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Innate sensing and cell-autonomous resistance pathways in *Legionella pneumophila* infection

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ABSTRACT

Legionella pneumophila is a facultative intracellular bacterium which can cause a severe pneumonia called Legionnaires' disease after inhalation of contaminated water droplets and replication in alveolar macrophages. The innate immune system is generally able to sense and – in most cases- control *L. pneumophila* infection. Comorbidities and genetic risk factors, however, can compromise the immune system and high infection doses might overwhelm its capacity, thereby enabling *L. pneumophila* to grow and disseminate inside the lung. The innate immune system mediates sensing of *L. pneumophila* by employing *e.g.* NOD-like receptors (NLRs), Toll-like receptors (TLRs), as well as the cGAS/STING pathway to stimulate death of infected macrophages as well as production of proinflammatory cytokines and interferons (IFNs). Control of pulmonary *L. pneumophila* infection is largely mediated by inflammasome-, TNFα- and IFN-dependent macrophage-intrinsic resistance mechanisms. This article summarizes the current knowledge of innate immune responses to *L. pneumophila* infection in general, and of macrophage-intrinsic defense mechanisms in particular.

1. Introduction

Many invasive bacterial pathogens exploit intracellular niches to hide from the hosts humoral immune response. While some bacteria escape into the host cell cytosol upon infection, others establish a membrane-bound compartment specified to enable intracellular bacterial replication (Santos and Enninga, 2016; Sherwood and Roy, 2013). However, as a result of co-evolution, host cells have in turn developed strategies to defend against intracellular pathogens in order to control infections (Randow et al., 2013). Studying such infections has greatly broadened our knowledge of intracellular innate immune sensing pathways and cell-autonomous defense mechanisms.

One bacterial pathogen which is able to replicate in specialized vacuoles inside alveolar macrophages is *Legionella pneumophila* (Horwitz and Silverstein, 1980; Nash et al., 1984). Naturally, *L. pneumophila* persists in the environment as a parasite of freshwater protozoans such as *Acanthamoeba castelanii* (Fields, 1996). Humans can get infected following inhalation of contaminated aerosols from *e.g.* cooling towers, hot and cold water systems and whirlpool spas (Cunha et al., 2016). While the majority of immunocompetent human individuals remain asymptomatic upon exposure or suffer only mild, flu-like Pontiac fever, infection can also develop into a severe pneumonia which is called Legionnaires' disease (Cunha et al., 2016).

The risk for developing pneumonia depends on the bacterial concentration in the aerosol, the virulence of the bacterial strain, and on the susceptibility of the host. For example, *L. pneumophila* serogroup 1 (mAb3/1 positive) strains appear to be particularly virulent as they account for approximately 65–90% of the reported cases of Legionnaires' disease, although other strains account for the majority of isolates obtained from environmental samples (Cunha et al., 2016). Host factors that predispose to acquisition of Legionnaire's disease include older age, smoking, chronic respiratory and cardiovascular diseases, diabetes, a history of cancer or hematologic malignancies and immunosuppression (Phin et al., 2014; von Baum et al., 2008). In addition, genetic factors that affect antibacterial innate immune responses may enhance susceptibility to *Legionella* infection (Berrington and Hawn, 2013).

Following inhalation of contaminated aerosols, *L. pneumophila* is phagocytized by alveolar macrophages. Inside their host cells, *L. pneumophila* prevents transport of the phagosome through the endocytic pathway, and actively transforms it into a endoplasmic reticulum (ER)-like replicative organelle called *Legionella*-containing vacuole (LCV)

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(Horwitz, 1983; Isberg et al., 2009). This process requires the *dot/icm*-encoded type IV secretion system (T4SS). The T4SS injects around 300 bacterial effector molecules into the host cytosol which target GTPases and other host factors which lead to the enrichment of secretory vesicles from the endoplasmic reticulum (ER) as well as increase of LCV-mitochondria contacts (Asrat et al., 2014; Chong et al., 2009; Escoll et al., 2017; Hubber and Roy, 2010). Upon LCV establishment, *L. pneumophila* starts to replicate to high numbers before host cells are lysed and bacteria are released. In addition to the T4SS, full virulence of *L. pneumophila* also depends on a type II secretion system (T2SS) (Cianciotto and White, 2017).

While *L. pneumophila* manipulates host cell processes in order to establish an intracellular niche for their survival and replication, the host has evolved defense mechanisms to restrict infection. The balance between bacterial load as well as virulence on the one side, and external as well as genetic host factors that alter the immune systeḿs ability to control infection on the other side, determines the outcome of such bacterial encounters.

2. Innate immunity sensing of *L. pneumophila*

The immune system detects *L. pneumophila* by various pattern recognition receptors such as Toll-like receptors (TLRs), NOD-like receptors (NLRs), and cytosolic nucleic acid sensors (Brown et al., 2017; Chaput et al., 2013; Massis and Zamboni, 2011; Opitz et al., 2010).

2.1. TLRs

TLR2 and TLR5 recognize *Legionella* cell wall components and flagellin, respectively, at the cell surface, whereas TLR9 senses bacterial DNA in *endo-*/phagosomes (Akamine et al., 2005; Archer and Roy, 2006; Fuse et al., 2007; Girard et al., 2003; Newton et al., 2007) (Fig. 1). It has been demonstrated that TLR5 and −9 are largely redundant for defense against *Legionella* in mice, while TLR2 contributes to production of NFκB-dependent proinflammatory mediators such as TNFα and various chemokines (Archer et al., 2009; Archer and Roy, 2006; Fuse et al., 2007; Hawn et al., 2006). Chemokines induce recruitment of neutrophils which act bactericidally and contribute to cytokine production (Casson et al., 2017). On the other hand, *Legionella* might be able to partly evade TLR-mediated sensing, as a recent study indicated that TLR2-dependent signaling is dampened by the T2SS system in human but not murine macrophages (Mallama et al., 2017). Moreover, while lack of TLR5 does not affect *Legionella* infection in mice, this receptor appears to play a prominent role in humans. Indeed, a common dominant stop codon polymorphism in the gene encoding TLR5 has been associated with susceptibility to Legionnaires' disease (Hawn et al., 2003).

2.2. NLRs and inflammasomes

Chemokine production during *L. pneumophila* infection additionally depends on the NLRs NOD1 and NOD2, which sense *Legionella* cell wall peptidoglycan. Mice deficient in both NLRs or RIP2, the shared signaling mediator downstream of NOD1 and −2, exhibit impaired neutrophil recruitment and reduced bacterial clearance during lung infection (Frutuoso et al., 2010; Shin et al., 2008). Other NLRs involved in sensing of *L. pneumophila* are NAIP5, NLRC4 and NLRP3 which form multiprotein complexes called inflammasomes.

Different alleles of NAIP5 have long been known to determine whether a mouse is resistant or (moderately) susceptible to *L. pneumophila* infection (Diez et al., 2003; Wright et al., 2003). NAIP5 heterodimerizes with NLRC4 to generate the NAIP5/NLRC4 inflammasome which recognizes bacterial flagellin that is delivered into the host cell cytosol by the T4SS (Lightfield et al., 2011; Mariathasan et al., 2004; Pereira et al., 2011b; Poyet et al., 2001; Ren et al., 2006; Zamboni et al., 2006). Similarly, human NAIP has been shown to detect bacterial flagellin in the host cell cytosol (Kortmann et al., 2015; Vinzing et al., 2008). The NAIP5/NLRC4 inflammasome activates caspase-1 to regulate production of IL-1β and IL-18, and to restrict *L. pneumophila* growth in macrophages and mice. This bacterial growth restriction depends on gasdermin D-dependent cell death called pyroptosis and on induction of LCV − lysosome fusion (Amer et al., 2006; Fortier et al., 2007; Molofsky et al., 2006; Ren et al., 2006; Shi et al., 2015; Zamboni et al., 2006). In addition, a recent study indicated that caspase-8 also associates with NAIP5/NLRC4 inflammasomes upon *L. pneumophila* infection, although its activation could only been observed upon caspase-1 and gasdermin D depletion (Mascarenhas et al., 2017). In contrast to *L. pneumophila*, the non-flagellated *Legionella* species *L. longbeachae* fails to trigger pyroptosis and is not restricted by the NAIP5/NLRC4 inflammasome (Cazalet et al., 2010; Pereira et al., 2011a). Moreover, the NLRP3 inflammasome contributes to production of IL-1β and IL-18 during *L. pneumophila* infection, but is less essential for controlling infection *in vivo* (Case et al., 2009; Casson et al., 2013).

L. pneumophila mutants lacking the T4SS effector SdhA additionally activate the AIM2 inflammasome, which is formed by the non-NLR DNA sensor AIM2. SdhA is required for maintaining LCV membrane integrity, and absence of SdhA results in leakage of bacterial DNA into the cytosol and activation of AIM2 (Ge et al., 2012). In addition to these canonical inflammasomes, *L. pneumophila* infection is also sensed by the non-canonical caspase-11 inflammasome (Aachoui et al., 2013; Case et al., 2013; Casson et al., 2013; Kayagaki et al., 2011). Caspase-11 as well as its human orthologues caspase-4 and −5 directly bind LPS in the cytosol (Shi et al., 2014). Guanylate-binding proteins (GBPs) are required for caspase-11 activation by promoting lysis of the LCV and bacterial access to the cytosol (Meunier et al., 2014) (Pilla et al., 2014). Activated caspase-11 and −4 in turn cleave gasdermin D to induce pyroptosis and to activate the NLRP3 inflammasome via K+ efflux (Aachoui et al., 2013; Case et al., 2013; Casson et al., 2013; Ruhl and Broz, 2015; Schmid-Burgk et al., 2015). Moreover, murine caspase-11 as well as its human orthologues caspase-4 and −5 have been shown to restrict *Legionella* replication by promoting fusion of LCVs with lysosomes (Akhter et al., 2012). Thus, both canonical inflammasomes as well as non-canonical inflammasomes contribute to macrophage-intrinsic defense against *L. pneumophila*.

2.3. Cytokines, chemokines and immune cells

IL-1α and IL-1β released by *L. pneumophila*-infected alveolar macrophages activate non-infected bystander macrophages, neutrophils as well as non-hematopoietic cells via the IL-1 receptor (IL-1R) to produce proinflammatory cytokines and chemokines, the latter of which critically contribute to neutrophil recruitment (Barry et al., 2013; Copenhaver et al., 2015; LeibundGut-Landmann et al., 2011). Neutrophils produce antibacterial reactive oxygen species and cytokines such as TNFα (Ziltener et al., 2016). Another important producer of TNFα are monocytes which are CCL2-dependently recruited to the lung upon infection (Casson et al., 2017; Ziltener et al., 2016). Recruited monocyte-derived cells are also main producers of IL-12, which is required for induction of IFNγ production by e.g. NK cells and T cells (Brown et al., 2016; Casson et al., 2017). IFNγ is an important mediator of macrophage-intrinsic defense against intracellular pathogens such as *L. pneumophila* (see below).

2.4. Nucleic acid sensing and type I IFNs

Another important pathway mediating innate immune detection of *L. pneumophila* is activated by bacterial nucleic acids in the cytosol. We and others previously showed that production of type I IFNs (IFNα/β) upon *L. pneumophila* infection depends on the adapter molecule STING (stimulator of interferon genes), the transcription factor IRF3 (interferon regulatory factor 3), the bacterial T4SS and cytosolic sensing of bacterial DNA (Lippmann et al., 2011; Lippmann et al., 2008; Opitz