwaik, Y. A. (2009). Molecular mimicry by an F-box effector of *Legionella pneumophila* hijacks a conserved polyubiquitination machinery within macrophages and protozoa. *PLoS Pathog.* 5, e1000704. doi: 10.1371/journal.ppat.1000704.

Radtke, A. L., and O'Riordan, M. X. (2006). Intracellular innate resistance to bacterial pathogens. *Cell. Microbiol.* 8, 1720–1729.

Rogers, J., Dowsett, A. B., Dennis, P. J., Lee, J. V., and Keevil, C. W. (1994). Influence of plumbing materials on biofilm formation and growth of *Legionella pneumophila* in potable water systems. *Appl. Environ. Microbiol.* 60, 1842–1851.

Rovera, G., Santoli, D., and Damsky, C. (1979). Human promyelocytic leukemia cells in culture differentiate into macrophage-like cells when treated with a phorbol diester. *Proc. Natl. Acad. Sci. U.S.A.* 76, 2779–2783.

Rowbotham, T. J. (1980). Preliminary report on the pathogenicity of *Legionella pneumophila* for freshwater and soil amoebae. *J. Clin. Pathol.* 33, 1179–1183.

Rowbotham, T. J. (1986). Current views on the relationships between amoebae, legionellae and man. *Isr. J. Med. Sci.* 22, 678–689.

Santic, M., Asare, R., Doric, M., and Abu Kwaik, Y. (2007). Host-dependent trigger of caspases and apoptosis by *Legionella pneumophila*. *Infect. Immun.* 75, 2903–2913.

Santic, M., Molmeret, M., and Abu Kwaik, Y. (2005). Maturation of the *Legionella pneumophila*-containing phagosome into a phagolysosome within gamma interferon-activated macrophages. *Infect. Immun.* 73, 3166–3171.

Schmeck, B., N'Guessan, P. D., Ollomang, M., Lorenz, J., Zahlten, J., Opitz, B., Flieger, A., Suttorp, N., and Hippenstiel, S. (2007). *Legionella pneumophila*-induced NF-kappaB- and MAPK-dependent cytokine release by lung epithelial cells. *Eur. Respir. J.* 29, 25–33.

Sethi, K. K., and Brandis, H. (1983). Direct demonstration and isolation of *Legionella pneumophila* (serogroup 1) from bathroom water specimens in a hotel. *Zentralbl. Bakteriol. Mikrobiol. Hyg. B* 177, 402–405.

Spitalny, K. C., Vogt, R. L., Orciari, L. A., Witherell, L. E., Etkind, P., and Novick, L. F. (1984). Pontiac fever associated with a whirlpool spa. *Am. J. Epidemiol.* 120, 809–817.

Swanson, M. S., and Isberg, R. R. (1995). Association of *Legionella pneumophila* with the macrophage endoplasmic reticulum. *Infect. Immun.* 63, 3609–3620.

Takahashi, R., Deveraux, Q., Tamm, I., Welsh, K., Assa-Munt, N., Salvesen, G. S., and Reed, J. C. (1998). A single BIR domain of XIAP sufficient for inhibiting caspases. *J. Biol. Chem.* 273, 7787–7790.

Takeda, K., Kaisho, T., and Akira, S. (2003). Toll-like receptors. *Annu. Rev. Immunol.* 21, 335–376.

Turner, M. W. (1996). Mannose-binding lectin: the pluripotent molecule of the innate immune system. *Immunol. Today* 17, 532–540.

Underhill, D. M., Ozinsky, A., Smith, K. D. and Aderem, A. (1999). Toll-like receptor-2 mediates mycobacteria-induced proinflammatory signaling in macrophages. *Proc. Natl. Acad. Sci. U.S.A.* 96, 14459–14463.

Vinzing, M., Eitel, J., Lippmann, J., Hocke, A. C., Zahlten, J., Slevogt, H., N'guessan, P. D., Günther, S., Schmeck, B., Hippenstiel, S., Flieger, A., Suttorp, N., and Opitz, B. (2008). NAIP and Ipaf control *Legionella pneumophila* replication in human cells. *J. Immunol.* 180, 6808–6815.

Viswanathan, V. K., Edelstein, P. H., Pope, C. D., and Cianciotto, N. P. (2000). The *Legionella pneumophila* iraAB locus is required for iron assimilation, intracellular infection, and virulence. *Infect. Immun.* 68, 1069–1079.

Walsh, J. G., Cullen, S. P., Sheridan, C., Luthi, A. U., Gerner, C., and Martin, S. J. (2008). Executioner caspase-3 and caspase-7 are functionally distinct proteases. *Proc. Natl. Acad. Sci. U.S.A.* 105, 12815–12819.

Watnick, P., and Kolter, R. (2000). Biofilm, city of microbes. *J. Bacteriol.* 182, 2675–2679.

Winn, W. C. Jr., and Myerowitz, R. L. (1981). The pathology of the *Legionella* pneumonias. A review of 74 cases and the literature. *Hum. Pathol.* 12, 401–422.

Wright, E. K., Goodart, S. A., Growney, J. D., Hadinoto, V., Endrizzi, M. G., Long, E. M., Sadigh, K., Abney, A. L., Bernstein-Hanley, I., and Dietrich, W. F. (2003). Naip5 affects host susceptibility to the intracellular pathogen *Legionella pneumophila*. *Curr. Biol.* 13, 27–36.

Yu, V. L., Plouffe, J. F., Pastoris, M. C., Stout, J. E., Schousboe, M., Widmer, A., Summersgill, J., File, T., Heath, C. M., Paterson, D. L., and Chereshsky, A. (2002). Distribution of *Legionella* species and serogroups isolated by culture in patients with sporadic community-acquired legionellosis: an international collaborative survey. *J. Infect. Dis.* 186, 127–128.

Ziegler-Heitbrock, H. W., Thiel, E., Futterer, A., Herzog, V., Wirtz, A., and Riethmuller, G. (1988). Establishment of a human cell line (Mono Mac 6) with characteristics of mature monocytes. *Int. J. Cancer* 41, 456–461.

Zink, S. D., Pedersen, L., Cianciotto, N. P., and Abu-Kwaik, Y. (2002). The Dot/Icm type IV secretion system of *Legionella pneumophila* is essential for the induction of apoptosis in human macrophages. *Infect. Immun.* 70, 1657–1663.

The Nlrc4 Inflammasome contributes to restriction of pulmonary infection by flagellated *Legionella* spp. that trigger pyroptosis

Marcelo S. F. Pereira, Gabriel G. Marques, José Eduardo DelLama and Dario S. Zamboni*

Department of Cell Biology, School of Medicine of Ribeirão Preto, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, São Paulo, Brazil

The Nlrc4 inflammasome is triggered in response to contamination of the host cell cytoplasm with bacterial flagellin, which induces pyroptosis, a form of cell death that accounts for restriction of bacterial infections. Although induction of pyroptosis has been extensively investigated in response to *Salmonella typhimurium* and *Legionella pneumophila*, little is known regarding the role of the inflammasome for restriction of non-pneumophila *Legionella* species. Here, we used five species of the *Legionella* genus to investigate the importance of the inflammasome for restriction of bacterial infection *in vivo*. By infecting mice deficient for inflammasome components, we demonstrated that caspase-1 and Nlrc4, but not Asc, contribute to restriction of pulmonary infection with *L. micdadei*, *L. bozemanii*, *L. gratiana*, and *L. rubrilucens*. *L. longbeachae*, a non-flagellated bacterium that fails to trigger pyroptosis, was not restricted by the inflammasome and induced death in the infected mice. In contrast to *L. longbeachae*, flagellin mutants of *L. pneumophila* did not induce mice death; therefore, besides bypassing the Nlrc4 inflammasome, *L. longbeachae* may employ additional virulence strategies to replicate in mammalian hosts. Collectively, our data indicate that the Nlrc4 inflammasome plays an important role in host protection against opportunistic pathogenic bacteria that express flagellin.

Keywords: *Legionella*, pulmonary infection, caspase-1, Nlrc4, inflammasome

INTRODUCTION

Innate immune cells employ germ line encoded pattern recognition receptors (PRRs) that are important for recognition of pathogen-associated molecular patterns (PAMPs), conserved structures present in microbes and pathogens (Janeway and Medzhitov, 2002). Extensively studied PRRs are the Toll-like receptors (TLRs), Rig-like receptors (RLRs), and Nod-like receptors (NLRs); this trinity of PRR plays an important role in recognition of bacterial, viruses, fungi, and parasitic infections (Kawai and Akira, 2009; Schroder and Tschopp, 2010). Whereas TLR are transmembrane proteins that signal for expression of inflammatory genes via transcriptional factors, the NLRs are cytosolic proteins that trigger diverse host cell processes.

Activation of NLRs, such as Nod1 and Nod2 turns on signaling cascades that culminate in activation of NF-κB and MAPK. Nod1 and Nod2 signaling requires the protein kinase Rip2, leading to expression of inflammatory genes, such as those of cytokines and chemokines (Franchi et al., 2008). Accordingly, mice deficient for Nod/Rip2 signaling were defective for recruitment of additional phagocytes for the sites of infection (Masumoto et al., 2006; Archer et al., 2010; Berrington et al., 2010; Frutuoso et al., 2010; Silva et al., 2010). Other members of the NLR family do not play an evident role in regulation of gene expression. Instead, they participate in the activation of the pro-caspase-1, which will lead to the formation of a multimeric complex called inflammasome (Schroder and Tschopp, 2010). Several inflammasomes have been described, among them the Nlrp3, Nlrc4, Nlrp1, and AMI2 inflammasomes (Pedra et al., 2009; Schroder and Tschopp, 2010). Nlrp3 inflammasome is possibly the most studied; it requires the adaptor protein Asc and leads to a strong inflammatory response mediated by the secretion of inflammatory cytokines, such as IL-1β and IL-18. The Nlrp3 inflammasome is triggered in response to diverse stimuli including several crystals, skin irritants, microbial PAMPs, microbial toxins, etc. (Schroder and Tschopp, 2010). The diverse range of stimulus that are able to trigger the Nlrp3 inflammasome led to the statement that Nlrp3 is a general sensor of host cell stress.

Regardless of the Nlrp3 inflammasome, the Nlrc4 inflammasome is another platform that also plays important roles for induction of inflammation and restriction of bacterial infection (Sutterwala and Flavell, 2009). The Nlrc4 inflammasome may be composed by another NLR called Naip5/Birc1e and may not require the adaptor protein Asc (Zamboni et al., 2006; Lightfield et al., 2008; Case et al., 2009). The Nlrc4 inflammasome was originally described as important for caspase-1 activation and host response against intracellular bacterial pathogens such as *Salmonella enterica* serotype Typhimurium (*S. typhimurium*) and *Legionella pneumophila* (Mariathasan et al., 2004; Amer et al., 2006; Franchi et al., 2006; Miao et al., 2006; Zamboni et al., 2006). It was shown that flagellin from *S. typhimurium* and *L. pneumophila* was required and sufficient to trigger the Nlrc4 inflammasome in macrophages (Franchi et al., 2006; Miao et al., 2006; Lightfield et al., 2008; Silveira and Zamboni, 2010; Whitfield et al., 2010). Furthermore, engagement of the Nlrc4 inflammasome contributed to pore formation, pyroptosis, and restriction of *L. pneumophila* replication both in macrophages and *in vivo* (Amer et al., 2006; Zamboni et al., 2006; Lightfield et al., 2008; Case et al., 2009; Miao et al., 2010; Silveira

and Zamboni, 2010; Whitfield et al., 2010). Therefore, the Nlrc4 plays important role in recognition, response, and resolution of the infection by flagellated pathogens. Although activation of the Nlrc4 inflammasome has being extensively investigated in response to several bacteria, little is known about the role of the inflammasome in restriction of the infection by opportunistic bacterial pathogens such as non-pneumophila *Legionella* spp.

Besides *L. pneumophila*, a number of *Legionella* species are found parasitizing ameba in freshwater environments, soil, and man-made water distribution system. About 20 *Legionella* species have being described as human pathogens based in isolation from clinical material (Muder and Yu, 2002; Gobin et al., 2009a). *L. pneumophila* may account to more than 90% of the human infection by *Legionella*. However, this estimative may be biased because appropriate diagnostic kits for non-pneumophila species are not often used. It is estimated that non-pneumophila species, such as *L. longbeachae, L. micdadei,* and *L. bozemanii,* account for approximately 2–7% of the infections worldwide (Muder and Yu, 2002; Gobin et al., 2009a). Other species such as *L. feeleii, L. dumoffii, L. wadsworthii,* and *L. anisa* were not rarely reported, but their frequency may increase with the development of more accurate kits for diagnostic. We and others have previously demonstrated that the flagellin expression is variable among non-pneumophila species of *Legionella* (Cazalet et al., 2010; Kozak et al., 2010; Silveira and Zamboni, 2010; Whitfield et al., 2010). Whereas species, such as *L. longbeachae* and *L. wadsworthii* fail to express flagellin, some species behave similarly to *L. pneumophila*: are flagellated and trigger Nlrc4-dependent responses in macrophages (Cazalet et al., 2010; Kozak et al., 2010; Silveira and Zamboni, 2010). Herein, we used four flagellated *Legionella* spp. and the non-flagellated *L. longbeachae* to assess the role of the inflammasome in restriction of bacterial infection *in vivo*. We found that Nlrc4, but not Asc, accounts for restriction of *in vivo* infection by the flagellated bacteria such as *L. micdadei, L. bozemanii, L. gratiana,* and *L. rubrilucens*. In contrast, *L. longbeachae* bypasses the Nlrc4 inflammasome-mediated growth restriction and may employ additional virulence strategies to survive in mammalian hosts.

MATERIALS AND METHODS
BACTERIAL STRAINS
Bacterial strains used were *L. gratiana* (ATCC 49413), *L. micdadei* (ATCC 33218), *L. bozemanii* (ATCC 33217), *L. rubrilucens* (ATCC 35304), and *L. longbeachae* (ATCC 33462). *L. pneumophila* used were JR32 and isogenic mutant for the flagellin (*flaA*) gene (Case et al., 2009). All bacteria grew in buffered charcoal-yeast extract (CYE) agar [1% yeast extract, 1% *N*-(2 acetamido)-2-aminoethanesulfonic (ACES), pH 6.9, 3.3 mM L-cysteine, 0.33 mM Fe(NO$_3$)$_3$, 1.5% Bacto agar, and 0.2% activated charcoal] at 37°C (Feeley et al., 1979). Before infection, bacteria were resuspended in sterile water and diluted in PBS to an appropriate MOI according to optical density (OD) at 600 nm.

MICE AND *IN VIVO* INFECTIONS
Mice were bred and maintained in the Animal Facility of the University of São Paulo at Ribeirão Preto. C57BL/6 mice were from institutional animal facility and caspase-1$^{-/-}$, Nlrc4$^{-/-}$, and

Asc$^{-/-}$ mice were previously described (Kuida et al., 1995; Lara-Tejero et al., 2006). Mice deficient for Nlrc4 or Caspase-1 were backcrossed to BL/6 mice for eight (caspase-1 and Nlrc4) or nine (Asc) generations. For *in vivo* infections, male or female mice from 10 to 14 weeks of age were anesthetized with 2,2,2-tribromoethanol (Sigma) by intraperitoneal administration followed by intranasal (i.n.) inoculation of 40 μl of PBS containing the bacteria at the indicated MOI. Mice were euthanized at the indicated time points for determination of CFU per lung. The lungs were harvested and homogenized in 10 ml of sterile water for 30 s using a tissue homogenizer (Fisher Scientific). Lung homogenates were diluted in water, plated on CYE agar plates and incubated at 37°C for determination of CFU. The limit of the detection of the assay was 10^2 CFU/lung. Survival rates were determined by daily examination of the infected animals. The mice protocols were previously approved by the institutional ethics committee for animal care and research (CETEA-FMRP/USP).

Statistical analysis
Statistical significance was calculated using the unpaired Student's *t*-test or one-way ANOVA, followed by multiple comparisons according to Tukey's procedure using GraphPad Prism v5 for Windows (GraphPad Software). The Kaplan–Meier method was used to compare survival curves. Differences were considered statistically significant if the *P* value was <0.05, indicated with an asterisk in the figures.

RESULTS
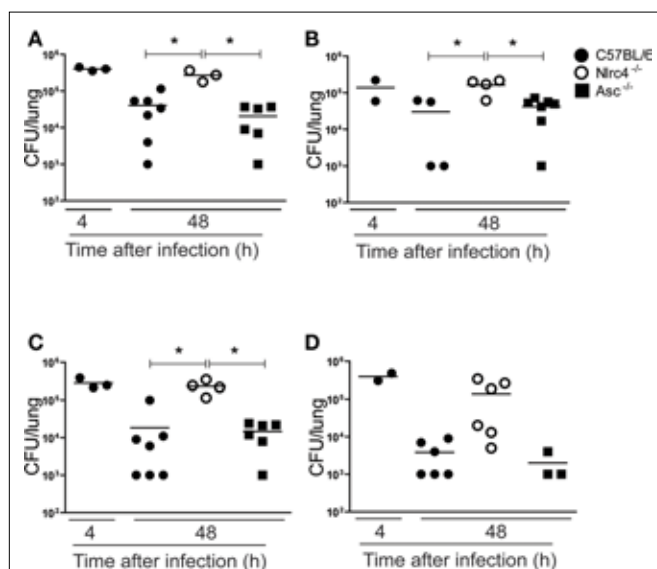### CASPASE-1 CONTRIBUTES FOR RESTRICTION OF PULMONARY INFECTION WITH *LEGIONELLA* spp. THAT TRIGGER PYROPTOSIS
We have previously demonstrated that similarly to *L. pneumophila*, other *Legionella* species such as *L. micdadei, L. bozemanii, L. gratiana,* and *L. rubrilucens* express flagellin and trigger pyroptosis in macrophages (Silveira and Zamboni, 2010). To investigate whether the inflammasome contributes to growth restriction of these species in a murine model of pulmonary infection, we infected C57BL/6 and caspase-1$^{-/-}$ mice with *L. micdadei, L. bozemanii, L. gratiana, and L. rubrilucens* and measured the CFU in the lungs 4, 48, and 96 h after infection. By comparing mice infected for 4, 48, and 96 h, we found that whereas wild-type mice restricted bacterial growth, the caspase-1-deficient mice showed impaired restriction of bacterial replication in the mice lungs (**Figure 1**).

Nlrc4, BUT NOT Asc, CONTRIBUTES FOR GROWTH RESTRICTION OF *LEGIONELLA* spp. THAT TRIGGER PYROPTOSIS
To investigate which inflammasome was responsible for caspase-1--dependent restriction of infection with *L. micdadei, L. bozemanii, L. gratiana,* and *L. rubrilucens*, we infected mice deficient for Nlrc4 or Asc and measured the CFU in the lungs 48 h after infection. We found that, for these four species, mice deficient for Nlrc4 restricted pulmonary infection less effectively than wild-type and mice deficient for Asc (**Figure 2**). These results indicate that the Nlrc4 inflammasome, but not an inflammasome-dependent on the adaptor protein Asc, contributed for growth restriction of flagellated *Legionella* species that trigger pyroptosis. Although statistically
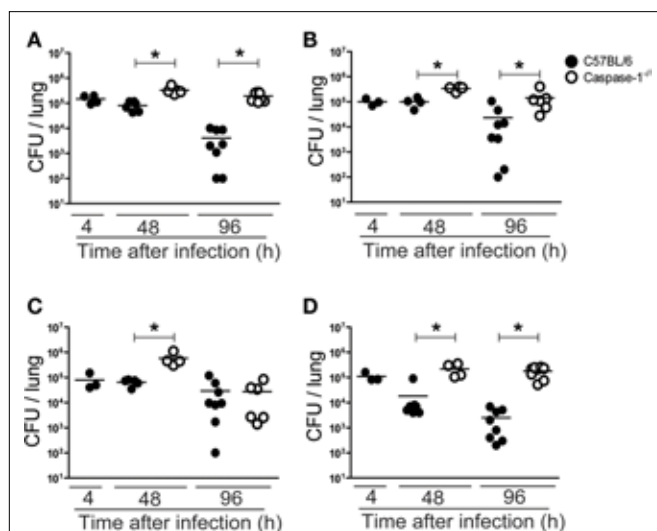
FIGURE 1 | Caspase-1 contributes for restriction of *in vivo* infection with *L. micdadei*, *L. bozemanii*, *L. gratiana,* and *L. rubrilucens*. C57BL/6 (closed circles) and caspase-1−/− (open circles) mice were infected intranasally with 1.0 × 10⁶ *Legionella* spp. per mouse. **(A)** *L. micdadei*. **(B)** *L. bozemanii*. **(C)** *L. gratiana*. **(D)** *L. rubrilucens*. Mice were sacrificed 4, 48, and 96 h after infection and dilutions of the lung homogenates were added to CYE agar plates for CFU determination. Each dot represents a single animal and the horizontal lines represent the average. Six to 10 mice were used per group. Data are representative of those found in four independent experiments. An asterisk indicates a *P* value of <0.05.


FIGURE 2 | Nlrc4, but not Asc, contributes for restriction of *in vivo* infection with *L. micdadei*, *L. bozemanii*, *L. gratiana,* and *L. rubrilucens*. C57BL/6 (closed circles), Nlrc4−/− (open circles), and Asc−/− (closed squares) mice were infected intranasally with 1.0 × 10⁶ *Legionella* spp. per mouse. **(A)** *L. micdadei*. **(B)** *L. bozemanii*. **(C)** *L. gratiana*. **(D)** *L. rubrilucens*. Mice were sacrificed 4 or 48 h after infection and dilutions of the lung homogenates were added to CYE agar plates for CFU determination. Each dot represents a single animal and the horizontal lines represent the average. Three to 10 mice were used per group. Data are representative of those found in three independent experiments. An asterisk indicates a *P* value of <0.05.

significant differences were not detected for *L. rubrilucens*, the data show a trend for increased bacterial numbers in the lungs of Nlrc4−/− mice (**Figure 2D**).

THE INFLAMMASOME DOES NOT PARTICIPATE IN THE RESTRICTION OF INFECTION BY *L. longbeachae IN VIVO*

Next, we investigated whether caspase-1 was important for *in vivo* restriction of the *L. longbeachae* replication, a non-flagellated bacteria that lacks genes important for flagellum biosynthesis (Cazalet et al., 2010; Kozak et al., 2010). To test if the inflammasome participated in the control of *L. longbeachae* infection *in vivo*, wild-type mice and those deficient for caspase-1 were infected for 48 h and the bacterial CFU in the lungs were counted. We found that caspase-1 deficiency did not influence bacterial replication in the lungs (**Figure 3A**). Furthermore, *L. longbeachae* was able to effectively multiply in the lungs of mice from 4 to 48 h of infection (**Figure 3A**). This information is consistent with previous reports showing that *L. longbeachae* is lethal for mice (Asare et al., 2007; Gobin et al., 2009b). To further evaluate the role of caspase-1 in mouse resistance, we performed infections in wild-type and caspase-1−/− mice with different amounts of *L. longbeachae* and evaluated the survival of the infected animals. We found that infections with 10⁵ bacteria per mice is sub-lethal and induce mice death at variable rates (from 0 to approximately 60%). In contrast, infections with 10⁶ or 10⁷ *L. longbeachae* per mice induced death in 100% of the mice in less than 10 days after infection (**Figures 3B–D**). Importantly, in all the MOI used, caspase-1−/− mice died similarly
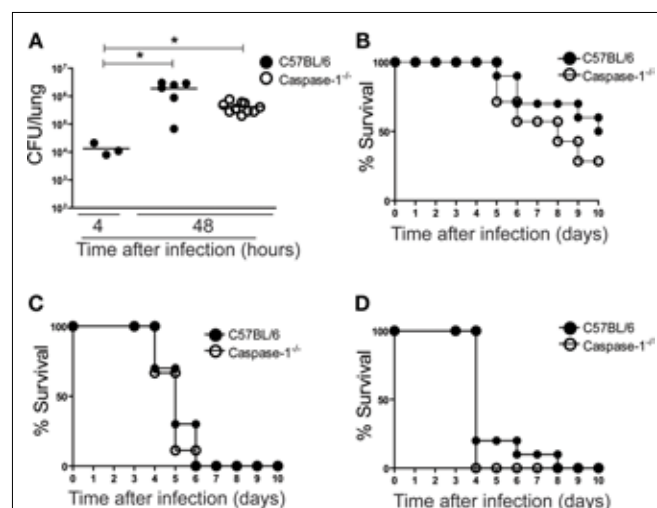
FIGURE 3 | Caspase-1 does not influence pulmonary infection by *L. longbeachae*. C57BL/6 (closed circles) and caspase-1−/− (opened circles) mice were infected intranasally with *Legionella longbeachae* for determination of CFU in the lungs **(A)** and mice survival **(B–D)**. **(A)** Mice were sacrificed after 4 or 48 h after infection and dilutions of the lung homogenates were added to CYE agar plates for CFU determination. Each dot represents a single animal and the horizontal lines represent the average. Six to 10 mice were used per group. **(B–D)** Survival of mice inoculated with 10⁵ **(B)**; 10⁶ **(C)**; or 10⁷ **(D)** *L. longbeachae* per mice over a period of 10 days. Ten mice were used per group. Data are representative of those found in four independent experiments. An asterisk indicates a *P* value of <0.05.

to the wild-type mice, indicating that caspase-1 may not play an important role for host resistance against *L. longbeachae* infection (**Figures 3B–D**).

BYPASSING THE INFLAMMASOME IS NOT SUFFICIENT TO INDUCE MICE DEATH IN RESPONSE TO *LEGIONELLA* INFECTION

Activation of the inflammasome is an important host defense against pulmonary infections by pathogenic bacteria including *L. pneumophila* (Amer et al., 2006; Molofsky et al., 2006; Zamboni et al., 2006; Miao et al., 2010). Since *L. longbeachae* bypasses the inflammasome activation and is lethal for mice, we investigated whether failure to trigger the Nlrc4 inflammasome is sufficient to *Legionella* to induce mice death. To test this hypothesis, we infected C57BL/6 mice with different MOIs of *L. longbeachae* and compared to infections using *L. pneumophila* and isogenic mutants for flagellin (*flaA*). Flagellin mutants were used because they are known to bypass Nlrc4-mediated growth restriction and are virulent for mice infections as the CFU numbers in the lungs increased over the first 48 h of mice infection (Amer et al., 2006; Molofsky et al., 2006; Ren et al., 2006; Case et al., 2009; Silveira and Zamboni, 2010). We found that whereas infections with *L. longbeachae* induced death of mice at MOIs higher than 10^5, *flaA* mutants and wild-type *L. pneumophila* did not induce mice death (**Figure 4**). These data suggests that bypassing the Nlrc4 inflammasome is not sufficient to induce mice death. Therefore, *L. longbeachae* may employ additional virulence strategies to multiply in mammalian hosts.
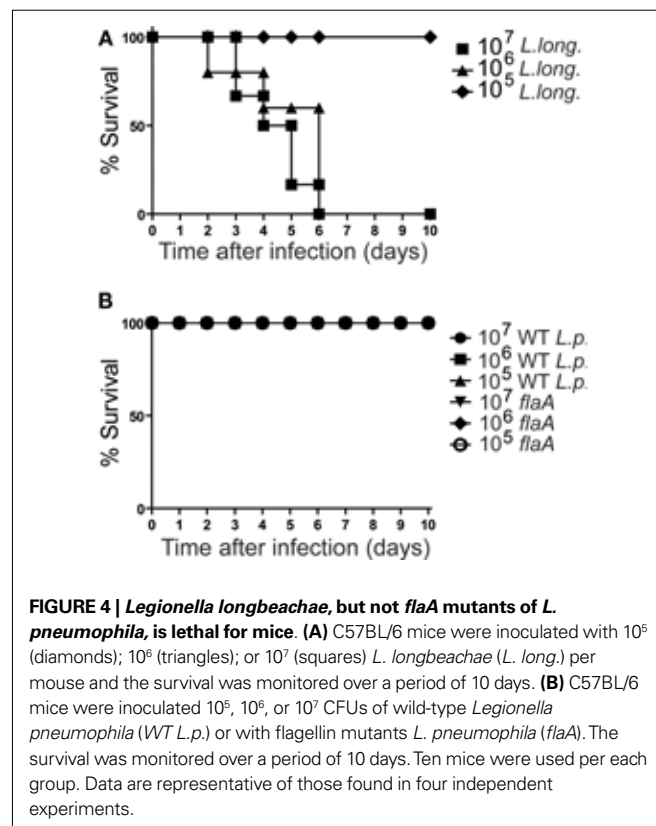
DISCUSSION

The inflammasomes are important platforms that account for recognition and restriction of the infection by pathogenic microbes. Herein, we show that the inflammasome accounts for restriction of pulmonary infection of four species of non-pneumophila *Legionella*: *L. micdadei, L. bozemanii, L. gratiana,* and *L. rubrilucens* (**Figure 1**). Among the non-pneumophila *Legionella* that cause infection in humans, the most prevalent species are *L. longbeachae, L. micdadei,* and *L. bozemanii,* accounting for 2–7% of the infections worldwide (Muder and Yu, 2002). In this context, the demonstration that the Nlrc4 inflammasome accounts for restriction of *in vivo* infection by these species contributes to our understanding of the mechanisms by which host immune responses restrict infections by frequent pneumonia-causing bacteria such as *L. micdadei* and *L. bozemanii.*

It is worth noting that from the 52 species of the genus *Legionella,* only four species are prevalent causative agents of human infection (Gobin et al., 2009a). In this scenario, it is possible that the Nlrc4 inflammasome represents an important line of defense during pulmonary infection with these flagellated species of *Legionella.* Interestingly, although only *L. pneumophila, L. longbeachae, L. bozemanii,* and *L. micdadei* are frequent causative agents of pulmonary infections in humans, more than 25 species have been reported to induce disease in immunocompromised individuals (Gobin et al., 2009a). In this context, it is important to investigate whether the immunocompromised individuals that develop disease caused by infrequent *Legionella* spp. are defective for any component of the Nlrc4 inflammasome.

We have previously demonstrated that *L. micdadei, L. bozemanii, L. gratiana,* and *L. rubrilucens* express flagellin and trigger pyroptosis in isolated macrophages (Silveira and Zamboni, 2010). These previous observations are in agreement with the data presented here showing an important role of Nlrc4 inflammasome for restriction of pulmonary infection by these bacteria. Importantly, the Asc-containing inflammasome played no role in restriction of the infection by *Legionella* spp. (**Figure 2**). These data support the hypothesis that Nlrc4 and Asc proteins participate in distinct inflammasome (Case et al., 2009). However, this is still a controversial issue since macrophages from Asc$^{-/-}$ mouse are defective for caspase-1 activation, processing, and secretion of IL-1β in response to *L. pneumophila* infection (Zamboni et al., 2006; Case et al., 2009). These features had lead to the speculation that Asc protein does participate in the Nlrc4 inflammasome (Pedra et al., 2009). Alternatively, it is possible that Nlrc4 participates in the assembly of two distinct inflammasomes in response to *L. pneumophila*: one independent of Asc (which would be responsible for *Legionella* spp. growth restriction, pore formation, and pyroptosis) and one dependent on Asc (that would be required for robust caspase-1 activation and IL-1β secretion). In this context, further experiments may elucidate the complex interaction of Asc and Nlrc4 in the assembly of the pathogen-induced inflammasomes.

As opposed to flagellated *Legionella* spp., *L. longbeachae* does not encode genes for flagellum biosynthesis and thus does not express flagellin (Cazalet et al., 2010; Kozak et al., 2010; Silveira and Zamboni, 2010). Accordingly, experiments performed with

FIGURE 4 | *Legionella longbeachae,* but not *flaA* mutants of *L. pneumophila,* is lethal for mice. **(A)** C57BL/6 mice were inoculated with 10^5 (diamonds); 10^6 (triangles); or 10^7 (squares) *L. longbeachae* (*L. long.*) per mouse and the survival was monitored over a period of 10 days. **(B)** C57BL/6 mice were inoculated 10^5, 10^6, or 10^7 CFUs of wild-type *Legionella pneumophila* (*WT L.p.*) or with flagellin mutants *L. pneumophila* (*flaA*). The survival was monitored over a period of 10 days. Ten mice were used per each group. Data are representative of those found in four independent experiments.

L. longbeachae indicated that this bacterium does not trigger caspase-1 activation and fails to trigger pore formation and pyroptosis (Asare et al., 2007; Silveira and Zamboni, 2010). Here, we demonstrated that the inflammasome proteins might not substantially account for restriction of the infection by *L. longbeachae in vivo* (**Figure 3**). Importantly, *L. longbeachae* were lethal for mice infections *in vivo*, a feature not observed for other *Legionella* spp. (Alli et al., 2003; Asare et al., 2007; Gobin et al., 2009b). This feature has lead to speculation that *L. longbeachae* is highly virulent in mouse models because it bypasses the inflammasome-mediated growth restriction. To address this hypothesis we compared mortality curves of mice infected with *L. longbeachae* and *L. pneumophila* mutants for *flaA*, a gene that encodes flagellin. Although *flaA* mutants bypassed Nlrc4-dependent growth restriction, the *flaA* mutants failed to trigger death in infected mice even at high infection doses (**Figure 4**). These data suggests that bypassing the inflammasome is not sufficient to induce mouse mortality upon infection with virulent strains of *L. pneumophila*. Therefore, our data support the idea that *L. longbeachae* employ additional virulence strategies

besides bypassing the activation of the Nlrc4 inflammasome. In this context, the recent elucidation of *L. longbeachae* genome revealed that the bacteria contain genes predicted to encode proteins for capsule formation and several genes encoding proteins with predicted eukaryotic-like motifs that are not shared with *L. pneumophila* (Cazalet et al., 2010; Kozak et al., 2010). We envisage that in the next few years, investigation of these putative virulence genes might reveal novel molecular mechanisms that explain the high virulence of *L. longbeachae* in mammalian models of experimental infection.

ACKNOWLEDGMENTS

REFERENCES

Alli, O. A., Zink, S., von Lackum, N. K., and Abu-Kwaik, Y. (2003). Comparative assessment of virulence traits in *Legionella* spp. *Microbiology* 149, 631–641.

Amer, A., Franchi, L., Kanneganti, T. D., Body-Malapel, M., Ozoren, N., Brady, G., Meshinchi, S., Jagirdar, R., Gewirtz, A., Akira, S., and Nunez, G. (2006). Regulation of *Legionella* phagosome maturation and infection through flagellin and host Ipaf. *J. Biol. Chem.* 281, 35217–35223.

Archer, K. A., Ader, F., Kobayashi, K. S., Flavell, R. A., and Roy, C. R. (2010). Cooperation between multiple microbial pattern recognition systems is important for host protection against the intracellular pathogen *Legionella pneumophila*. *Infect. Immun.* 78, 2477–2487.

Asare, R., Santic, M., Gobin, I., Doric, M., Suttles, J., Graham, J. E., Price, C. D., and Abu Kwaik, Y. (2007). Genetic susceptibility and caspase activation in mouse and human macrophages are distinct for *Legionella longbeachae* and *L. pneumophila*. *Infect. Immun.* 75, 1933–1945.

Berrington, W. R., Iyer, R., Wells, R. D., Smith, K. D., Skerrett, S. J., and Hawn, T. R. (2010). NOD1 and NOD2 regulation of pulmonary innate immunity to *Legionella pneumophila*. *Eur. J. Immunol.* 40, 3519–3527.

Case, C. L., Shin, S., and Roy, C. R. (2009). Asc and Ipaf Inflammasomes direct distinct pathways for caspase-1 activation in response to *Legionella pneumophila*. *Infect. Immun.* 77, 1981–1991.

Cazalet, C., Gomez-Valero, L., Rusniok, C., Lomma, M., Dervins-Ravault, D., Newton, H. J., Sansom, F. M., Jarraud, S., Zidane, N., Ma, L., Bouchier, C., Etienne, J., Hartland, E. L., and Buchrieser, C. (2010). Analysis of the *Legionella longbeachae* genome and transcriptome uncovers unique strategies to cause Legionnaires' disease. *PLoS Genet.* 6, e1000851. doi: 10.1371/journal.pgen.1000851

Feeley, J. C., Gibson, R. J., Gorman, G. W., Langford, N. C., Rasheed, J. K., Mackel, D. C., and Baine, W. B. (1979). Charcoal-yeast extract agar: primary isolation medium for *Legionella pneumophila*. *J. Clin. Microbiol.* 10, 437–441.

Franchi, L., Amer, A., Body-Malapel, M., Kanneganti, T. D., Ozören, N., Jagirdar, R., Inohara, N., Vandenabeele, P., Bertin, J., Coyle, A., Grant, E. P., and Núñez, G. (2006). Cytosolic flagellin requires Ipaf for activation of caspase-1 and interleukin 1beta in salmonella-infected macrophages. *Nat. Immunol.* 7, 576–582.

Franchi, L., Park, J. H., Shaw, M. H., Marina-Garcia, N., Chen, G., Kim, Y. G., and Nunez, G. (2008). Intracellular NOD-like receptors in innate immunity, infection and disease. *Cell. Microbiol.* 10, 1–8.

Frutuoso, M. S., Hori, J. I., Pereira, M. S., Junior, D. S., Sonego, F., Kobayashi, K. S., Flavell, R. A., Cunha, F. Q., and Zamboni, D. S. (2010). The pattern recognition receptors Nod1 and Nod2 account for neutrophil recruitment to the lungs of mice infected with *Legionella pneumophila*. *Microbes Infect.* 12, 819–827.

Gobin, I., Newton, P. R., Hartland, E. L., and Newton, H. J. (2009a). Infections caused by non-pneumophila species of *Legionella*. *Rev. Med. Microbiol.* 20, 1–11.

Gobin, I., Susa, M., Begic, G., Hartland, E. L., and Doric, M. (2009b). Experimental *Legionella longbeachae* infection in intratracheally inoculated mice. *J. Med. Microbiol.* 58, 723–730.

Janeway, C. A. Jr., and Medzhitov, R. (2002). Innate immune recognition. *Annu. Rev. Immunol.* 20, 197–216.

Kawai, T., and Akira, S. (2009). The roles of TLRs, RLRs and NLRs in pathogen recognition. *Int. Immunol.* 21, 317–337.

Kozak, N. A., Buss, M., Lucas, C. E., Frace, M., Govil, D., Travis, T., Olsen-Rasmussen, M., Benson, R. F., and Fields, B. S. (2010). Virulence factors encoded by *Legionella longbeachae* identified on the basis of the genome sequence analysis of clinical isolate D-4968. *J. Bacteriol.* 192, 1030–1044.

Kuida, K., Lippke, J. A., Ku, G., Harding, M. W., Livingston, D. J., Su, M. S., and Flavell, R. A. (1995). Altered cytokine export and apoptosis in mice deficient in interleukin-1 beta converting enzyme. *Science* 267, 2000–2003.

Lara-Tejero, M., Sutterwala, F. S., Ogura, Y., Grant, E. P., Bertin, J., Coyle, A. J., Flavell, R. A., and Galan, J. E. (2006). Role of the caspase-1 inflammasome in *Salmonella typhimurium* pathogenesis. *J. Exp. Med.* 203, 1407–1412.

Lightfield, K. L., Persson, J., Brubaker, S. W., Witte, C. E., von Moltke, J., Dunipace, E. A., Henry, T., Sun, Y. H., Cado, D., Dietrich, W. F., Monack, D. M., Tsolis, R. M., and Vance, R. E. (2008). Critical

function for Naip5 in inflammasome activation by a conserved carboxy-terminal domain of flagellin. *Nat. Immunol.* 9, 1171–1178.

Mariathasan, S., Newton, K., Monack, D. M., Vucic, D., French, D. M., Lee, W. P., Roose-Girma, M., Erickson, S., and Dixit, V. M. (2004). Differential activation of the inflammasome by caspase-1 adaptors ASC and Ipaf. *Nature* 430, 213–218.

Masumoto, J., Yang, K., Varambally, S., Hasegawa, M., Tomlins, S. A., Qiu, S., Fujimoto, Y., Kawasaki, A., Foster, S. J., Horie, Y., Mak, T. W., Núñez, G., Chinnaiyan, A. M., Fukase, K., and Inohara, N. (2006). Nod1 acts as an intracellular receptor to stimulate chemokine production and neutrophil recruitment in vivo. *J. Exp. Med.* 203, 203–213.

Miao, E. A., Alpuche-Aranda, C. M., Dors, M., Clark, A. E., Bader, M. W., Miller, S. I., and Aderem, A. (2006). Cytoplasmic flagellin activates caspase-1 and secretion of interleukin1beta via Ipaf. *Nat. Immunol.* 7, 569–575.

Miao, E. A., Leaf, I. A., Treuting, P. M., Mao, D. P., Dors, M., Sarkar, A., Warren, S. E., Wewers, M. D., and Aderem, A. (2010). Caspase-1-induced pyroptosis is an innate immune effector mechanism against intracellular bacteria. *Nat. Immunol.* 11, 1136–1142.

Molofsky, A. B., Byrne, B. G., Whitfield, N. N., Madigan, C. A., Fuse, E. T., Tateda, K., and Swanson, M. S. (2006). Cytosolic recognition of flagellin by mouse macrophages restricts *Legionella pneumophila* infection. *J. Exp. Med.* 203, 1093–1104.

Muder, R. R., and Yu, V. L. (2002). Infection due to *Legionella* species other than L. *pneumophila*. *Clin. Infect. Dis.* 35, 990–998.

Pedra, J. H., Cassel, S. L., and Sutterwala, F. S. (2009). Sensing pathogens and danger signals by the inflammasome. *Curr. Opin. Immunol.* 21, 10–16.

Ren, T., Zamboni, D. S., Roy, C. R., Dietrich, W. F., and Vance, R. E. (2006). Flagellin-deficient *Legionella* mutants evade caspase-1- and Naip5-mediated macrophage immunity. *PLoS Pathog.* 2, e18. doi: 10.1371/journal.ppat.0020018

Schroder, K., and Tschopp, J. (2010). The inflammasomes. *Cell* 140, 821–832.

Silva, G. K., Gutierrez, F. R., Guedes, P. M., Horta, C. V., Cunha, L. D., Mineo, T. W., Santiago-Silva, J., Kobayashi, K. S., Flavell, R. A., Silva, J. S., and Zamboni, D. S. (2010). Cutting edge: nucleotide-binding oligomerization domain 1-dependent responses account for murine resistance against *Trypanosoma cruzi* infection. *J. Immunol.* 184, 1148–1152.

Silveira, T. N., and Zamboni, D. S. (2010). Pore formation triggered by *Legionella* spp. is an Nlrc4 inflammasome-dependent host cell response that precedes pyroptosis. *Infect. Immun.* 78, 1403–1413.

Sutterwala, F. S., and Flavell, R. A. (2009). NLRC4/IPAF: a CARD carrying member of the NLR family. *Clin. Immunol.* 130, 2–6.

Whitfield, N. N., Byrne, B. G., and Swanson, M. S. (2010). Mouse macrophages are permissive to motile *Legionella* species that fail to trigger pyroptosis. *Infect. Immun.* 78, 423–432.

Zamboni, D. S., Kobayashi, K. S., Kohlsdorf, T., Ogura, Y., Long, E. M., Vance, R. E., Kuida, K., Mariathasan, S., Dixit, V. M., Flavell, R. A., Dietrich, W. F., and Roy, C. R. (2006). The Birc1e cytosolic pattern-recognition receptor contributes to the detection and control of *Legionella pneumophila* infection. *Nat. Immunol.* 7, 318–325.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Asc-dependent and independent mechanisms contribute to restriction of *Legionella pneumophila* infection in murine macrophages

Dalia H. A. Abdelaziz[1,2†], Mikhail A. Gavrilin[1†], Anwari Akhter[1], Kyle Caution[1], Sheetal Kotrange[1], Arwa Abu Khweek[1], Basant A. Abdulrahman[1,2], Zeinab A. Hassan[2], Fathia Z. El-Sharkawi[2], Simranjit S. Bedi[1], Katherine Ladner[3], M. Elba Gonzalez-Mejia[4], Andrea I. Doseff[4], Mahmoud Mostafa[1], Thirumala-Devi Kanneganti[5], Dennis Guttridge[3], Clay B. Marsh[1], Mark D. Wewers[1] and Amal O. Amer[1]*

[1] Division of Pulmonary, Allergy, Critical Care, and Sleep Medicine, Center for Microbial Interface Biology and the Department of Internal Medicine, Ohio State University, Columbus, OH, USA
[2] Faculty of Pharmacy, Department of Biochemistry and Molecular Biology, Helwan University, Helwan, Egypt
[3] Human Cancer Genetics Program, Ohio State University, Columbus, OH, USA
[4] Department of Molecular Genetics, Davis Heart and Lung Research Institute, The Ohio State University, Columbus, OH, USA
[5] Department of Immunology, St Jude Children's Research Hospital, Memphis, TN, USA

The apoptosis-associated speck-like protein containing a caspase recruitment domain (Asc) is an adaptor molecule that mediates inflammatory and apoptotic signals. *Legionella pneumophila* is an intracellular bacterium and the causative agent of Legionnaire's pneumonia. *L. pneumophila* is able to cause pneumonia in immuno-compromised humans but not in most inbred mice. Murine macrophages that lack the ability to activate caspase-1, such as caspase-1$^{-/-}$ and Nlrc4$^{-/-}$ allow *L. pneumophila* infection. This permissiveness is attributed mainly to the lack of active caspase-1 and the absence of its down stream substrates such as caspase-7. However, the role of Asc in control of *L. pneumophila* infection in mice is unclear. Here we show that caspase-1 is moderately activated in Asc$^{-/-}$ macrophages and that this limited activation is required and sufficient to restrict *L. pneumophila* growth. Moreover, Asc-independent activation of caspase-1 requires bacterial flagellin and is mainly detected in cellular extracts but not in culture supernatants. We also demonstrate that the depletion of Asc from permissive macrophages enhances bacterial growth by promoting *L. pneumophila*-mediated activation of the NF-κB pathway and decreasing caspase-3 activation. Taken together, our data demonstrate that *L. pneumophila* infection in murine macrophages is controlled by several mechanisms: Asc-independent activation of caspase-1 and Asc-dependent regulation of NF-κB and caspase-3 activation.

Keywords: inflammasome, caspase-1, *Legionella pneumophila*, Asc

INTRODUCTION

The apoptosis-associated speck-like protein containing a caspase recruitment domain (Asc encoded by the *Pycard* gene) is an adaptor molecule that mediates inflammatory and apoptotic signals and is predominantly expressed in monocytes and mucosal epithelial cells (Taniguchi and Sagara, 2007; Hasegawa et al., 2009). Asc contains an N-terminal pyrin/PAAD (PYD) death domain and a C-terminal CARD protein–protein interaction domain (CARD; Masumoto et al., 1999, 2001; Liepinsh et al., 2003; Stehlik et al., 2003). Both domains enable Asc to recruit other PYD and CARD-containing proteins through homotypic protein–protein interactions (Fernandes-Alnemri et al., 2007; Mariathasan, 2007). Proteins with pyrin and/or caspase recruitment domains have roles in inflammation, apoptosis, and innate immunity. Many pyrin domain proteins, such as Asc, modulate NF-κB activity. Asc also participates in the assembly of multiprotein complexes called "inflammasomes" (Srinivasula et al., 2002; Fernandes-Alnemri et al., 2007; Fernandes-Alnemri and Alnemri, 2008). Within the inflammasome, Asc is able to link caspase-1 to NOD-like receptors (NLRs) via its CARD domain, leading to the

activation of caspase-1 (Srinivasula et al., 2002; Fernandes-Alnemri et al., 2007; Fernandes-Alnemri and Alnemri, 2008). NLRs act as intracellular sensors to stress, and foreign molecules like microbial components (Kanneganti et al., 2007; Martinon and Tschopp, 2007). Once activated, caspase-1 subsequently cleaves pro-IL-1β/IL-18 and accompanies the active cytokines when secreted out of the cell (Stehlik et al., 2002, 2003; Liepinsh et al., 2003; Hasegawa et al., 2005, 2007, 2009; Sarkar et al., 2006; Bedoya et al., 2007). Caspase-1 also activates caspase-7 in response to flagellin or LPS (Franchi et al., 2008; Lamkanfi et al., 2008, 2009; Akhter et al., 2009; Lamkanfi and Kanneganti, 2010; Shaw et al., 2010). Many Gram-negative bacteria, such as *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Shigella flexneri*, and *Legionella pneumophila*, are recognized in murine macrophages by the NLR Nlrc4/Ipaf leading to caspase-1 activation through the inflammasome (Abdelaziz et al., 2010; Amer, 2010).

Furthermore, when Asc is over expressed, it cooperates with Nlrp3 (cryopyrin) and Nlrp12 (Pypaf7) in order to promote NF-κB activity in an over expression system (Masumoto et al., 2003; Hasegawa et al., 2005). However, other reports show that Asc is

a suppressor of NF-κB activity and that it uses its CARD interaction not only to induce caspase-1 activation, but also to down regulate NF-κB signaling (Sarkar et al., 2006; Bedoya et al., 2007). Additionally, other studies demonstrate that Asc can be either an inducer or an inhibitor of NF-κB depending on expression level and location (Stehlik et al., 2002; Yu et al., 2006). Thus, Asc regulates the inflammasome and other signaling complexes depending on the stoichiometry of its expression and also whether certain other PYD family proteins are expressed upon activation. On the other hand, Asc mediates apoptosis by serving as an adaptor molecule for Bax and regulates a p53-Bax mitochondrial pathway of apoptosis resulting in the activation of caspase-2 and -3 (Ohtsuka et al., 2004; Hasegawa et al., 2007; Fernandes-Alnemri and Alnemri, 2008; Motani et al., 2010).

Legionella pneumophila is an intracellular bacterium and the causative agent of Legionnaire's pneumonia (Horwitz and Silverstein, 1981, 1983; Nash et al., 1984). The ability of *L. pneumophila* to cause pneumonia in humans is dependent on its capability to evade the immune system and multiply within human monocytes and derived macrophages (Horwitz and Silverstein, 1981, 1983; Nash et al., 1984). In murine macrophages, *L. pneumophila* activates the Nlrc4 inflammasome leading to the production of active caspase-1 and IL-1β (Amer et al., 2006; Ren et al., 2006; Zamboni et al., 2006; Coers et al., 2007; Lightfield et al., 2008; Akhter et al., 2009; Abdelaziz et al., 2010; Amer, 2010; Vance, 2010). Then, Naip5 mediates caspase-7 activation downstream of caspase-1 which restricts the intracellular survival of the organism (Akhter et al., 2009; Abdelaziz et al., 2010). Therefore, mice and their derived macrophages lacking Nlrc4, caspase-1, or caspase-7 allow *L. pneumophila* growth and are ideal models to study *L. pneumophila* pathogenesis (Amer et al., 2006; Ren et al., 2006; Zamboni et al., 2006; Coers et al., 2007; Lightfield et al., 2008; Akhter et al., 2009; Abdelaziz et al., 2010; Amer, 2010; Vance, 2010).

Here we demonstrate that murine macrophages control *L. pneumophila* infection through an Asc-dependent mechanism by decreasing NF-κB activation and an Asc-independent mechanism by modestly activating caspase-1 in the cytosol. Together, our data show that Asc controls *L. pneumophila* infection in the absence of caspase-1.

RESULTS

Asc CONTROLS *L. PNEUMOPHILA* INFECTION IN MURINE MACROPHAGES LACKING CASPASE-1

WT mouse macrophages effectively activate and release caspase-1 in response to *L. pneumophila* infection. This is accompanied by the fusion of the *L. pneumophila*-containing phagosome with the lysosome, bacterial degradation, and elimination (Amer et al., 2006; Akhter et al., 2009). Consistent with that, caspase-1 knockout (⁻/⁻) macrophages are permissive to *L. pneumophila* replication. Asc is an adaptor molecule involved in caspase-1 activation in response to a variety of agents. However, its role during *L. pneumophila* infection is not well established. To characterize the role of Asc during *L. pneumophila* infection, Asc was down regulated in both caspase-1⁻/⁻ (**Figure 1A**) and WT macrophages (**Figure 1B**) using Asc specific siRNA. After transfection, Asc protein level declined in both sets of cells, while other components of the inflammasome, such as Nlrc4 and pro-caspase-1, were not affected (**Figures 1A,B**). After depletion

of Asc, cells were infected with *L. pneumophila* and the bacterial replication was assessed by counting colony-forming units (CFU). Depletion of Asc in caspase-1⁻/⁻ cells supported significantly more *L. pneumophila* replication compared to caspase-1⁻/⁻ cells treated with control siRNA (**Figure 1C**). In WT mouse macrophages, depletion of Asc did not have an effect on the bacterial growth compared to that of cells treated with control siRNA or untreated cells (**Figure 1D**). Therefore, Asc controls *L. pneumophila* replication in murine macrophages in the absence of caspase-1.

Asc REDUCES NF-κB ACTIVATION INDUCED BY *L. PNEUMOPHILA* INFECTION

It has been shown by several reports that *L. pneumophila* triggers NF-κB activation in TLR5 dependent and independent manner depending on the stage of the infection (Bartfeld et al., 2009; Losick et al., 2010). Nevertheless, the role of Asc in NF-κB modulation depends on its protein levels and its location within the cells (Stehlik et al., 2002; Sarkar et al., 2006; Yu et al., 2006; Bedoya et al., 2007). Our results showed that Asc controls *L. pneumophila* replication in a caspase-1-independent manner. To determine if the mechanism by which Asc restricts *L. pneumophila* (independently of caspase-1) involves the NF-κB pathway, Asc was depleted in caspase-1⁻/⁻ macrophages by siRNA against Asc. Then, cells were infected with *L. pneumophila* and NF-κB activation was assessed in nuclear extracts by electrophoretic mobility shift assay (EMSA). We found that caspase-1⁻/⁻ macrophages depleted for Asc allowed more NF-κB activation at 1, 4, and 8 h of infection compared with cells treated with control siRNA (**Figure 2A**). These results suggest that Asc decreased NF-κB during *L. pneumophila* infection. To confirm these findings, Asc⁻/⁻ and WT macrophages were infected with *L. pneumophila* and EMSA assay was performed. Initially, WT and Asc⁻/⁻ macrophages showed NF-κB activation within 1 h after infection. This activation declined in WT macrophages by 8 h infection whereas NF-κB pathway remained activated in Asc⁻/⁻ 8 h after *L. pneumophila* infection (**Figure 2B**). Thus, in the context of *L. pneumophila* infection, Asc hinders NF-κB activation, and decreases cell survival.

CASPASE-1 IS ACTIVATED IN THE CYTOSOL OF MURINE MACROPHAGES LACKING Asc

The involvement of Asc in Nlrc4 inflammasome is still unclear (Zamboni et al., 2006). To discern the role of Asc in *L. pneumophila*-mediated caspase-1 activation, Asc⁻/⁻ macrophages were left untreated or infected with *L. pneumophila* at a low multiplicity of infection (MOI). Then, cleaved caspase-1 was examined in cellular extracts and culture supernatants of Asc⁻/⁻ macrophages and of their WT counterparts. Notably, cells lacking Asc still allowed the cleavage of caspase-1 within their cytosols when infected with *L. pneumophila* (**Figure 3A**). The amount of cleaved caspase-1 in Asc⁻/⁻ cell lysates was less than that of WT macrophages (**Figure 3A**). Cleaved caspase-1 was detected in culture supernatants of infected WT macrophages but not that of infected Asc⁻/⁻ macrophages (**Figure 3B**). This data indicates that in the absence of Asc, a fraction of caspase-1 is cleaved by a yet unknown mechanism.

To further investigate the role of Asc in caspase-1 activation during *L. pneumophila* of WT macrophages, Asc was depleted in WT murine macrophages, as in **Figure 1B** and then infected with *L. pneumophila*. The amount of cleaved caspase-1 detected
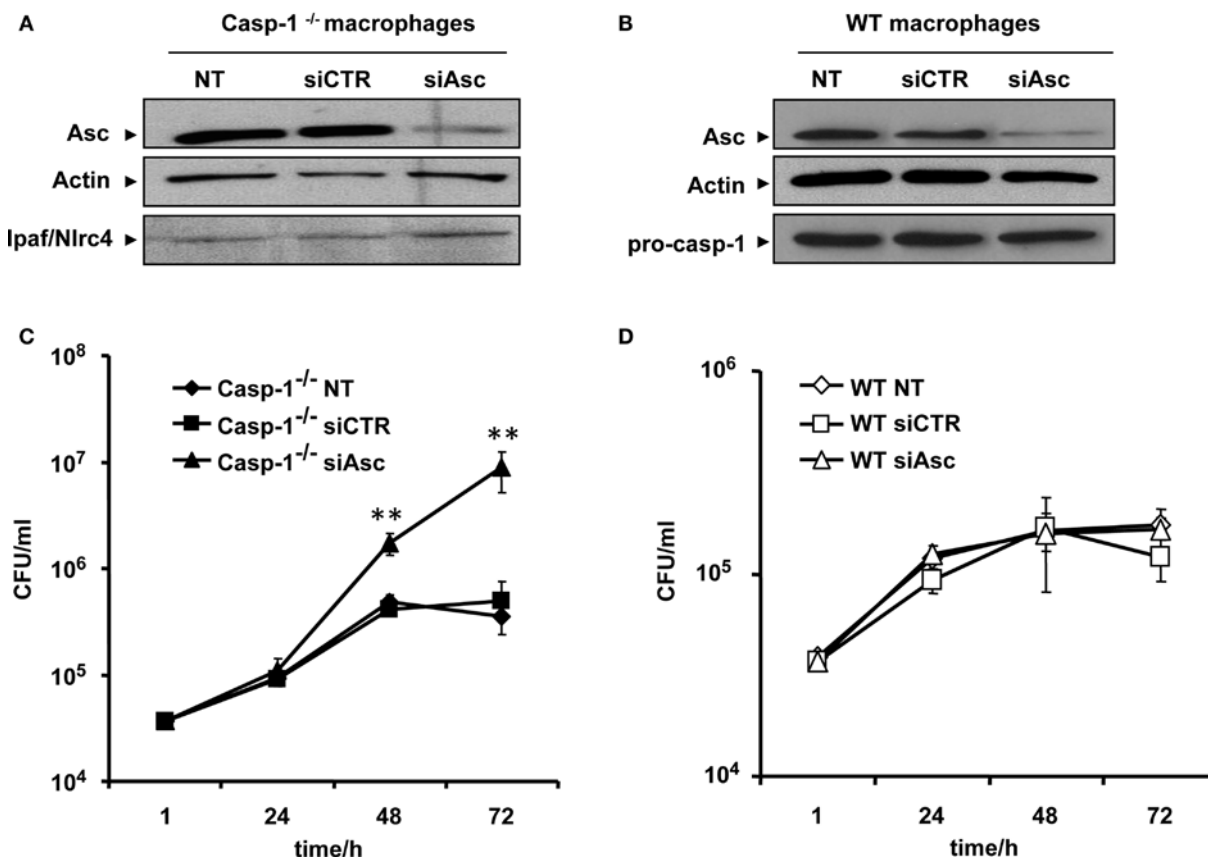
FIGURE 1 | Asc controls *Legionella pneumophila* replication in caspase-1⁻/⁻ macrophages. (A) Caspase-1⁻/⁻ (casp-1⁻/⁻) macrophages and **(B)** WT macrophages were either left untreated (NT) or transfected with Asc specific siRNA (siAsc) or control siRNA (siCTR). After 48 h transfection Asc levels were assessed using Western blotting. Actin was used as a loading control. Casp-1⁻/⁻ macrophages **(C)** and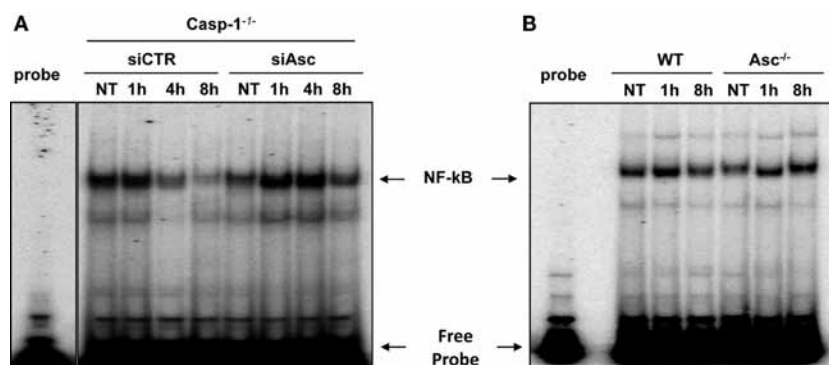 wild type macrophages **(D)** were transfected or not (NT) with siAsc or siCTR and 48 h after transfection cells were infected with *L. pneumophila* (Leg) at MOI = 0.1 and the bacterial replication was assessed by counting the CFU after 1, 24, 48, and 72 h. Results are displayed as mean ± SD of three independent wells. **$P \leq 0.01$. The data shown in **(C,D)** are representative of four independent experiments showing the same results.

in extracts of cells treated with Asc specific siRNA was less than that in cells treated with control siRNA (**Figure 4A**). Accordingly, the release of active caspase-1 from siAsc treated cells was diminished (**Figure 4B**). In support of this data, total cleaved caspase-1 in combined cell extracts and culture supernatants was less in Asc heterozygote macrophages than that of WT ones (**Figure A1** of Appendix). Furthermore, IL-1β release from macrophages was impeded when Asc was depleted (**Figure 4C**). Therefore, Asc contributes to the activation of a portion of pro-caspase-1, while the rest of caspase-1 pro-form is cleaved independently of Asc.

Asc-INDEPENDENT ACTIVATION OF CASPASE-1 WITHIN MACROPHAGE CYTOSOLS REQUIRES FLAGELLIN AND CONTRIBUTES TO THE RESTRICTION OF *L. PNEUMOPHILA* INFECTION

The activation of caspase-1 requires flagellin, thereby promoting the restriction of *L. pneumophila* infection (Amer et al., 2006; Ren et al., 2006; Zamboni et al., 2006; Lamkanfi et al., 2007; Akhter et al., 2009; Case et al., 2009; Amer, 2010; Kang et al., 2010). Thus, we next examined the growth of *L. pneumophila* in WT, caspase-1⁻/⁻, and Asc⁻/⁻ macrophages. As previously reported by our group and by others, caspase-1⁻/⁻ macrophages were permissive, whereas WT and
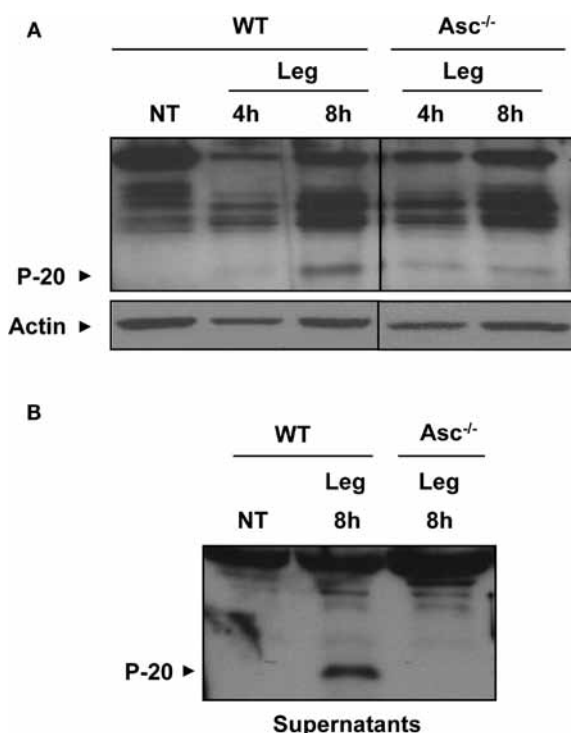
Asc⁻/⁻ macrophages restricted *L. pneumophila* replication (Amer et al., 2006; Ren et al., 2006; Zamboni et al., 2006; Lamkanfi et al., 2007; Akhter et al., 2009; Case et al., 2009; Amer, 2010; Kang et al., 2010). However, our data demonstrate that caspase-1 is cleaved in the cytosol of Asc⁻/⁻ macrophages, therefore, we examined if this cytosolic cleavage required flagellin and if it is sufficient for restriction of *L. pneumophila* infection. WT macrophages were infected with the *L. pneumophila* mutant lacking flagellin (Fla), then, caspase-1 activation in cell extracts was examined by Western blots. **Figure 5** shows that the Fla mutant did not activate caspase-1 within the cytosols of macrophages (**Figure 5A**). Because caspase-1 activation requires flagellin and is accompanied with restriction to *L. pneumophila* infection, we examined the growth of *L. pneumophila* mutants lacking flagellin (Fla) in macrophages lacking Asc in comparison to WT cells, since both cells lack caspase-1 activation in response to Fla (data not shown). Specifically, **Figure 5B** demonstrates that the growth of Fla mutants in Asc⁻/⁻ macrophages exceeds that exhibited in WT cells. Yet, NF-κB activation during Fla was similar in WT and Asc⁻/⁻ macrophages (**Figure 5C**). Therefore, there must be another pathway mediated by Asc that maintains the replication of Fla mutant under control in WT macrophages and

FIGURE 2 | Asc hinders the activation of NF-κB induced by *L. pneumophila*.
(A) Caspase-1⁻/⁻ (casp-1⁻/⁻) macrophages were transfected with Asc specific
siRNA (siAsc) or control siRNA (siCTR) and 48 h after transfection cells were
infected or not (NT) with *L. pneumophila* (Leg) at MOI = 0.5 for 1, 4, and 8 h.

Afterward, NF-κB activation was examined using electrophoretic mobility shift
assay (EMSA). **(B)** WT and Asc⁻/⁻ mouse macrophages were infected or not (NT)
with *L. pneumophila* (Leg) at MOI = 0.5 for 1 and 8 h. Subsequently, NF-κB
activation was examined using EMSA.

**FIGURE 3 | A fraction of caspase-1 is activated in the cytosol of murine
macrophages lacking Asc. (A)** WT and Asc⁻/⁻ mouse macrophages were
either not treated (NT) or infected with *L. pneumophila* (Leg) at MOI = 0.5 for
4 and 8 h. Then active caspase-1 (p-20) was detected in the cell extracts **(A)**
and the supernatants **(B)** by Western blotting. Actin was used as a loading
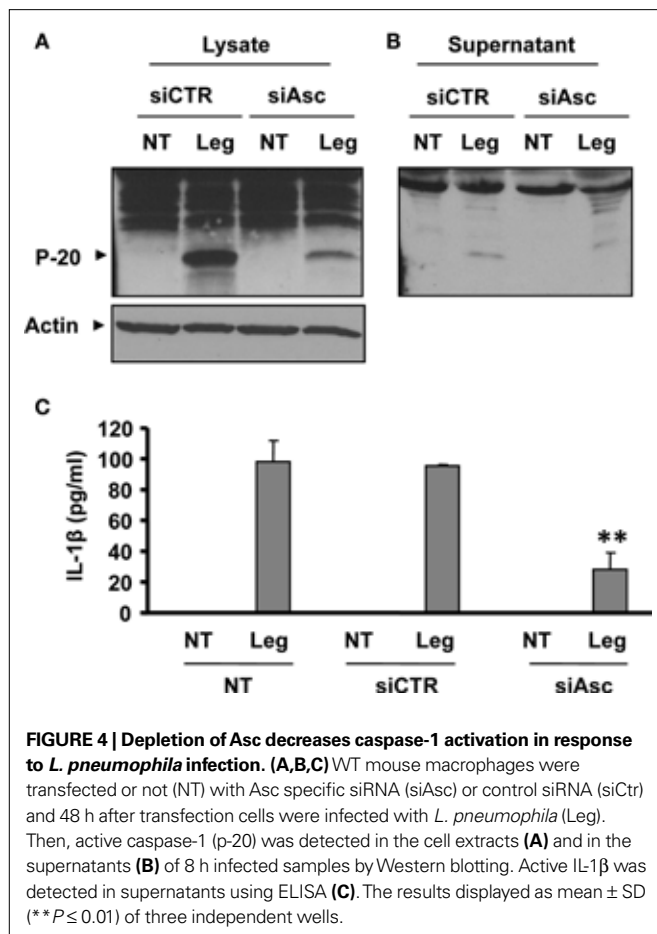control.

activated during *L. pneumophila* infection (Molmeret et al., 2004).
First, we tested if the Fla mutant activates caspase-3 in wild type
macrophages. Until 4 h post infection only WT *L. pneumophila*
activated caspase-3 (**Figure 6A**). However, at 5 h post infection,
the Fla mutant activated caspase-3 in WT macrophages but not in
Asc⁻/⁻ macrophages (**Figure 6B**). Taken together, these data suggest
that Asc is involved in caspase-3 activation which mediates cell
death in WT macrophages at later stages of Fla infection.

LEGIONELLA PNEUMOPHILA MODULATES THE EXPRESSION OF Nlrc4 INFLAMMASOME COMPONENTS IN WILD TYPE MOUSE MACROPHAGES

In WT murine macrophages *L. pneumophila* flagellin is detected by
Nlrc4 with subsequent activation of caspase-1 (Amer et al., 2006;
Ren et al., 2006; Zamboni et al., 2006; Lamkanfi et al., 2007; Akhter
et al., 2009; Case et al., 2009; Amer, 2010; Kang et al., 2010). This
response is completely lacking in human phagocytes. In contrast
to murine phagocytes, human phagocytes which are permissive
to *L. pneumophila* do not activate caspase-1 in response to the
pathogen (Abdelaziz et al., 2011). This lack of activation is due to
the down regulation of ASC in human cells upon infection with
L. pneumophila (Abdelaziz et al., 2011). To characterize the effect
of *L. pneumophila* on the expression of the Nlrc4 inflammasome
components in the mouse, murine WT macrophages were infected
with *L. pneumophila* and the expression of caspase-1, IL-1β, Nlrc4,
and Asc was assessed on both mRNA and protein levels. The expres-
sion of both caspase-1 and IL-1β proteins were induced signifi-
cantly within 4 h after infection and remained up regulated for
24 h of infection (**Figures A2A,B** of Appendix). Subsequently,
both caspase-1 and IL-1β were activated early upon infection
(**Figures A2A,B** lower panel of Appendix) and their active forms
were released into the supernatant (Data not shown). Remarkably,
in contrast to mouse macrophages, the expression of Asc was
enhanced 24 h after infection (**Figure A2C**). As for Nlrc4, mRNA,
and protein levels were decreased later in infection (**Figure A2D**
lower panel of Appendix). We found that this decrease in Nlrc4
protein levels is due to its release with the rest of the inflamma-
some components into the media (data not shown). Therefore,
L. pneumophila differentially modulates the expression of Asc in
murine and in human phagocytes.

is missing in Asc⁻/⁻ macrophages. To answer this question, we next
determined the levels of LDH release in culture supernatants of
WT and Asc⁻/⁻ macrophages infected with *L. pneumophila* or the
Fla mutant. We found that the absence of Asc allows more host cell
survival during Fla infection. To further understand the mecha-
nism by which Asc modulates cell survival, we examined the activa-
tion of caspase-3, a caspase involved in apoptosis and know to be

FIGURE 4 | Depletion of Asc decreases caspase-1 activation in response to *L. pneumophila* infection. (A,B,C) WT mouse macrophages were transfected or not (NT) with Asc specific siRNA (siAsc) or control siRNA (siCtr) and 48 h after transfection cells were infected with *L. pneumophila* (Leg). Then, active caspase-1 (p-20) was detected in the cell extracts **(A)** and in the supernatants **(B)** of 8 h infected samples by Western blotting. Active IL-1β was detected in supernatants using ELISA **(C)**. The results displayed as mean ± SD (**$P \leq 0.01$) of three independent wells.

DISCUSSION

Asc is an adaptor molecule necessary for the assembly and activation of several inflammasome complexes in response to stress signals and microbial molecules (Taniguchi and Sagara, 2007; Hasegawa et al., 2009). Asc is required for caspase-1 activation downstream of Nlrc4/Ipaf during *Salmonella* or *Shigella* infections, yet its role during *L. pneumophila* infection has been controversial (Zamboni et al., 2006; Akhter et al., 2009; Case et al., 2009). In this study, we demonstrate that a fraction of caspase-1 is activated within the cytosol of infected macrophages independently of Asc. Data from our group and others indicate that restriction of *L. pneumophila* infection is mediated by several mechanisms. This can occur either via caspase-1-dependent or independent mechanisms. Moreover, caspase-1 dependent control of *L. pneumophila* infection can be either Asc-dependent or independent.

First, caspase-1-dependent restriction of *L. pneumophila* is by modulation of pyroptosis and phagosome–lysosome fusion. Several studies have demonstrated that at high MOIs with *L. pneumophila*, the activation of caspase-1 leads to pyroptotic cell death, which contributes to resistance to infection (Zamboni et al., 2006; Case et al., 2009). Other reports have shown that during extreme MOIs of *L. pneumophila*, murine caspase-1 is activated independently of Nlrc4 (Case et al., 2009). This is at odds with other data that clearly demonstrates that the activation of caspase-1 during physiological levels of infection requires Nlrc4 (Amer et al., 2006; Ren et al., 2006; Zamboni et al., 2006; Coers et al., 2007; Lightfield et al., 2008;

Akhter et al., 2009; Abdelaziz et al., 2010; Amer, 2010; Vance, 2010). It seems that these discrepancies are largely due to different infection doses. Consequently, it is plausible to suggest that the activation of caspase-1 by high bacterial burdens overrides the signaling pathways controlling their activation at physiological levels of infection. Yet, our data suggest that Asc is required for the recruitment and cleavage of at least a part of pro-caspase-1 protein into its active form since the amount of cleaved p20 detected in the cell extracts of Asc$^{-/+}$ macrophages was much less than that of WT macrophages.

Secondly, macrophages deploy an Asc-dependent restriction mechanism to control *L. pneumophila* infection by controlling NF-κB pathway. Dixit and colleagues demonstrated that macrophages from Asc$^{-/-}$ mice are markedly resistant to *Salmonella typhimurium* induced cell death (Mariathasan et al., 2004). However, in their study, cell death was mainly attributed to caspase-1 mediated pyroptosis while other pathways such as NF-κB activation were not examined. Several reports by Isberg and Abu Kwaik showed that *L. pneumophila* activates NF-κB in two phases to extend the host cell survival to permit intracellular growth (Losick and Isberg, 2006; Abu-Zant et al., 2007; Bartfeld et al., 2009). They clearly demonstrated that NF-κB activation is absolutely required for *L. pneumophila* growth within macrophages. *L. pneumophila* promotes two phases of NF-κB activation, a TLR5-dependent and a TLR5-independent activation at an early and later stages of infection respectively (Losick and Isberg, 2006; Bartfeld et al., 2009). The role of Asc in *L. pneumophila*-induced NF-κB activation was not explored previously. A number of studies suggest that Asc promotes NF-κB activation (Chamaillard et al., 2003; Masumoto et al., 2003; Hasegawa et al., 2005). Conversely, several studies by Wewers and Reed showed that THP-1 cells treated with small interfering RNA for human ASC decreased their caspase-1 activity while enhancing their NF-κB signal (Stehlik et al., 2002; Sarkar et al., 2006; Yu et al., 2006). The later studies showed that the interaction of caspase-1 and RIP2 mediates NF-κB activation which is prevented by human ASC as it hinders their interaction. Here, we report that mouse Asc deters the activation of NF-κB during *L. pneumophila* infection since the depletion of Asc allowed more NF-κB activation and additional *L. pneumophila* growth.

Importantly, murine Asc is induced in response to *L. pneumophila* infection (**Figure A2**). This is in stark contrast to human ASC, which is down regulated in human monocytes upon infection with *L. pneumophila* (Abdelaziz et al., 2011). The down regulation of human ASC contributed to the permissiveness to *L. pneumophila* growth. These findings are among the first reports clarifying the mechanism of permissiveness of human monocytes to *L. pneumophila* (Abdelaziz et al., 2011). Thus, regulating Asc availability in human phagocytes could be a mechanism employed by *L. pneumophila* to modulate caspase-1, NF-κB, and pyroptosis.

The detection of cleaved caspase-1 in supernatants of cultured cells has been widely used as the hallmark for caspase-1 activation. Few studies have shown that Nlrc4/Ipaf$^{-/-}$ and Asc$^{-/-}$ macrophages fail to produce cleaved caspase-1 in response to *L. pneumophila*. The absence of active caspase-1 promoted *L. pneumophila* growth. Yet, it is unclear as to why Asc$^{-/-}$ macrophages do not allow *L. pneumophila* replication. Here we show that in Asc$^{-/-}$ murine macrophages, a small portion of caspase-1 is cleaved during *L. pneumophila* infection but is not detected in culture supernatants. However, we cannot exclude that cleaved caspase-1 was released in amounts below our detection
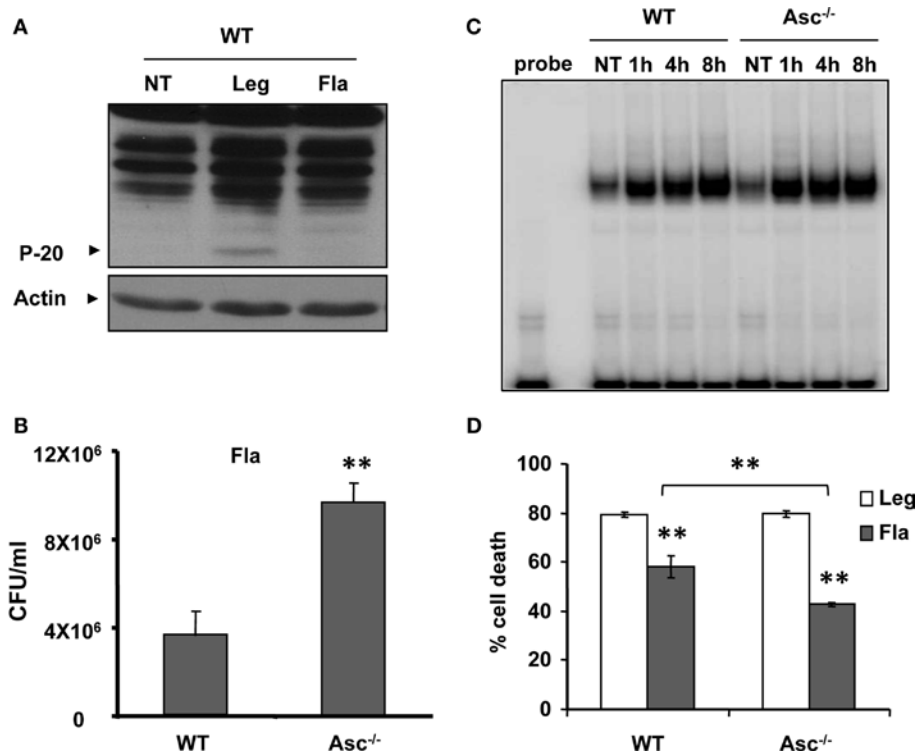
FIGURE 5 | Asc⁻/⁻ macrophages are permissive to flagellin mutant. (A) WT macrophages were infected with *L. pneumophila* (Leg), with corresponding flagellin mutant (Fla), or left untreated (NT). Active caspase-1 (p-20) was then detected in the cell extracts. **(B)** WT and Asc⁻/⁻ macrophages were infected with Fla mutant and CFUs were scored 72 h after infection. **(C)** WT and Asc⁻/⁻ macrophages were infected with Fla mutant for 1, 4, and 8 h then, nuclear extracts were processed for determination of NF-κB activation using electrophoretic mobility shift assay (EMSA). **(D)** WT and Asc macrophages were infected with Leg or Fla for 24 h then LDH release was determined and presented as percent cell death on the *Y* axis.

threshold. Our data agree with recent work by Monack group although the interpretation differs. They suggested that caspase-1 can be active without being cleaved and independently of Asc as suggested by the absence of cleaved caspase-1 in culture supernatants (Broz et al., 2010). However, it is possible that caspase-1 was modestly cleaved within the cytosol (which was not examined) and not released in supernatants. Irrespective of its release, the active cytosolic caspase-1 is required and sufficient to restrict *L. pneumophila* growth in Asc⁻/⁻ macrophages.

On the other hand, the *L. pneumophila* mutant lacking flagellin did not activate cytosolic caspase-1 in macrophages and replicated efficiently. These findings support the idea that cytosolic cleavage of caspase-1 is sufficient to restrict *L. pneumophila* growth within macrophages and requires flagellin. The flagellin mutant replicated more effectively in Asc⁻/⁻ macrophages than in WT ones although both cells lacked cytosolic caspase-1 activation and activated NF-κB at comparable levels. However, Asc⁻/⁻ macrophages survive more that WT ones allowing more time for Fla replication. This was because caspase-3 was activated in WT macrophages during late stages of Fla infection but not in Asc⁻/⁻ macrophages. It is possible that *L. pneumophila* activates caspase-3 directly or through the Bax/Bak pathway which is governed by Asc (Abu-Zant et al., 2005; Fischer et al., 2006). Taken together, these data suggest that Asc is involved in caspase-3 activation and apoptosis induction during *L. pneumophila* infection. Altogether, our data demonstrate that the host employs more than one mechanism to prevent *L. pneumophila* infection and that many of these are governed by Asc.
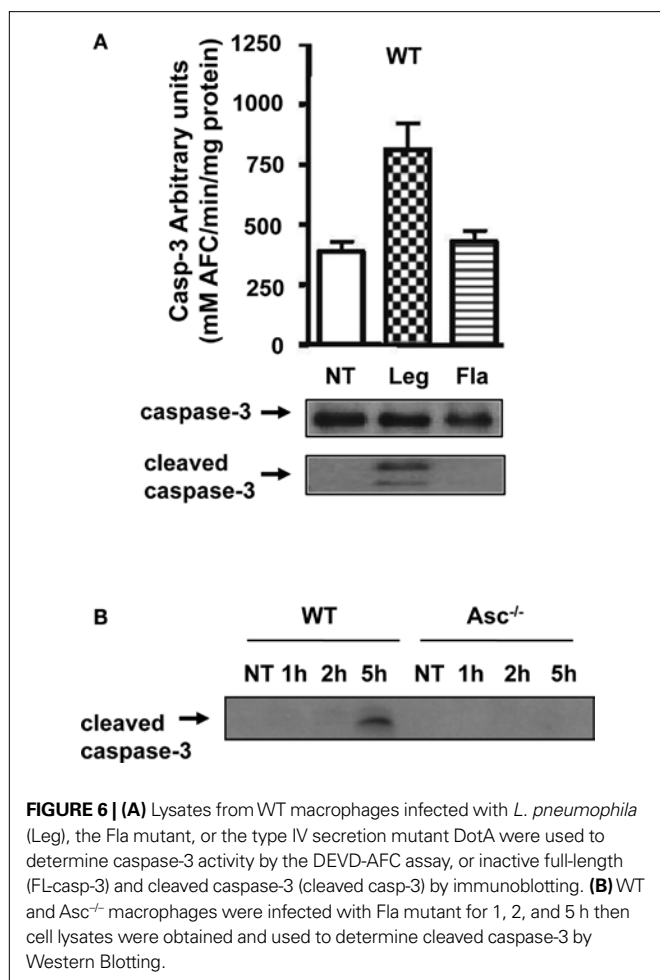
MATERIALS AND METHODS

MICE AND MACROPHAGES

Wild type C57BL/6 (B6) and Asc⁻/⁻ mice were previously described (Mariathasan et al., 2004). Caspase-1⁻/⁻ mice were from Dr. Amy Hise at Case Western University. All knockout mice were in a C57BL/6 background. Bone marrow-derived macrophages (BMDMs) were isolated from femurs of 6- to 12-week-old mice and were cultured in IMDM containing 10% heat-inactivated FBS, 20% L cell-conditioned medium, 100 U/ml penicillin, and 100 mg/ml streptomycin at 37°C in a humidified atmosphere containing 5% CO_2. After 5 days of incubation, cells were collected and plated in 6-well plates or in 24-well plates in IMDM containing 10% heat-inactivated FBS (Stanley, 1997; Amer et al., 2005, 2006; Akhter et al., 2009).

BACTERIAL STRAINS

Legionella pneumophila strain Lp02, is a thymine auxotrophic derivative of Philadelphia-1 (Brenner et al., 1979; McDade and Shepard, 1979). *L. pneumophila* flagellin (Fla) mutant was previously described (Albert-Weissenberger et al., 2010). *L. pneumophila* was cultured as described previously (Sturgill-Koszycki and Swanson, 2000; Akhter et al., 2009) All experiments were performed at a low MOI of 0.5, followed by centrifugation and rinsing of the wells after 30 min except when otherwise indicated (Derre and Isberg, 2004).

FIGURE 6 | (A) Lysates from WT macrophages infected with *L. pneumophila* (Leg), the Fla mutant, or the type IV secretion mutant DotA were used to determine caspase-3 activity by the DEVD-AFC assay, or inactive full-length (FL-casp-3) and cleaved caspase-3 (cleaved casp-3) by immunoblotting. **(B)** WT and Asc−/− macrophages were infected with Fla mutant for 1, 2, and 5 h then cell lysates were obtained and used to determine cleaved caspase-3 by Western Blotting.

INTRACELLULAR GROWTH OF *L. PNEUMOPHILA*

All experiments were performed at an MOI ranging 0.1–1, followed by centrifugation and rinsing of the wells after 30 min except when otherwise indicated (Derre and Isberg, 2004). All experiments were performed in the absence of ferric nitrate and L-cysteine from the monocytes or macrophage culture medium, to allow *L. pneumophila* multiplication only intracellularly. At designated time points, macrophages were lysed and plated on AYE plates for CFUs (Amer et al., 2006; Abdelaziz et al., 2010). The quantification of the CFU *in vitro* was performed more than four independent times as described (Amer et al., 2006; Abdelaziz et al., 2010).

IMMUNOBLOTTING

Cell extracts of macrophages were prepared and immunoblotted with an antibody that recognizes Nlrc4, Asc (Alexis Biochemicals), caspase-1 (Santa Cruz), IL-1β (National Cancer Institute), caspase-3 (Cell Signalling), actin (Abcam), followed by appropriate secondary antibody as described (Amer et al., 2006; Abdelaziz et al., 2010).

NF-κB DNA BINDING ACTIVITY ASSAY

Nuclear extracts of *L. pneumophila* treated or untreated BMDMs from WT and Asc−/− mice were prepared as previously described (Akhter et al., 2009). EMSA was used to measure NF-κB DNA binding activity as described (Sarkar et al., 2006).

QUANTITATIVE PCR

Total RNA was extracted from cells lysed in Trizol (Invitrogen Life Technologies) and 1–2 μg of the RNA was converted to cDNA by ThermoScript RNase H− Reverse Transcriptase (Invitrogen, Life Technologies). 20–60 ng of the converted cDNA was then used for quantitative PCR with SYBR Green I PCR Master Mix using the StepOne Plus Real Time PCR System (Applied Biosystems). The target gene C_t values were normalized to the C_t values of two housekeeping genes (mouse GAPDH and CAP-1) and expressed as relative copy number (RCN), as described earlier (Gavrilin et al., 2006; Zakharova et al., 2010). Primers used in RT-PCR are presented in **Table A1** of Appendix. We also evaluated expression of an around 600 genes with Open Array Mouse Inflammatory Panel (BioTrove, Life Technologies). All individual C_t values were normalized to the average of 18 housekeeping genes used in this array, and also expressed as RCN.

CASPASE-3 ACTIVITY ASSAY

Active caspase-3 was determined by the AFC assay, as previously described (Gonzalez-Mejia et al., 2010). Lysates were incubated in a cyto-buffer (10% glycerol, 50 mM Pipes, pH 7.0, 1 mM EDTA, containing 1 mM DTT) containing 20 mM of the tetrapeptide substrate DEVD-AFC. The tetrapeptide was obtained from Enzyme Systems Products (Livermore, CA, USA). Release of free AFC was determined using a Cytofluor 4000 fluorometer (Perspective Company, Framingham, MA, USA; Filters: excitation; 400 nm, emission; 508 nm).

LDH RELEASE ASSAY

LDH release into cell culture medium was used as an indicator of cell death using NAD+ reduction assay, according to the manufacturer (Roche Applied Science). Cells were plated in 12-well plate at the density 0.5×10^6, and 0.5 MOI of both wild type *L. pneumophila* (Leg) and its flagellin mutant (Fla) were added. Cell culture medium was collected 24 h post infection; clarified from floating bacteria by centrifugation; and used for LDH assay. To determine spontaneous cell death, referred as a negative control, we collected medium from cells incubated the same time without bacteria. To measure total LDH content in cells, referred as a positive control, cells were lysed by adding TritonX-100 (1% final concentration) to the well. Media alone was used as a blank. LDH concentration in the medium was detected at OD 490 nm. Cell death was calculated by the following formula: cytotoxicity (%) = (sample−blank/positive control−blank) × 100.

STATISTICAL ANALYSIS

Data are displayed as mean of three independent experiments ± SD. *P* value ≤0.05 was considered significant.

REFERENCES

Abdelaziz, D. H., Amr, K., and Amer, A. O. (2010). Nlrc4/Ipaf/CLAN/CARD12: more than a flagellin sensor. *Int. J. Biochem. Cell Biol.* 42, 789–791.

Abdelaziz, D. H., Gavrilin, M. A., Akhter, A., Caution, K., Kotrange, S., Khweek, A. A., Abdulrahman, B. A., Grandhi, J., Hassan, Z. A., Marsh, C., Wewers, M. D., and Amer, A. O. (2011). ASC controls *Legionella pneumophila* infection in human monocytes. *J. Biol. Chem.* 286, 3203–3208.

Abu-Zant, A., Jones, S., Asare, R., Suttles, J., Price, C., Graham, J., and Kwaik, Y. A. (2007). Anti-apoptotic signalling by the Dot/Icm secretion system of *L. pneumophila*. *Cell Microbiol.* 9, 246–264.

Abu-Zant, A., Santic, M., Molmeret, M., Jones, S., Helbig, J., and Abu Kwaik, Y. (2005). Incomplete activation of macrophage apoptosis during intracellular replication of *Legionella pneumophila*. *Infect. Immun.* 73, 5339–5349.

Akhter, A., Gavrilin, M. A., Frantz, L., Washington, S., Ditty, C., Limoli, D., Day, C., Sarkar, A., Newland, C., Butchar, J., Marsh, C. B., Wewers, M. D., Tridandapani, S., Kanneganti, T. D., and Amer, A. O. (2009). Caspase-7 activation by the Nlrc4/Ipaf inflammasome restricts *Legionella pneumophila* infection. *PLoS Pathog.* 5, e1000361. doi: 10.1371/journal.ppat.1000361

Albert-Weissenberger, C., Sahr, T., Sismeiro, O., Hacker, J., Heuner, K., and Buchrieser, C. (2010). Control of flagellar gene regulation in *Legionella pneumophila* and its relation to growth phase. *J. Bacteriol.* 192, 446–455.

Amer, A., Franchi, L., Kanneganti, T. D., Body-Malapel, M., Ozoren, N., Brady, G., Meshinchi, S., Jagirdar, R., Gewirtz, A., Akira, S., and Nunez, G. (2006). Regulation of *Legionella* phagosome maturation and infection through flagellin and host ipaf. *J. Biol. Chem.* 281, 35217–35223.

Amer, A. O. (2010). Modulation of caspases and their non-apoptotic functions by *Legionella pneumophila*. *Cell. Microbiol.* 12, 140–147.

Amer, A. O., Byrne, B. G., and Swanson, M. S. (2005). Macrophages rapidly transfer pathogens from lipid raft vacuoles to autophagosomes. *Autophagy* 1, 53–58.

Bartfeld, S., Engels, C., Bauer, B., Aurass, P., Flieger, A., Bruggemann, H., and Meyer, T. F. (2009). Temporal resolution of two-tracked NF-kappaB activation by *Legionella pneumophila*. *Cell. Microbiol.* 11, 1638–1651.

Bedoya, F., Sandler, L. L., and Harton, J. A. (2007). Pyrin-only protein 2 modulates NF-kappaB and disrupts ASC:CLR interactions. *J. Immunol.* 178, 3837–3845.

Brenner, D. J., Steigerwalt, A. G., and McDade, J. E. (1979). Classification of the Legionnaires' disease bacterium: *Legionella pneumophila*, genus novum, species nova, of the family Legionellaceae, familia nova. *Ann. Intern. Med.* 90, 656–658.

Broz, P., von Moltke, J., Jones, J. W., Vance, R. E., and Monack, D. M. (2010). Differential requirement for caspase-1 autoproteolysis in pathogen-induced cell death and cytokine processing. *Cell Host Microbe* 8, 471–483.

Case, C. L., Shin, S., and Roy, C. R. (2009). Asc and ipaf inflammasomes direct distinct pathways for caspase-1 activation in response to *Legionella pneumophila*. *Infect. Immun.* 77, 1981–1991.

Chamaillard, M., Hashimoto, M., Horie, Y., Masumoto, J., Qiu, S., Saab, L., Ogura, Y., Kawasaki, A., Fukase, K., Kusumoto, S., Valvano, M. A., Foster, S. J., Mak, T. W., Nunez, G., and Inohara, N. (2003). An essential role for NOD1 in host recognition of bacterial peptidoglycan containing diaminopimelic acid. *Nat. Immunol.* 4, 702–707.

Coers, J., Vance, R. E., Fontana, M. F., and Dietrich, W. F. (2007). Restriction of *Legionella pneumophila* growth in macrophages requires the concerted action of cytokine and Naip5/Ipaf signalling pathways. *Cell. Microbiol.* 9, 2344–2357.

Derre, I., and Isberg R. R. (2004). Macrophages from mice with the restrictive Lgn1 allele exhibit multifactorial resistance to *Legionella pneumophila*. *Infect. Immun.* 72, 6221–6229.

Fernandes-Alnemri, T., and Alnemri E. S. (2008). Assembly, purification, and assay of the activity of the ASC pyroptosome. *Meth. Enzymol.* 442, 251–270.

Fernandes-Alnemri, T., Wu, J., Yu, J. W., Datta, P., Miller, B., Jankowski, W., Rosenberg, S., Zhang, J., and Alnemri, E. S. (2007). The pyroptosome: a supramolecular assembly of ASC dimers mediating inflammatory cell death via caspase-1 activation. *Cell Death Differ.* 14, 1590–1604.

Fischer, S. F., Vier, J., Muller-Thomas, C., and Hacker, G. (2006). Induction of apoptosis by *Legionella pneumophila* in mammalian cells requires the mitochondrial pathway for caspase activation. *Microbes Infect.* 8, 662–669.

Franchi, L., Park, J. H., Shaw, M. H., Marina-Garcia, N., Chen, G., Kim, Y. G., and Nunez, G. (2008). Intracellular NOD-like receptors in innate immunity, infection and disease. *Cell. Microbiol.* 10, 1–8.

Gavrilin, M. A., Bouakl I. J., Knatz, N. L., Duncan, M. D., Hall, M. W., Gunn, J. S., and Wewers, M. D. (2006).

Internalization and phagosome escape required for *Francisella* to induce human monocyte IL-1beta processing and release. *Proc. Natl. Acad. Sci. U.S.A.* 103, 141–146.

Gonzalez-Mejia, M. E., Voss, O. H., Murnan, E. J., and Doseff, A. I. (2010). Apigenin-induced apoptosis of leukemia cells is mediated by a bimodal and differentially regulated residue-specific phosphorylation of heat-shock protein-27. *Cell Death Dis.* 1, e64.

Hasegawa, M., Imamura, R., Kinoshita, T., Matsumoto, N., Masumoto, J., Inohara, N., and Suda, T. (2005). ASC-mediated NF-kappaB activation leading to interleukin-8 production requires caspase-8 and is inhibited by CLARP. *J. Biol. Chem.* 280, 15122–15130.

Hasegawa, M., Imamura, R., Motani, K., Nishiuchi, T., Matsumoto, N., Kinoshita, T., and Suda, T. (2009). Mechanism and repertoire of ASC-mediated gene expression. *J. Immunol.* 182, 7655–7662.

Hasegawa, M., Kawase, K., Inohara, N., Imamura, R., Yeh, W. C., Kinoshita, T., and Suda, T. (2007). Mechanism of ASC-mediated apoptosis: bid-dependent apoptosis in type II cells. *Oncogene* 26, 1748–1756.

Horwitz, M. A., and Silverstein, S. C. (1981). Interaction of the Legionnaires' disease bacterium (*Legionella pneumophila*) with human phagocytes. I. L. pneumophila resists killing by polymorphonuclear leukocytes, antibody, and complement. *J. Exp. Med.* 153, 386–397.

Horwitz, M. A., and Silverstein, S. C. (1983). Intracellular multiplication of Legionnaires' disease bacteria (*Legionella pneumophila*) in human monocytes is reversibly inhibited by erythromycin and rifampin. *J. Clin. Invest.* 71, 15–26.

Kang, T. J., Lee G. S., Kim, S. K., Jin, S. H., and Chae, G. T. (2010). Comparison of two mice strains, A/J and C57BL/6, in caspase-1 activity and IL-1beta secretion of macrophage to *Mycobacterium leprae* infection. *Mediators Inflamm.* 2010, 708713.

Kanneganti, T. D., Lamkanfi, M., and Nunez, G. (2007). Intracellular NOD-like receptors in host defense and disease. *Immunity* 27, 549–559.

Lamkanfi, M., Amer, A., Kanneganti, T. D., Munoz-Planillo, R., Chen, G., Vandenabeele, P., Fortier, A., Gros, P., and Nunez, G. (2007). The Nod-like receptor family member Naip5/Birc1e restricts *Legionella pneumophila* growth independently of caspase-1 activation. *J. Immunol.* 178, 8022–8027.

Lamkanfi, M., and Kanneganti, T. D. (2010). Caspase-7: a protease involved in apoptosis and inflammation. *Int. J. Biochem. Cell Biol.* 42, 21–24.

Lamkanfi, M., Kanneganti T. D., Van Damme, P., Vanden Berghe, T., Vanoverberghe, I., Vandekerckhove, J., Vandenabeele, P., Gevaert, K., and Nunez, G. (2008). Targeted peptide-centric proteomics reveals caspase-7 as a substrate of the caspase-1 inflammasomes. *Mol. Cell Proteomics* 7, 2350–2363.

Lamkanfi, M., Moreira, L. O., Makena, P., Spierings, D. C., Boyd, K., Murray, P. J., Green, D. R., and Kanneganti, T. D. (2009). Caspase-7 deficiency protects from endotoxin-induced lymphocyte apoptosis and improves survival. *Blood* 113, 2742–2745.

Liepinsh, E., Barbals, R., Dahl, E., Sharipo, A., Staub, E., and Otting, G. (2003). The death-domain fold of the ASC PYRIN domain, presenting a basis for PYRIN/PYRIN recognition. *J. Mol. Biol.* 332, 1155–1163.

Lightfield, K. L., Persson, J., Brubaker, S. W., Witte, C. E., von Moltke, J., Dunipace, E. A., Henry, T., Sun, Y. H., Cado, D., Dietrich, W. F., Monack, D. M., Tsolis, R. M., and Vance, R. E. (2008). Critical function for Naip5 in inflammasome activation by a conserved carboxy-terminal domain of flagellin. *Nat. Immunol.* 9, 1171–1178.

Losick, V. P., Haenssler, E., Moy, M. Y., and Isberg, R. R. (2010). LnaB: a *Legionella pneumophila* activator of NF-kappaB. *Cell. Microbiol.* 12, 1083–1097.

Losick, V. P., and Isberg, R. R. (2006). NF-kappaB translocation prevents host cell death after low-dose challenge by *Legionella pneumophila*. *J. Exp. Med.* 203, 2177–2189.

Mariathasan, S. (2007). ASC, ipaf and cryopyrin/Nalp3: bona fide intracellular adapters of the caspase-1 inflammasome. *Microbes Infect.* 9, 664–671.

Mariathasan, S., Newton, K., Monack, D. M., Vucic, D., French, D. M., Lee, W. P., Roose-Girma, M., Erickson, S., and Dixit, V. M. (2004). Differential activation of the inflammasome by caspase-1 adaptors ASC and Ipaf. *Nature* 430, 213–218.

Martinon, F., and Tschopp, J. (2007). Inflammatory caspases and inflammasomes: master switches of inflammation. *Cell Death Differ.* 14, 10–22.

Masumoto, J., Dowds, T. A., Schaner, P., Chen, F. F., Ogura, Y., Li, M., Zhu, L., Katsuyama, T., Sagara, J., Taniguchi, S., Gumucio, D. L., Nunez, G., and Inohara, N. (2003). ASC is an activating adaptor for NF-kappa B and caspase-8-dependent apoptosis. *Biochem. Biophys. Res. Commun.* 303, 69–73.

Masumoto, J., Taniguchi, S., Ayukawa, K., Sarvotham, H., Kishino, T., Niikawa, N., Hidaka, E., Katsuyama, T., Higuchi, T., and Sagara, J. (1999). ASC, a novel 22-kDa protein, aggregates during apoptosis of human promyelocytic

leukemia HL-60 cells. *J. Biol. Chem.* 274, 33835–33838.

Masumoto, J., Taniguchi, S., Nakayama, K., Ayukawa, K., and Sagara, J. (2001). Murine ortholog of ASC, a CARD-containing protein, self-associates and exhibits restricted distribution in developing mouse embryos. *Exp. Cell Res.* 262, 128–133.

McDade, J. E., and Shepard, C. C. (1979). Virulent to avirulent conversion of Legionnaires' disease bacterium (*Legionella pneumophila*) – its effect on isolation techniques. *J. Infect. Dis.* 139, 707–711.

Molmeret, M., Zink S. D., Han, L., Abu-Zant, A., Asari, R., Bitar, D. M., and Abu Kwaik, Y. (2004). Activation of caspase-3 by the Dot/Icm virulence system is essential for arrested biogenesis of the *Legionella*-containing phagosome. *Cell. Microbiol.* 6, 33–48.

Motani, K., Kawase, K., Imamura, R., Kinoshita, T., Kushiyama, H., and Suda, T. (2010). Activation of ASC induces apoptosis or necrosis, depending on the cell type, and causes tumor eradication. *Cancer Sci.* 101, 1822–1827.

Nash, T. W., Libby, D. M., and Horwitz, M. A. (1984). Interaction between the legionnaires' disease bacterium (*Legionella pneumophila*) and human alveolar macrophages. Influence of antibody, lymphokines, and hydrocortisone. *J. Clin. Invest.* 74, 771–782.

Ohtsuka, T., Ryu, H., Minamishima, Y. A., Macip, S., Sagara, J., Nakayama, K. I., Aaronson, S. A., and Lee, S. W. (2004).

ASC is a Bax adaptor and regulates the p53-Bax mitochondrial apoptosis pathway. *Nat. Cell Biol.* 6, 121–128.

Ren, T., Zamboni, D. S., Roy, C. R., Dietrich, W. F., and Vance, R. E. (2006). Flagellin-deficient Legionella mutants evade caspase-1- and Naip5-mediated macrophage immunity. *PLoS Pathog.* 2, e18. doi: 10.1371/journal.ppat.0020018

Sarkar, A., Duncan, M., Hart, J., Hertlein, E., Guttridge, D. C., and Wewers, M. D. (2006). ASC directs NF-kappaB activation by regulating receptor interacting protein-2 (RIP2) caspase-1 interactions. *J. Immunol.* 176, 4979–4986.

Shaw, P. J., Lamkanfi, M., and Kanneganti, T. D. (2010). NOD-like receptor (NLR) signaling beyond the inflammasome. *Eur. J. Immunol.* 40, 624–627.

Srinivasula, S. M., Poyet, J. L., Razmara, M., Datta, P., Zhang, Z., and Alnemri, E. S. (2002). The PYRIN-CARD protein ASC is an activating adaptor for caspase-1. *J. Biol. Chem.* 277, 21119–21122.

Stanley, E. R. (1997). Murine bone marrow-derived macrophages. *Methods Mol. Biol.* 75, 301–304.

Stehlik, C., Fiorentino, L., Dorfleutner, A., Bruey, J. M., Ariza, E. M., Sagara, J., and Reed, J. C. (2002). The PAAD/PYRIN-family protein ASC is a dual regulator of a conserved step in nuclear factor kappaB activation pathways. *J. Exp. Med.* 196, 1605–1615.

Stehlik, C., Krajewska, M., Welsh, K., Krajewski, S., Godzik, A., and Reed, J. C. (2003). The PAAD/PYRIN-only

protein POP1/ASC2 is a modulator of ASC-mediated nuclear-factor-kappa B and pro-caspase-1 regulation. *Biochem. J.* 373(Pt 1), 101–113.

Sturgill-Koszycki, S., and Swanson, M. S. (2000). *Legionella pneumophila* replication vacuoles mature into acidic, endocytic organelles. *J. Exp. Med.* 192, 1261–1272.

Taniguchi, S., and Sagara, J. (2007). Regulatory molecules involved in inflammasome formation with special reference to a key mediator protein, ASC. *Semin. Immunopathol.* 29, 231–238.

Vance, R. E. (2010). Immunology taught by bacteria. *J. Clin. Immunol.* 30, 507–511.

Yu, J. W., Wu, J., Zhang, Z., Datta, P., Ibrahimi, I., Taniguchi, S., Sagara, J., Fernandes-Alnemri, T., and Alnemri, E. S. (2006). Cryopyrin and pyrin activate caspase-1, but not NF-kappaB, via ASC oligomerization. *Cell Death Differ.* 13, 236–249.

Zakharova, E., Grandhi, J., Wewers, M. D., and Gavrilin, M. A. (2010). Mycoplasma suppression of THP-1 Cell TLR responses is corrected with antibiotics. *PLoS ONE* 5, e9900. doi: 10.1371/journal.pone.0009900

Zamboni, D. S., Kobayashi, K. S., Kohlsdorf, T., Ogura, Y., Long, E. M., Vance, R. E., Kuida, K., Mariathasan, S., Dixit, V. M., Flavell, R. A., Dietrich, W. F., and Roy, C. R. (2006). The Birc1e cytosolic pattern-recognition receptor contributes to the detection and control of *Legionella pneumophila* infection. *Nat. Immunol.* 7, 318–325.

APPENDIX

FIGURE A1 | (A) Wild type (WT) mouse macrophages and heterozygous Asc (Asc^{−/+}) were infected or not (NT) with *L. pneumophila* (Leg) at MOI = 0.5 for 1, 2, and 4 h. Total active caspase-1 (p-20) was detected in combined lysate and supernatants by Western blotting. **(B)** WT and caspase-1^{−/−} macrophages were infected with *L. pneumophila* (Leg) at MOI = 0.1 and the bacterial replication was assessed by counting the colony-forming units (CFU) after 1, 24, 48, and 72 h. Results are displayed as mean ± SD of three independent wells. **P ≤ 0.01.

FIGURE A2 | *Legionella pneumophila* differentially regulates the expression of several components of the inflammasome. WT mouse macrophages were infected or not (NT) with *Legionella pneumophila* (Leg) at MOI = 0.5 for 4 and 24 h. then the expressions of **(A)** pro-caspase-1, **(B)** pro-Il-1β, **(C)** Pycard (Asc), and **(D)** Nlrc4 (Ipaf) were then assessed on both mRNA (upper panels) and protein levels (lower panels) using RT-PCR and Western blots, respectively. The data of RT-PCR are displayed as mean relative copy numbers (RCN) ± SD of three independent experiments. *P ≤ 0.05, **P ≤ 0.01. Actin was used as loading control.

Table A1 | Mouse primers used in RT-PCR.

Gene	Size		Sequence
Casp1	145	F	ACCCTCAAGTTTTGCCCTTT
		R	CCCTCGGAGAAAGATGTTGA
Cap1	96	F	GAAGGCGGTGATTTTAACGA
		R	TCCAGCGATTTCTGTCACTG
Gapdh	128	F	TGGCATTGTGGAAGGGCTCA
		R	TGGATGCAGGGATGATGTTCT
Pycard	173	F	GCTCACAATGACTGTGCTTAG
		R	TGACCCTGGCAATGAGTGCT
Il 1b	153	F	CCTGAACTCAACTGTGAAATGC
		R	GTGCTGCTGTGAGATTTGAAG
Nlrc4	152	F	AGGACTTGCCAAACTTGGATT
		R	TGAAGTAAAGCCATCCGTCAC