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Legionella secreted effectors and innate immune responses

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Summary

Legionella pneumophila is a facultative intracellular pathogen capable of replicating in a wide spectrum of cells. Successful infection by Legionella requires the Dot/Icm type IV secretion system, which translocates a large number of effector proteins into infected cells. By co-opting numerous host cellular processes, these proteins function to establish a specialized organelle that allows bacterial survival and proliferation. Even within the vacuole, *L. pneumophila* triggers robust immune responses. Recent studies reveal that a subset of Legionella effectors directly target some basic components of the host innate immunity systems such as phagosome maturation. Others play essential roles in engaging the host innate immune surveillance system. This review will highlight recent progress in our understanding of these interactions and discuss implications for the study of the immune detection mechanisms.

Keywords

Type IV secretion; phagosome maturation; NFκB; Interferon Induction

Introduction

The innate immune system functions to provide the first line of defense against pathogen attack and to signal the adaptive immune system of the presence of potential infection. This system is composed of two major components. The first is the scavenging function by phagocytic cells such as macrophages that engulf and digest incoming pathogens. Phagocytosis and subsequent lysosomal digestion is one of the first challenges met by pathogens. The second component is signaling pathways consisting of germline-encoded receptors that are highly conserved across different kingdoms of organisms and, which sense pathogen-associated molecular patterns (PAMPs). These receptors, called pattern recognition receptors (PRRs), can be divided into two families: the Toll-like receptors (TLRs), which recognize ligands on the cell surface or those associated with the lysosome/endosome, and the Nod-like receptors (NLRs), which detect bacterial and viral molecules in the cytoplasm (Akira *et al.*, 2006).

L. pneumophila was first recognized as a pathogen following an outbreak of pneumonia among attendees of the 1976 American Legion convention in Philadelphia (Fields *et al.*, 2002). This bacterium is ubiquitous in aquatic environments as a parasite of freshwater protozoa. This habitat is believed to provide the evolutionary pressure for the acquisition of the traits necessary for survival and replication in phagocytes (Swanson *et al.*, 2000). Development of this potentially fatal disease occurs when susceptible individuals inhale contaminated aerosols. Instead of being digested, *L. pneumophila* engulfed by alveolar macrophages undergoes robust intracellular growth, leading to tissue damage and

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subsequent development of the disease (Fields *et al.*, 2002). Largely due to the progress in our understanding of the cell biological and biochemical basis for its intracellular life cycle (Isberg *et al.*, 2009), *L. pneumophila* has become an attractive model in the study of bacterial pathogenesis as well as the mechanisms of pathogen detection by the immune system,.

The single most important pathogenic determinant of *L. pneumophila* is the Dot/Icm type IV secretion, which translocates effector proteins into host cells (Ensminger *et al.*, 2009). These effectors have some highly intriguing features: First, the repertoire is extremely large and more than 270 experimentally verified substrates have been identified (Luo *et al.*, 2004, Heidtman *et al.*, 2008, Burstein *et al.*, 2009, Huang *et al.*, 2010, Zhu *et al.*, 2011); this number is close to 10% of the predicted protein encoding genes in the genome of *L. pneumophila* (Cazalet *et al.*, 2004, Chien *et al.*, 2004). Second, the structures of these proteins are highly diverse. Whereas some proteins contain readily detectable signatures suggestive of potential biochemical activity (e.g. RalF (Nagai *et al.*, 2002)), most proteins are unique to Legionella and do not show significant homology to other proteins in the current database (Zhu *et al.*, 2011). Third, deletion of a single one of these genes rarely leads to defects in Legionella intracellular growth in standard experimental conditions (Isberg *et al.*, 2009). Finally, host processes targeted by these proteins are very diverse and in some cases the potential benefit of such modulation is not yet understood (Dorer *et al.*, 2006, Shin *et al.*, 2008b). These effectors not only play essential roles in the biogenesis of the specialized replicative organelle but also function as messengers that trigger host immune responses. The latter activity has been exploited as a highly effective tool to dissect the mechanisms used by the host to distinguish pathogenic microorganisms from non-harmful ones.

Here, I will discuss the roles of Dot/Icm effectors directly engaging the host innate immune system. The immune responses mediated by TLRs in response to Legionella PAMP molecules such as LPS and the detection of Legionella flagellin by pathways involved the NLR family protein Naip5 and Ipaf have recently been reviewed (Neild *et al.*, 2004, Vance, 2010) and will not be covered here.

Inhibition of phagosome maturation

After phagocytosis, the Legionella containing vacuole (LCV) bypasses the default phagosome maturation pathway. At least in the first 10 hours after uptake, LCVs maintain a neutral pH and are devoid of typical lysosomal proteins such as LAMP-1 (Sturgill-Koszycki *et al.*, 2000). Lysosomal killing is one of the basic components of innate immunity and is the first challenge *L. pneumophila* faces after phagocytosis. As a parasite of freshwater amoeba, *L. pneumophila* has evolved sophisticated mechanisms to escape the delivery of its phagosome into the lysosomal network. Active remodeling of the phagosomal membranes to mimic those of resident organelles is a common strategy used by intravacuolar pathogens (Blander *et al.*, 2006). Within minutes of its formation, membranes of the LCV are loaded with some resident proteins of the endoplasmic reticulum (ER), such as calnexin and Bip, indicating that the LCV interacts with the ER network (Swanson *et al.*, 1995, Tilney *et al.*, 2001). Further, inhibition of the activity of proteins such as the small GTPases Sar1 and Rab1, which regulate vesicle budding and trafficking from the ER leads to the arrest of intracellular bacterial growth (Kagan *et al.*, 2002). Based on these observations, Roy and colleagues proposed that *L. pneumophila* redirects the trafficking of ER-originated vesicles to remodel the LCV membranes (Kagan *et al.*, 2002). This model was supported by the fact that the LCV is surrounded by small smooth vesicles shortly after its formation (Tilney *et al.*, 2001, Robinson *et al.*, 2006), and was greatly substantiated by the discovery of Dot/Icm effectors that directly manipulate activities of Arf1 and Rab1, small GTPases essential for

regulation of vesicle trafficking between the ER and the Golgi apparatus (Nagai *et al.*, 2002, Machner *et al.*, 2006, Murata *et al.*, 2006).

Oscillating between an active GTP-bound form and an inactive GDP-bound form, Rab1 regulates several important steps of vesicle transport, most notably, the anterograde transport between the ER and the Golgi apparatus (Stenmark, 2009). *L. pneumophila* hijacks Rab1 activity with proteins that mimic the functions of its regulatory proteins. Among these, SidM/DrrA is a protein of multiple activities. Anchoring on the LCV by binding to phosphatidylinositol-4-phosphate (Brombacher *et al.*, 2009), SidM/DrrA extracts Rab1 from the GDP association inhibitor (GDI) and recruits and activates it on the bacterial phagosome by its a guanine nucleotide exchange factor (GEF) activity (Murata *et al.*, 2006, Machner *et al.*, 2006, Ingmundson *et al.*, 2007, Machner *et al.*, 2007) (Fig. 1). The bacterial GTPase activation protein (GAP) LepB completes the activation cycle by inducing the GTPase activity of Rab1, leading to its subsequent removal from the LCVs (Ingmundson *et al.*, 2007) (Table 1). Activated Rab1 is believed to facilitate the recruitment of membrane vesicles to the LCV, thus contributing to the membrane remodeling process (Machner *et al.*, 2006).

It is intriguing to note that the activity of Rab1 recruited to the LCV is under another layer of regulation, which, strikingly, is imposed by SidM/DrrA. Using a catalytic mechanism similar to glutamine synthetase adenylyl transferase, SidM/DrrA posttranslationally modifies tyrosine 77 of Rab1 by AMPylation, locking the GTPase into its active form which cannot be induced by LepB (Muller *et al.*, 2010). This paradox was recently resolved by the identification of the bacterial deAMPylase SidD, which reverses the modification, making Rab1 accessible to the GAP protein (Neunuebel *et al.*, 2011, Tan *et al.*, 2011). Despite the interesting mechanisms of regulation and the apparent importance of temporal control of Rab1 activity during *L. pneumophila* infection, it is not clear why it is necessary to lock this small GTPase in its active form during the several hours of its association with the LCVs. It is possible that AMPylation prevents the interference of Rab1 activity by host or bacterial factors. Remarkably, a recent study showed that Rab1 is phosphorylcholinated by AnkX at Ser_{76} (Mukherjee *et al.*, 2011). Although the effect of this posttranslational modification on Rab1 is not clear, it is possible that AMPylation may prevent the potentially negative effect of AnkX on Rab1 at certain cellular location or phase of infection. In any case, the extensive and precise regulation of the activity of a host protein critical for vesicle transport highlights the importance of efficient membrane remodeling in meeting the challenges posed by the host innate immunity system.

It is worth noting that deletion of any of the genes involved in modulating Rab1 activity did not cause defects in *L. pneumophila* intracellular growth (Isberg *et al.*, 2009), indicating that modulation of Rab1 activity does not entirely represent the factors involved in membrane remodeling of the LCV. In fact, Dot/Icm effectors such as SidJ and LegK2, which are important for efficient acquisition of ER specific proteins to the LCVs, have been identified (Liu *et al.*, 2007, Hervet *et al.*, 2011) (Table 1). However, their mechanisms of action are unknown. Similarly, many effectors capable of interfering with membrane trafficking in the model system yeast have been identified but the mechanism of action of these proteins remains to be determined (de Felipe *et al.*, 2008, Heidtman *et al.*, 2008). Detailed analysis of their functions will very likely reveal new layers of regulation in membrane trafficking and membrane remodeling of the LCV.

Nascent phagosomes mature by sequential fusion with endosomes and ultimately with lysosomes, which allows for terminal degradation of the engulfed particles or the killing of the phagocytosized microorganisms. A notable feature associated with matured phagosomes is low luminal pH (about 4.5), which is important for their digestive capacity because the

optimal activity of many phagosomal hydrolytic enzymes requires an acidic environment (Greenberg *et al.*, 2002). Regulation of phagosomal pH is mediated by the vacuolar ATPase (v-ATPase), a multi-component machinery responsible for proton transport across membranes in a process powered by ATP hydrolysis (Forgac, 2007). After phagocytosis, wild type *L. pneumophila* maintains a neutral phagosomal pH (Sturgill-Koszycki *et al.*, 2000). Unexpectedly, given the absence of other endosomal markers, v-ATPase was found on membranes of LCVs (Urwyler *et al.*, 2009), suggesting the necessity to inhibit the proton transporter. By screening for Legionella genes capable of causing the neutral pH-sensitive phenotype exhibited by yeast v-ATPase mutants, Xu et al identified SidK, a Dot/Icm substrate that specifically inhibits v-ATPase activity by binding to VatA, a component of the peripheral V_1 domain involved in ATP hydrolysis (Xu *et al.*, 2010) (Fig. 1). Macrophages loaded with SidK were impaired in the digestion of internalized nonpathogenic bacteria (Xu *et al.*, 2010), highlighting the role of v-ATPase in lysosomal killing and the importance of SidK and other yet unidentified Legionella effectors for modulating the function of the proton transporter.

Regulatory factors such as members of the Rab small GTPase family and proteins involved in modulating lipid composition of the LCV membrane are believed to contribute to the prevention of the bacterial phagosome from entering the lysosomal pathway (Russell *et al.*, 2006). For example, kinases involved in the metabolism of phosphatidylinositol-3-phosphate (PI3P) may be important because this lipid directly affects localization and function of proteins involved in phagosome maturation (Fratti *et al.*, 2001). In fact, anchoring of two effectors, SidC and SidM to the LCV is mediated by binding to PI4P (Weber *et al.*, 2006, Brombacher *et al.*, 2009). However, effectors directly responsible for the presumed enrichment of phosphatidylinositol on the vacuole surface have not been reported. Interestingly, AnkX also targets Rab35, a small GTPase involved in endosomal trafficking (Mukherjee *et al.*, 2011) (Fig. 1). In light of our current appreciation of the redundant mechanisms used by Legionella to regulate its membrane remodeling, it is attempting to predict the existence of more effectors involved in these processes. Future identification and functional characterization of these effectors will surely lead to better understanding of mechanisms of phagosome maturation and its contribution to host defense against intravacuolar pathogens.

Activation of the NFκ pathway

The second component of the innate immune response is mediated by the activation of specific signaling pathways at the transcriptional level, leading to the production of antimicrobial molecules such as cytokines and the activation of the adaptive immune system. Infection by Legionella triggers the classical TLRs-dependent immune responses (Archer *et al.*, 2009). It also activates caspase 1 by the Naip5/Birc1e and Ipaf-dependent pathway involved in the detection of flagellin released into the host cytosol by the Dot/Icm transporter (Vance, 2010). Microarray analyses with bacterial flagellin mutants using macrophages derived from mice defective in the TLR pathways have revealed important roles for the Dot/Icm system in the induction of several innate immunity pathways, including the NFκB pathway, MAP kinase pathway and host stress response (Losick *et al.*, 2006, Shin *et al.*, 2008a, Fontana *et al.*, 2011).

The master regulator NFκB plays an essential role in the mammalian innate immunity response by controlling the expression of a large number of genes (Hayden *et al.*, 2006). Signals capable of activating NFκB are very diverse, ranging from classical PAMP molecules like LPS to cellular insults such as ER stress and disruption of the actin cytoskeleton (Akira *et al.*, 2006, Bruno *et al.*, 2009). In most cases, these molecules trigger a signaling cascade that causes the release of NFκB from members of the IκB inhibitor family and its subsequent nuclear translocation (Akira *et al.*, 2006). Phosphorylation of IκB by the

IκB kinases (IKKs) leads to K48–linked ubiquitination and degradation by the proteasome (Akira *et al.*, 2006). Thus, any activity that leads to IκB phosphorylation will cause an NFκB-mediated response. The importance of the Dot/Icm system in the activation NFκB suggests the existence of substrates capable of causing IκB phosphorylation. Using NFκB-specific luciferase reporters, two Dot/Icm substrates, LegK1 and LnaB, which potently activate NFκB have been identified Ge *et al.*, 2009, Losick *et al.*, 2010) (Fig. 1). LegK1 is a Ser/Thr kinase, which *in vitro* directly phosphorylates some members of IκB family, including IκBα and p105, leading to the activation of the noncanonical NFκB pathway (Ge *et al.*, 2009). However, the mechanism of action of LnaB is not known (Table 1). Further, the significance of LegK1 and LnaB in the activation of NFκB during *L. pneumophila* infection is unclear because mutants lacking either of these proteins exhibited very marginal defects, if any, in the activation of this pathway (Ge *et al.*, 2009, Losick *et al.*, 2010). Of note is that a number of Dot/Icm effectors that moderately activate the NFκB reporters were identified in these screenings (Ge *et al.*, 2009, Losick *et al.*, 2010; Haenssler *et al.*, 2011). It is possible that the collective effects of some of these proteins contribute significantly to the NFκB activation during bacterial infection.

Fontana et al revealed a novel mechanism of immune regulation during *L. pneumophila* infection by demonstrating the requirement of five effectors involved in inhibiting host protein synthesis in the production of distinctive cytokines such as IL23a and Csf2, (Fontana *et al.*, 2011). Compared to infections with the mutant lacking all five translation inhibitors (Table 1), a minimum of a 2 hr delay in IκB re-synthesis was observed in macrophages infected with the wild type strain (Fontana *et al.*, 2011). Because of its labile property, delayed re-synthesis of IκB after its turnover can lead to prolonged NFκB activation, which induces the expression of a set of immune genes (Fig. 1). Thus, activity that prevents efficient production of IκB can activate NFκB. These results indicate that in addition to specific microbial molecules that activate TLRs or cytosolic pattern recognition receptors, a pathogen-encoded activity can be sensed by host immune surveillance mechanisms. It is important to note that mice infected by the Δ5 mutant were not defective in cytokine production nor did IL23a$^{-/-}$ mice exhibit detectably higher bacterial burden (Fontana *et al.*, 2011). Such discrepancies may result from the many redundant pathways that recognize *L. pneumophila in vivo* (Fontana *et al.*, 2011, Archer *et al.*, 2010)

Because the death of a host cell will lead to premature termination of infection, induction of apoptotic pathways is considered a mechanism of innate immunity. In addition to the production of cytokines, NFκB activates the expression of many anti-apoptotic genes during *L. pneumophila* infection (Losick *et al.*, 2006, Abu-Zant *et al.*, 2007, Shin *et al.*, 2008a). Two lines of evidence indicate that reprogramming of the host cell death pathway by NFκB activation is important for bacterial intracellular growth. First, in macrophages lacking the anti-apoptotic gene plasminogen activator inhibitor-2 (PAI-2),*L. pneumophila* infection caused more extensive cell death and a reduction in bacterial replication (Losick *et al.*, 2006). Second, blocking NFκB nuclear translocation by genetic or pharmaceutical agents caused extensive cell death upon low dose bacterial challenge (Losick *et al.*, 2006). The activities act in concert with effectors such as SidF and SdhA, which inhibit host cell death by distinctive mechanisms (Laguna *et al.*, 2006, Banga *et al.*, 2007).

Activation of the MAP kinase pathway

Infection by *L. pneumophila* also strongly induces the MAP kinase pathway, which is involved in innate immunity and regulation of cell death in parallel with the NFκB pathway (Haenssler *et al.*, 2011). Unlike NFκB, which only exists in mammalian cells and is not believed to play a significant role in shaping the pathogenicity of *L. pneumophila*, the MAP kinase pathway is an important signaling conduit in its natural amoeba host. The NFκB and MAP kinase pathways, however share some common properties: First, in both cases, the

stimulus is relayed by specific kinases, which form the step-wise signaling cascade. Second, like the NFκB pathway, stress response signals and stimuli engaged by TLRs or NLRs also induce the MAP kinase pathway (Huang *et al.*, 2009, Ting *et al.*, 2010). Regulation of MAPK signaling is mainly achieved by negative feedback loops via members of the dual specificity phosphatases (DUSPs) (Jeffrey *et al.*, 2007). Analogous to the control of IκB expression by NFκB, transcription of DUSPs is directly regulated by MAPK signaling. Dot/Icm-dependent induction of the MAP kinase pathway occurs in both amoeba and mammalian hosts (Losick *et al.*, 2006, Farbrother *et al.*, 2006, Shin *et al.*, 2008a). For example, an amoeba mutant lacking DupA failed to support maximal intracellular bacterial growth (Li *et al.*, 2009). However, the lack of DupA caused constitutive activation of MAPK signaling, which led to significant changes in host gene expression, making it difficult to pinpoint the host proteins directly responsible for this phenotype (Li *et al.*, 2009). The role of the MAPK signaling cascade in Legionella infection of mammalian hosts is unknown.

Although the involvement of the Dot/Icm transporter in the induction of the MAP kinase pathway is well established, effectors specifically targeting components of this pathway have not been identified. Under infection conditions, the putative kinases LegK1, LegK2 and LegK3 translocated by the Dot/Icm system do not appear to be important in MAPK signaling (Shin *et al.*, 2008a). However, whether any one of these proteins is capable of activating this pathway when over-expressed is not known. Future studies using suitable MAPK-dependent reporters may lead to identification of such genes. It is also possible that the induction is initiated by indirect effects resulting from the perturbation of host processes by subsets of these effectors. Identification of *L. pneumophila* mutants unable to induce the MAP kinase pathway will provide excellent tools to probe its regulation. For example, if the mutant missing all five translation inhibitors no longer stimulates the induction, it would suggest the involvement of one or more labile proteins akin to IκB in the regulation of the MAPK signaling.

Whereas the role of the Dot/Icm transporter or its effectors in the induction of the NFκB pathway and the MAP kinase pathway has been established, it is important to note that in both cases, maximal and sustained induction of immune responses cannot be achieved by activity conferred by Dot/Icm alone. Instead, one or more pathways engaged by TLRs or NLRs are required for maximal immune responses. In fact, it has been suggested that NFκB activation during *L. pneumophila* infection is a two-phase process in which the classical TLR-dependent pathway occurs immediately after bacterial contact, whereas the Dot/Icm-dependent activation persists during the entire replication cycle (Bartfeld *et al.*, 2009). For examples, maximal induction of IL23a and Csf2 by macrophages only occurs in the presence of protein synthesis agents such as ExoA and Pam3 CSK4, and ligands for TLR2 and/or Nod2 ligands (Fontana *et al.*, 2011). Similarly, full induction of some interleukins such as Il1a, Il1b, Il6, and Il12, requires an active Dot/Icm system and the TLR pathway mediated by MyD88 and/or Rip2 (Shin *et al.*, 2008a).

Modulation of type I Interferon induction

L. pneumophila infection leads to transcriptional activation of type I interferon (IFN) genes and restriction of bacterial replication. This observation is reminiscent to the role of type I Interferon in the innate immune response against viral infection (Akira *et al.*, 2006). Several groups have contributed to our understanding of the mechanism of the induction of type I IFNs by *L. pneumophila* infected macrophages: First, this process is independent of the TLR pathway; second, a functional Dot/Icm type IV secretion system is indispensible for the induction; third, the response does not require the pathway involved the sensing of flagellin by Naip5/Nlrc4 (Opitz *et al.*, 2006, Stetson *et al.*, 2006, Monroe *et al.*, 2009). However, the nature of the ligand and receptor involved in this signaling cascade is less clear. Based on

the observation that IRF-3 or IPS-1 is essential for *L. pneumophila*-induced IFNβ expression, it has been suggested that bacterial DNA released by the Dot/Icm transporter is the signal sensed by the cell (Opitz *et al.*, 2006, Stetson *et al.*, 2006). On the other hand, another study showed that 5′-ppp RNA products generated by the host RNA polymerase III from bacterial DNA trigger IFNβ induction through the RIG-I pathway (Chiu *et al.*, 2009). In contrast, more recent evidence indicates that in mouse macrophages, the RIG-I and Mda5-dependent type I IFN response occurs by sensing RNA molecules presumably directly released by the Dot/Icm transporter (Monroe *et al.*, 2009). This conclusion is consistent with the fact that IPS-1/MAVS-KO mouse cells exhibited very marginal reduction in type I IFN response to the B form of DNA delivered by transfection (Kumar *et al.*, 2006). Of greater interest, Monroe *et al.*, found that the Dot/Icm substrate SdhA functions to suppress the type I IFN induction triggered by *L. pneumophila* infection (Monroe *et al.*, 2009) (Fig. 1). SdhA also plays a role in protecting infected cells from cell death (Laguna *et al.*, 2006), a function that appears to be independent of the suppression of type I IFN induction (Monroe *et al.*, 2009). The suppression by SdhA protects the intracellular bacteria from the detrimental effect of type I IFN production.

The role of type I IFN induction in defense against *L. pneumophila* infection is a subject of debate. Whereas earlier studies suggested that mice deficient in type I interferon signaling alone are not more susceptible to Legionella infection (Ang *et al.*, 2010, Monroe *et al.*, 2009), a recent report showed that both type I and II IFNs pr tect mice during lung infections, because higher bacterial burdens were observed in mice deficient in receptors for IFNα(IFNAR$^{-/-}$) or INFγIFNGR$^{-/-}$) or both (Lippmann *et al.*, 2011).

Dot/Icm-dependent transfer of DNA has been well established (Vogel *et al.*, 1998), but the transfer of RNA by this transporter has not yet been documented. Thus, the potential discrepancy regarding the receptors involved in the type I interferon pathway may be due to the uncertainty of the ligands and the cell types used. It is possible that a subset of the many Dot/Icm effectors or the collective effects of subsets of them serve as the signal (Monroe *et al.*, 2009). As exemplified by the activation of NFκB by bacterial proteins involved in inhibiting host protein synthesis (Fontana *et al.*, 2011), such proteins may not directly target the RNA sensing pathways. Instead they may interfere with one or more as yet unrecognized host processes, leading to indirect activation of the pathway. In light of the emerging mechanisms for Dot/Icm effector-mediated regulation of the activity of other effectors (Kubori *et al.*, 2010, Luo, 2011, Neunuebel *et al.*, 2011, Tan *et al.*, 2011), SdhA may function to countercheck the activity of one of more effectors directly involved in type I IFN induction. Regardless of their chemical nature, identification of the effectors responsible for the type I IFN induction will be instrumental in addressing these important questions.

Concluding Remarks

Study of the function of Dot/Icm effectors in the last decade has allowed us to better understand the mechanisms employed by *L. pneumophila* to manipulate host processes. To some extent, this pathogen can be considered a brilliant cell biologist with the tools necessary to exploit the intricacy of host cell biology. On the other hand, in contrast to highly adapted mammalian pathogens that have evolved sophisticated strategies to avoid acute immune responses from the host. *L. pneumophila* is “naïve” in its interactions with the immune system of higher organisms. The many “accidents” caused by Dot/Icm effectors in mammalian macrophages are invaluable tools for analyzing patterns of pathogenesis or activity-dependent immune surveillance mechanisms, which function in concert with the classical receptor-mediated recognition to distinguish pathogens from nonpathogens (Vance *et al.*, 2009). Detailed analysis of the activity of the effectors involved and the cellular processes engaged by them should reveal unrecognized yet important interactions among signaling pathways that govern a variety of distinct cellular functions.

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Abbreviations

RIG-I	retinoic-acid-inducible protein 1
MDA-5	melanoma-differentiation-associated gene 5
MAVS	mitochondrial antiviral signaling
IPS-1	interferon-β promoter stimulator 1
IRF	interferon regulatory factor
IPS	IFN-β promoter stimulator
RIP-2	receptor interacting protein 2

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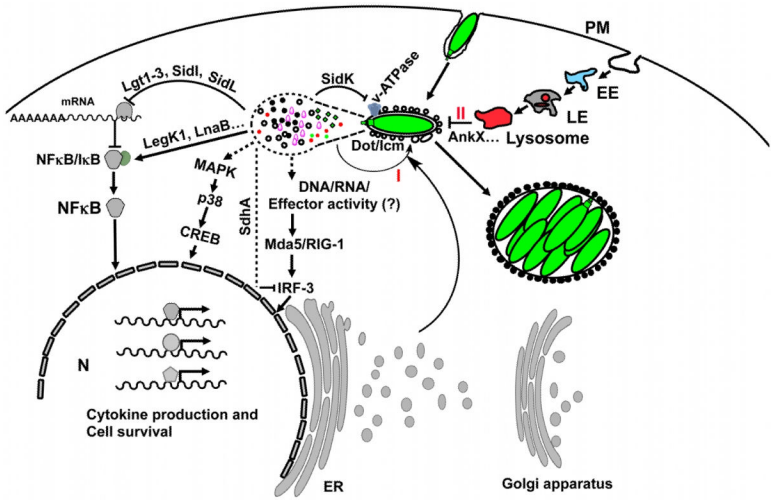

Figure 1. Innate immunity pathways modulated by Dot/Icm substrates

L. pneumophila translocates a large number of effectors into host cytosol via the Dot/Icm type IV secretion system to facilitate its intracellular replication. Host protein synthesis inhibition by 5 effectors, including three glucosyltransferases (Lgt1-3), SidL and SidL led to inefficient resynthesis of the NF-κB inhibitor IκB and NFκB activation. LegK1 and LnaB activate NF-κB, most likely by initiating the kinase cascade that ultimately causes phosphorylation and subsequent degradation of IκB. Nuclear translocation of NF-κB leads to cytokine production and the induction of anti-apoptotic genes. A yet unidentified set of effectors induces the MAP kinase pathway, leading to similar effects. Undefined effectors or DNA/RNA released by the Dot/Icm transporter induce expression of type I interferon, a process which is inhibited by the effector SdhA. Membrane remodeling of the bacterial phagosome (I) is achieved by a set of effectors, some of which are well-characterized, including RalF, SidM/DrrA, LepB, SidD, AnkX, SidJ and LegK2. Inhibition of phagolysosomal fusion (II) probably is mediated by AnkX, and very likely other yet unidentified effectors. Dashed lines indicate undefined effectors involved in the specified pathway. For clarity, pyroptotic cell death caused by flagellin, which reaches the cytosol probably via the Dot/Icm transporter is not shown. CREB, cAMP response element-binding protein; EE, early endosome; LE, late endosome; ER, endoplasmic reticulum; MAPK, mitogen-activated protein (MAP) kinases; N, nucleus; PM, plasma membrane.

Table 1

Dot/Icm effectors involved in the modulation of host innate immunity

Effectors (Lpg number)	synonym	Biochemical activity	Targeted host function	Role in innate immunity	Reference
Lpg2464	SidM/DrrA	GEF[a]/GDF/AMPylator	Vesicle trafficking	Membrane remodeling	Machner *et al.*, 2006, Murata *et al.*, 2006, Machner *et al.*, 2007, Muller *et al.*, 2010)
Lpg2464	LepB	GAP	Vesicle trafficking	Membrane remodeling	(Ingmundson *et al.*, 2007)
Lpg2465	SidD	DeAMPylase	Vesicle trafficking	Membrane remodeling	(Neunuebel *et al.*, 2011, Tan *et al.*, 2011)
Lpg0695	AnkX/LegA8	PC transferase	Vesicle trafficking	Membrane remodeling	(Mukherjee *et al.*, 2011)
Lpg1950	RalF	GEF	Vesicle trafficking	Membrane remodeling	(Nagai *et al.*, 2002)
Lpg2155	SidJ	Unknown	Vesicle trafficking	Membrane remodeling	(Liu *et al.*, 2005)
Lpl2066	LegK2	Protein kinase	Vesicle trafficking	Membrane remodeling	(Hervet *et al.*, 2011)
Lpg0968	SidK	Unknown	v-ATPase	Vacuolar acidification	(Xu *et al.*, 2010)
Lpg1483	LegK1	Protein kinase	NFκ-B activation	Cell survival/immune response	(Ge *et al.*, 2009)
Lpg2527	LnaB	Unknown	NFκ-B activation	Cell survival/immune response	(Losick *et al.*, 2010)
Lpg0376	SdhA	Unknown	Cell death and IFN induction	Cell survival/immune response	(Laguna *et al.*, 2006, Monroe *et al.*, 2009)
Lpg1368	Lgt1	glucosyltransferase	Protein translation	immune response	(Belyi *et al.*, 2006, Fontana *et al.*, 2011)
Lpg2862	Lgt2	glucosyltransferase	Protein translation	immune response	(Belyi *et al.*, 2008, Fontana *et al.*, 2011)
Lpg1488	Lgt3	glucosyltransferase	Protein translation	immune response	Belyi *et al.*, 2008, Fontana *et al.*, 2011)
Lpg2504	SidI	Unknown	Protein translation	immune response	(Shen *et al.*, 2009, Fontana *et al.*, 2011)

Effectors (Lpg number)	synonym	Biochemical activity	Targeted host function	Role in innate immunity	Reference
Lpg0437	SidL	Unknown	Protein translation	immune response	(Fontana *et al.*, 2011)

[a]Note: GEF, guanosine exchange factor

GDF, GDI-displacement factor (GDF)

GAP

GTPase activation protein