VIRULENCE
2021, VOL. 12, NO. 1, 1122–1144
https://doi.org/10.1080/21505594.2021.1903199

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REVIEW ARTICLE

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Pathogenicity and Virulence of *Legionella*: Intracellular replication and host response

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ABSTRACT

Bacteria of the genus *Legionella* are natural pathogens of amoebae that can cause a severe pneumonia in humans called Legionnaires' Disease. Human disease results from inhalation of *Legionella*-contaminated aerosols and subsequent bacterial replication within alveolar macrophages. *Legionella* pathogenicity in humans has resulted from extensive co-evolution with diverse genera of amoebae. To replicate intracellularly, *Legionella* generates a replication-permissive compartment called the *Legionella*-containing vacuole (LCV) through the concerted action of hundreds of Dot/Icm-translocated effector proteins. In this review, we present a collective overview of *Legionella* pathogenicity including infection mechanisms, secretion systems, and translocated effector function. We also discuss innate and adaptive immune responses to *L. pneumophila*, the implications of *Legionella* genome diversity and future avenues for the field.

ARTICLE HISTORY
Received 14 November 2020
Revised 5 March 2021
Accepted 10 March 2021

KEYWORDS
Legionella; pathogenicity; virulence; host-response; innate immunity

Introduction

Bacteria of the genus *Legionella* are natural pathogens of environmentally free-living eukaryotes that can cause respiratory illness in humans termed legionellosis. Legionellosis includes a fatal pneumonia called Legionnaires' Disease (LD) and a self-limiting illness called Pontiac Fever. LD primarily affects elderly and immunocompromised individuals, including those on immunosuppressive therapy[1]. *Legionella* bacteria were first isolated from an outbreak of atypical pneumonia at the 1976 American Legion Convention in Philadelphia, USA[2]. The etiological Gram-negative bacillus was identified and named *Legionella pneumophila* after the disease and its victims[3]. LD accounts for 2–9% of total community-acquired pneumonia; however, the worldwide prevalence of LD is difficult to quantify due to underdiagnosis, variation in diagnostic methods, awareness level, and reporting standards between countries[4]. It is predicted that less than 5% of cases are properly reported and diagnosed [1,5]. Pontiac Fever is a benign, febrile, non-pneumonic disease caused by exposure to *Legionella* bacteria, which is rarely diagnosed and does not require antimicrobial treatment[2]. *Legionella* carry a massive toolbox of virulence factors that facilitate its survival and robust intracellular replication [6,7]. This review provides an overarching discussion of *Legionella* pathogenicity and the interactions of these pathogens with the mammalian immune system.

Human disease is primarily a consequence of *Legionella* colonization of anthropomorphic freshwater environments, including air-conditioning cooling towers, building water systems and spa pools [8,9]. *Legionella* infection occurs almost exclusively from aspiration of contaminated water and person-to-person transmission is very rare[10]. The rarity of transmission between humans and co-evolution with unicellular amoebae has likely resulted in *Legionella*'s susceptibility to innate immune defenses (see below). Consequently, *Legionella* spp. are clinically important pathogens that additionally serve as valuable models to dissect mechanisms of both host–pathogen interactions and innate immune defense.

Approximately half of the 65 identified *Legionella* species have been associated with human disease; however, the overwhelming majority of clinical infections (~90%) are caused by a single species, *L. pneumophila*. The next most common etiological agents of LD are *L. longbeachae, L. bozemanii* and *L. micdadei*, which account for 2–7% of infections worldwide[11]. Interestingly, *L. longbeachae* is the leading cause of LD (~30%) in Australia and New Zealand and is the only species naturally found in soil [12,13]. In comparison to *L. pneumophila*, pneumonia caused by non-*pneumophila Legionella* (non-*Lpn*) species are rare and

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almost exclusively nosocomial[11]. Interestingly, *L. pneumophila* and non-*Lpn* species are found in similar habitats, including built freshwater environments; however, non-*Lpn* species contribute less to overall disease burden. This can be attributed to various factors such as difficulty in strain recovery from water samples, decreased fitness of some species in the sediment of aquatic systems, lack of serological test validation of non-*Lpn* spp., and high genetic diversity among *Legionella* species, which makes diagnosis difficult [11,14].

Legionella spp. are ubiquitous in the environment where they parasitize and replicate within free-living eukaryotic phagotrophs, primarily Amoebozoa [15,16]. Amoebae including *Acanthamoeba castellanii, Acanthamoeba polyphaga, Hartmannella vermiformis, Dictyostelium discoideum, and Naegleria* spp. are natural hosts of *Legionella* and are used to investigate *Legionella* host–pathogen interactions[17]. Amoebae serve a dual role for *Legionella* by providing both a niche for intracellular replication and protection from harsh conditions of the environment including antibiotics, chemicals, heat, and osmotic stress [18–21]. *Legionella*'s environmental persistence is also due to colonization of interspecies biofilms in natural and built freshwater environments [22,23]. Within biofilms *Legionella* are phenotypically heterogeneous, containing subpopulations of virulent non-growing bacteria[24]. The phenotypic variation in biofilms is controlled by *Legionella* quorum sensing (Lqs) system along with a transcription factor, LvbR, and the temperature[24]. The nongrowing cells or "persisters" are metabolically active, have high tolerance to antibiotics and express virulence genes. These virulent sessile persisters are highly infectious and replicate efficiently within permissive protozoan hosts[24]. *Legionella*'s extensive adaptation to persist and replicate within natural freshwater environments has resulted in efficient colonization of built environments and consequent human disease.

All sequenced *Legionella* species encode a highly conserved type IVB secretion system (T4SS) called Dot/Icm (defective for organelle trafficking/intracellular multiplication) [25,26]. The Dot/Icm T4SS is essential for intracellular replication, spans both bacterial membranes and functions to translocate hundreds of bacterial virulence factors, termed effector proteins, directly into host cells. Dot/Icm-translocated effectors have diverse functions and biochemical activities but broadly act to subvert lysosomal bacterial degradation and acquire nutrients from the host cell (see below). *L. pneumophila* encodes over 300 individual effector genes, which comprise 10% of open reading frames in the genome. Despite conservation of the Dot/Icm T4SS and relative abundance of effector genes, there is extensive interspecies variation in translocated effector repertoires[26]. *Legionella* spp. encode the greatest quantity and diversity of effectors of all intracellular pathogens characterized to date [25,26].

Intracellular lifecycle of *Legionella*

Attachment to host cells

The basic mechanism of *L. pneumophila* intracellular replication is consistent between natural (amoebae) and accidental hosts (mammalian macrophages). Following initial attachment and phagocytosis, *Legionella* intracellular replication is contingent on biogenesis and maintenance of its replicative niche, the *Legionella* containing vacuole (LCV), and temporal regulation of egress (Figure 1)[27].

Initial attachment of *L. pneumophila* to host cells is enhanced by several bacterial factors. The *rtxA locus, pilEL locus, ladC*, and *enhC*, which encode a type I secretion system, type IV pili, an inner membrane-associated protein and a periplasmic protein, respectively, facilitate *L. pneumophila* adherence and entry into both amoebae and mammalian cells [28–31]. *L. pneumophila* major outer membrane protein (MOMP), a porin, and Lcl, a collagen-like protein contribute to mammalian cell adhesion [32–34]. However, *L. pneumophila* also exploits host factors for its attachment and internalization, including the complement receptors CR1 and CR3, which are engaged by MOMP, and Fc receptors. Complement and Fc receptors are important for *L. pneumophila* entry into macrophages since antibody-mediated neutralization of CR1, CR3 or Fc receptors impairs phagocytosis [-35–38]. Opsonin-independent adherence to macrophages and lectin-mediated adherence to *A. castellanii* has been described; however, specific factors involved in this process have yet to be definitively characterized [37,39–42]. Together, initial attachment of *L. pneumophila* to host cells is facilitated by both host and bacterial factors.

Phagocytosis of *Legionella*

After attachment, *L. pneumophila* enters the host cell by either traditional phagocytosis or a specialized process called coiling phagocytosis [43,44]. In contrast to traditional phagocytosis, a symmetrical and circumferential uptake processes, coiling phagocytosis involves encircling of extracellular bacteria by unilateral pseudopods making this as an asymmetrical engulfment

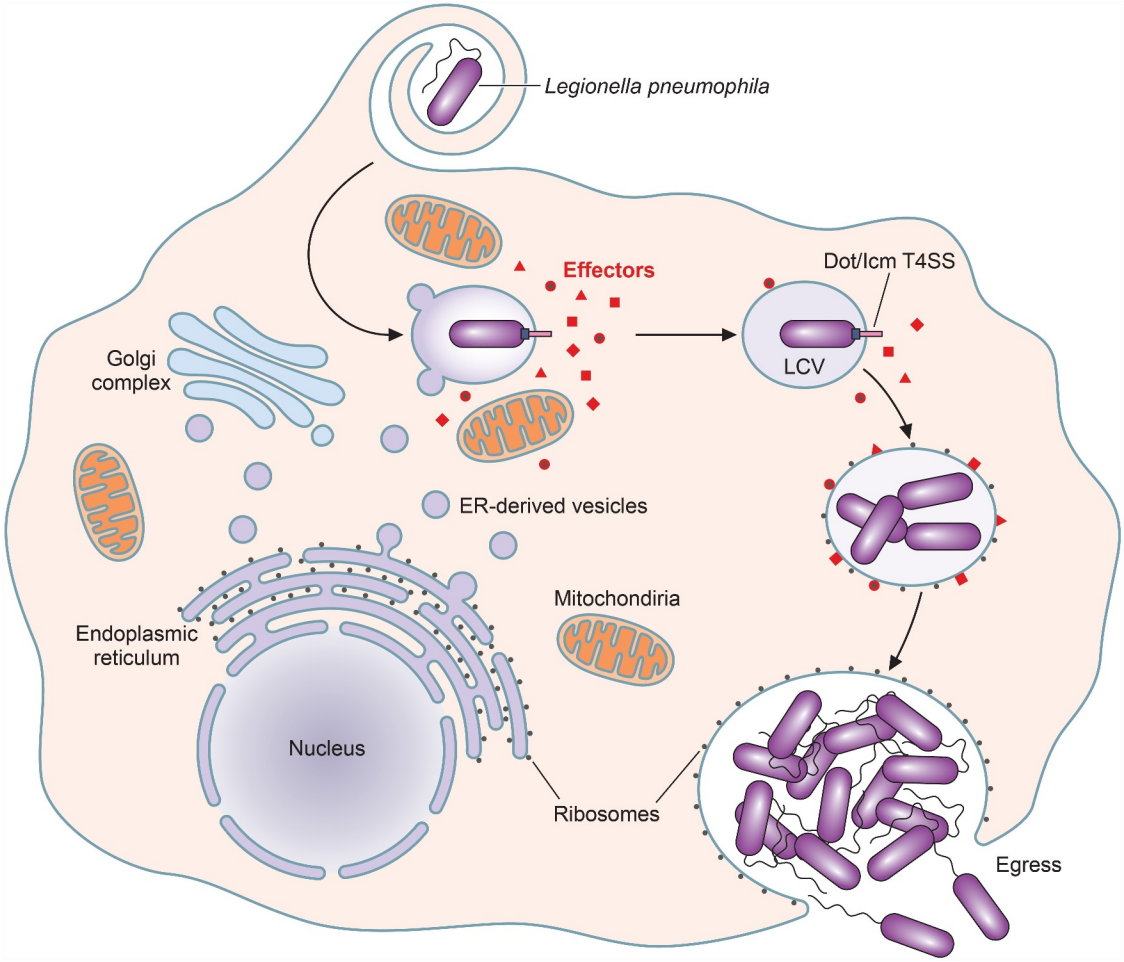

Figure 1. The life cycle of *Legionella pneumophila* within eukaryotic host cells. Bacterial uptake takes place by either coiling (shown) or conventional phagocytosis. Early after entry into the host cell, *L. pneumophila* loses its flagella and the *Legionella* containing vacuole (LCV) escapes the endocytic pathway via effector-mediated recruitment of endoplasmic reticulum (ER)-derived vesicles and transient association with mitochondria. Subsequently, the LCV becomes studded with ribosomes and effectors and exponential replication occurs (replicative phase; see text). Upon exhaustion of host nutrients, *L. pneumophila* become flagellated (transmissive phase; see text) and egress the host cell.

process (Figure 1)[45]. However, the biological relevance of coiling phagocytosis is unclear since it is neither necessary for intracellular replication nor a pathogen-driven process as heat-killed bacteria are also internalized by this mechanism [20,43,46].

Phagocytosis of *L. pneumophila* is an active process relying on phosphatidylinositol 3-kinase (PI3K)-mediated actin polymerization. Chemical inhibition of either PI3K (LY294002 or wortmannin) or actin polymerization (cytochalasin-D) impair *L. pneumophila* phagocytosis [39,47–49]. Actin binding proteins called coronins are also important for *L. pneumophila* phagocytosis. Coronins are transiently recruited to the phagocytic cup of U937 macrophage-like cells harboring live, but not dead, *L. pneumophila*, which supports a role for directed atypical phagocytosis in *L. pneumophila* pathogenicity[50]. Furthermore, the Dot/Icm T4SS is important for efficient *L. pneumophila* uptake by phagocytes and at least one translocated effector (SdeA/LaiA) contributes to this process[51]. Thus, *L. pneumophila* phagocytosis is a directed uptake process and is conserved at the molecular level among diverse host cells.

LCV biogenesis: Evasion of endocytic trafficking and vacuole acidification

L. pneumophila utilizes a myriad of virulence mechanisms to escape the endocytic pathway and establish the LCV. Canonically, phagocytosed bacteria are delivered to early endosomes where the early sorting process occurs. While some receptors are recycled back to the surface, the remaining cargo is transported to late

endosomes and lysosomes for degradation [52]. Thus, phagosomes rapidly undergo endocytic maturation and conversion to a phagolysosome. The acidic microenvironment of phagolysosomes along with lysosomal hydrolases efficiently degrade internalized particles, including bacteria[53]. However, the LCV evades endocytic maturation through effector-mediated subversion of vesicular trafficking, ribosomal localization, the ubiquitin-proteasome system, phosphoinositide metabolism and vacuolar acidification.

Rapid molecular remodeling of *Legionella*-containing phagosomes is essential for LCV biogenesis and bacterial intracellular replication (Figure 1)[54–56] *L. pneumophila* temporally regulates the pH of the LCV. Despite the importance of lysosomal evasion and maintenance of near neutral vacuolar pH at early stages of infection [<6 h post-infection (p.i.)] [55,56], vacuolar acidification at late time points is important at late stages. Concomitantly, at late time points during infection (>18 h p.i.), LCVs acidify and acquire late endosomal and lysosomal markers such as lysosomal-associated membrane protein 1 (LAMP-1)[56]. Treatment of infected macrophages with bafilomycin, a vacuolar(v)-ATPase inhibitor, which prevents acidification, impairs bacterial replication[56]. Dot/Icm-translocated effectors also temporally regulate LCV pH through subversion of the v-ATPase. The effector SidK is expressed at early stages of infection and prevents acidification through binding v-ATPase VatA, which impairs v-ATPase function[57]. WipB, a lysosome-targeted phosphatase, also interacts with components of the host v-ATPase but the influence of these interactions on v-ATPase function are unknown [58]. This suggest that regulation of v-ATPase activity is a key feature for intracellular replication of *L. pneumophila* in macrophages.

Initial studies imaging fixed *L. pneumophila*-infected cells revealed Dot/Icm-mediated recruitment of at least one mitochondrion to the LCV [59,60]. However, recent live-cell imaging revealed that mitochondrial association with the LCV is transient, highly dynamic and independent of the Dot/Icm T4SS [59,61–63]. Dot/Icm-dependent subversion of mitochondrial metabolism at early time points contributes to *L. pneumophila* intracellular replication. The effector LegG1 induces dynamin 1-like protein (DNM1L)-dependent mitochondrial fragmentation. The abrupt halt in mitochondrial respiration leads to a Warburg-like metabolism in macrophages, which favors bacterial replication [61,64].

Multiple *L. pneumophila* effectors modulate vesicular and organelle trafficking [65–69]. Many effectors that subvert host vesicular trafficking have been identified through yeast secretion assays and localization to either the Golgi apparatus or ER [66,68,70–72]. While the molecular mechanisms by which these effectors function have not been fully elucidated, recent advances have revealed a critical role for host phosphoinositide metabolism (reviewed in [73]), small GTPases (see below and reviewed in [74,75]) and the retromer complex [76,77].

Subsequently, ER-derived vesicles are replaced by ribosomes and the LCV transitions into a replication-permissive a rough-ER-like compartment [59,78]. Recruitment of ribosomes to the LCV is a conserved process in both amoeba and macrophages; however, the mechanism by which it occurs is still not clear [79,80]. *Legionella* effectors likely play a role in this process as *dot/icm* mutants do not establish an LCV [59,62]. Moreover, establishment of the LCV is conserved between *Legionella* species. Despite genomic differences, [81] the *L. longbeachae* LCV is similar to the *L. pneumophila* LCV as it avoids lysosomal fusion and Rab1 and Sec22b are recruited [81,82]. These findings highlight the conservation of essential pathways and phenotypic similarities among *Legionella* species.

L. pneumophila effectors regulate the function of several host small GTPases to facilitate LCV biogenesis [75,83]. Modulation of small GTPases is central to LCV biogenesis and several comprehensive reviews have been published on this topic [74,75,84,85]. Immediately following *L. pneumophila* entry, Rab1 and Arf1 are recruited to the LCV [54,86]. In macrophages, recruitment of Rab1 and Arf1 to LCVs is mediated by the effectors SidM/DrrA (defect in Rab1 recruitment) and RalF, respectively. LidA binds to the cytoplasmic face of the LCV and synergizes with SidM to recruit Rab1 [87,88]. The effector, VipD, has Rab5-dependent phospholipase A1 activity that inhibits endosomal fusion of LCV by catalyzing removal of PI(3)P from the endosomal membrane[89]. Rab5 and Rab7 play an important role in phagosome maturation and are required for cargo transition from early to late endosomes. These small GTPases also play a role in retrograde trafficking of endosomes to the Golgi-complex [90,91].

Effector-mediated subversion of the host retromer complex is important for LCV biogenesis. The effector RidL impedes retrograde trafficking pathway by binding to the retromer subunit Vps29 and the lipid phosphoinositol-3-phosphate [PI(3)P], which localizes retromer components to the LCV membrane [76,92]. This, in turn, modulates retromer function and promotes intracellular replication of *L. pneumophila*, likely through LCV acquisition of retrograde transport vesicles [77,93].

Biphasic lifecycle of *Legionella*

Legionella have a biphasic lifecycle that alternates between an infectious transmissive phase and a non-infective replicative phase. In nutrient-rich conditions, such as within host cells, *Legionella* undergoes exponential replication (replicative phase) and under scarcity of nutrients the bacteria enter into stationary (transmissive) phase. After exit from the nutrient deprived host, *Legionella* disperse into the environment and reestablish infection into a new host cell, which offers a protective intracellular niche favorable for replication[94].

The transition between growth phases is accompanied by major transcriptomic changes. Nearly half of *L. pneumophila*'s predicted genes have a drastic shift in expression from replicative to transmissive phase[95]. In the replicative phase, genes related to metabolism, amino acid degradation/breakdown, sugar assimilation, cell division and biosynthetic processes are upregulated. During transmissive phase, genes associated with host entry, virulence and survival, which include Dot/Icm-translocated effectors, motility machinery (flagellar and type IV pilus genes), enhanced entry proteins (Enh) and cyclic-di-GMP regulatory proteins are upregulated[95].

Legionella's transition from replicative to transmissive phase is a highly coordinated process that is initiated upon nutrient limitation. Amino acid starvation triggers synthesis and accumulation of guanosine 3,5-bispyrophosphate (ppGpp), which initiates stationary phase and consequent up-regulation of virulence genes[96]. Similar to other microbes, *L. pneumophila* ppGpp synthetases, RelA and SpoT, are activated when uncharged tRNAs bind to ribosomes[97]. Moreover, transition from replicative to transmissive phase is controlled by the sigma factors RpoS and FliA, activator protein LetE, and the LetA/S two-component system [98–100]. LetA/S signaling results in upregulation of two small non-coding RNAs, RsmY and RsmZ, which facilitate phase switching by repressing the global repressor CsrA [101–103].

Following differentiation into transmissive phase, *L. pneumophila* must egress the host cell. Mechanisms by which *L. pneumophila* temporally regulate egress are poorly understood. However, current data support a role for pore-formation and subsequent necrotic host cell death when *L. pneumophila* egress from macrophages and amoebae [104,105]. The Dot/Icm component IcmT contributes to pore-formation-dependent lysis of host cells; however, the mechanism by which IcmT functions is unknown[106]. The *Legionella* translocated effectors LepA and LepB also contribute to egress through non-lytic exocytosis from amoebae[107]. However, the mechanism by which Lep proteins facilitate release of *L. pneumophila* from protozoa is still unclear.

Genome diversity and conserved proteins in *Legionella* virulence

Variation in *Legionella* genomes and effector repertoires

A high degree of plasticity and diversity exists in the genomes of *Legionella* species. Recently, Gomez-Valero and colleagues sequenced the genomes of 58 *Legionella* species and performed a comparative analysis of genomes across 80 strains of *Legionella*[26]. *Legionella* genomes are highly diverse in size and content with genome size and GC content varying from 2.37 Mb to 4.88 Mb and 32.82% to 50.93%, respectively. The GC content of Legionella genomes is inversely correlated with genome size, suggesting that horizontal gene transfer, which results in AT-rich regions, drives *Legionella* genome size[26]. Moreover, out of 17,992 identified orthologous gene clusters, 5,832 (32%) were strain specific and only 1,008 genes (6%) comprised the core genome[26].

Despite diversity between *Legionella* genomes, the Dot/Icm T4SS is highly conserved and present in all species. However, the size and composition of *Legionella* effector repertoires is highly variable. For example, there is only a 50% overlap in effector repertoires of *L. pneumophila* and *L. longbeachae*[81]. No common set of effectors exists between strains that either cause human disease or replicate robustly in human macrophages[26]. Moreover, 18,000 unique translocated effectors are encoded by *Legionella* spp., which reflects the diversity of hosts and environmental adaptations evolved by *Legionella*[26].

Extensive co-evolution with diverse environmental phagotrophs has conferred on *Legionella* the ability to replicate within mammalian macrophages and cause human disease [7,35,108–110]. Using high-throughput genetic screening, Park and colleagues demonstrated that replication in *L. pneumophila* within mammalian cells is a consequence of combinatorial selection of virulence factors required for replication with phylogenetically diverse protozoan hosts[111]. The authors identified a subpopulation of effectors that are universally important for *L. pneumophila* replication within *A. castellanii, A. polyphaga, H. veriformis* and *N. gruberi*, and additional subpopulations important for replication within a single host genera or phylum. This study elegantly demonstrates how

L. pneumophila's broad host tropism has (1) driven evolution of the largest known repertoire of effector virulence factors discovered to date; and (2) how an environmental pathogen gained the ability to cause accidental human disease.

Eukaryotic-like domains and motifs in *Legionella* genomes

Legionella species encode genes containing 137 distinct eukaryotic domains/motifs [26]. Ankyrin repeats are most prevalent eukaryotic motifs present across *Legionella* effectors; however, eukaryotic F-box, U-box, small GTPase, Rab, and SET domains are also abundant. The SET domain, present in histone methyltransferases, is present in 46 out of 58 species of *Legionella*. The effector RomA contains a SET domain and represses host gene expression via methylation of host histone proteins. Thus, the prevalence of SET domains among *Legionella* spp. suggests that host chromatin manipulation is a common mechanism employed by this genus [26,112].

Another motif prevalent in the genomes of multiple *Legionella* species is the ergosterol reductase ERG4/ERG24 motif. Ergosterol is present in the cell membranes of yeast, mitochondria, filamentous fungi and amoeba and 31 species encode one or two genes with the ERG4/ERG24 motif. Further sequence analysis revealed high similarity among *Legionella* proteins containing ERG domain with amoeba suggesting that *Legionella* has acquired this domain and others from amoebae[26]. However, the role of the ERG4/ERG24 motifs in *L. pneumophila* pathogenicity are poorly understood.

Legionella species encode 184 predicted small GTPases and 149 of these are present exclusively in eukaryotes and *Legionella*[26]. Homology was uncovered by BlastP analysis of *Legionella* Rab domain-containing proteins against protozoans in the NCBI database. Moreover, a subset of *Legionella* Rab GTPases possessed additional domains such as U-box, F-box and ankyrin repeats. Notably, 16 of these Rab GTPase motif-containing proteins were translocated by the Dot/Icm T4SS suggesting that these proteins are actual substrates of T4SS and function in the host cell [26]. While modulation of GTPase function is critical for intracellular replication, the functions of the majority of these genes is elusive. However, it is tempting to speculate a role for these proteins in subversion of host vesicular trafficking.

Legionella eukaryotic-like proteins

In addition to proteins containing eukaryotic-like domains/motifs, *Legionella* additionally encode eukaryotic-like proteins [26,113]. Many of these proteins are confirmed or predicted Dot/Icm-translocated effectors. *Legionella* contains 2,196 eukaryotic-like proteins, representing 400 different orthologous groups with high similarity to eukaryotic proteins. The majority of these genes were likely acquired directly from protozoa, emphasizing the importance of host–pathogen interactions on *Legionella* genomes[26]. For example, *L. anisa* LanA0735 belongs to a FAD-dependent oxidoreductase family. This protein has a similarity to thioredoxin reductase, found in higher eukaryotes as two major isoenzymes: cytosolic and mitochondrial. In *Caenorhabditis elegans*, the cytosolic form has been reported to impede the lysosomal compartment acidification indicating a plausible role for LanA0735 in evasion of vacuole acidification during *Legionella* replication[114]. Moreover, *L. pneumophila* secretes eukaryote-like proteins PlcA and PlcB, which are phosphatidylcholine-hydrolyzing phospholipase C. Phosphatidylcholine is made by bacteria that interact closely with eukaryotes, such as *Brucella abortus* or *Francisella tularensis*. The synthesis of this phospholipid is essential for *L. pneumophila* virulence[115]. *Legionella* is unable to synthesize choline and these eukaryotic-like proteins likely aid in acquisition of choline from the host cell.

Conservation of the *Legionella* Dot/Icm T4SS and effectors

Despite plasticity in the genomes of *Legionella* spp., the Dot/Icm T4SS is highly conserved in all *Legionella* spp. analyzed to date [25,26,109]. Between sequenced *Legionella* strains, Dot/Icm apparatus proteins share >50% amino acid identity with DotB and IcmS exceeding 90% identity[25]. Conversely, Dot/Icm-translocated effectors share little identity and the predicted number of effectors is highly variable between species. Only 8 core effectors are conserved in all analyzed *Legionella* genomes: Lpg0103 (VipF), Lpg1017 (RavC), Lpg0140, Lpg1356/Lpg1310, Lpg2300 (LegA3/AnkH/AnkW), Lpg2815 (IroT/MavN), Lpg2832 and Lpg3000 [25,26]. Seven additional effectors are present in all strains with the exception of a few strains, suggesting important role of these effectors in infection. Intraspecies effectors are highly conserved (82–97%), further emphasizing the influence of the host environment on evolution of the

Legionella effector repertoire[25]. Together bacteria of the genus *Legionella* encode at least 18,000 effectors spanning 1,600 orthologous groups[26]. Thus, *Legionella* encode the greatest quantity and most diverse range of effectors among intracellular bacterial pathogens.

Effector translocation by the Dot/Icm type IV secretion system

Effector recognition by the Dot/Icm T4SS

The Dot/Icm T4SS is composed of 27 proteins, spans both bacterial membranes, and functions to translocate effector proteins directly into the host cell cytosol [-116–120]. Effector translocation by the Dot/Icm T4SS involves recognition of effectors and subsequent translocation of unfolded effector proteins into host cells [121–124]. The Dot/Icm T4SS is composed of two major complexes: the core transmembrane complex (CTMC) and the Dot/Icm type IVB coupling complex (T4CC). The CTMC forms a pore for effector translocation and composed of DotC, DotD, DotF, DotG, DotH and DotK. The T4CC functions to recruit effectors for translocation and is composed of DotL (IcmO), DotM, DotN (IcmJ), IcmS, IcmW, LvgA, DotY, and DotZ. Six hetero-pentameric units (DotLMNYZ) of the T4CC form an inner membrane channel for the delivery of effectors [123,125–130]. Effector recognition by the T4CC is essential for translocation through the core transmembrane complex and cryo-electron tomography studies have revealed the molecular architecture of this structure and how effectors are recognized as translocation substrates [125,130].

Effector translocation through the T4CC occurs either through interaction with DotL-IcmSW or DotL-IcmSW-LvgA complexes or by DotM-mediated recognition of a C-terminal secretion signal, termed the E-block motif. DotL is a VirD4 homolog with an N-terminus ATPase domain and a C-terminal extension (CTE) that binds effectors in complex with IcmSW or IcmSW-LvgA [125,126,129,131]. The current model for IcmSW-mediated translocation is that effectors bound to IcmSW are delivered to the DotL channel where DotL ATPase activity may direct both effector unfolding and transport [125,126]. Some effectors are additionally recognized by LvgA, which binds IcmSW and copurifies together with the T4CC. Although many effectors are translocated independently of IcmSW or IcmSW-LvgA, *L. pneumophila* intracellular replication is severely attenuated by loss-of-function mutation in either *icmS, icmW* or *lvgA* [128,132]. DotM engages effectors that are translocated independently of IcmSW through recognition of a C-terminal Glu-rich region (E-block motif) [125,133]. While the C-terminal ~25 amino acids comprising the E-block motif are generally rich in Glu residues, residues with similar biochemical properties contribute more to translocation than Glu residues at specific positions[134]. Basic patches on DotM engage E-block-containing effectors through electrostatic interactions. This interaction is hypothesized to alleviate the requirement for IcmSW effector recognition [133]. Interestingly, the effector SidJ possesses an internal translocation signal, in addition to a C-terminal translocation signal, that aids in IcmSW-dependent Dot/Icm translocation[135]. Multiple signal sequences may provide an additional layer of effector translocation regulation. Thus, translocation signals are likely more complex than previously appreciated and may contribute to spatiotemporal regulation of effector translocation. How signal sequences and chaperone engagement contribute to translocation efficiency and hierarchy has yet to be elucidated.

Spatiotemporal regulation of effector translocation

L. pneumophila effectors are translocated hierarchically during specific growth phases. Broadly, effectors are expressed either before/upon infection, early in infection, late in infection or during whole intracellular life cycle. Several effectors, including the SidE family, SidC and RalF, are accumulated during post-exponential phase, suggesting importance very early in infection [136–138]. In general, effector translocation hierarchy is thought to correlate with gene expression, but regulation of translocation is likely far more complex. Temporal regulation of effector translocation is important for *Legionella* intracellular replication and much remains to be discovered about how *Legionella* regulate translocation of effectors.

Effector-mediated modulation of host autophagy, protein translation and ubiquitin homeostasis

Regulation of host autophagy

Autophagy is an essential cellular process that is central to cellular survival and cell-autonomous defense against intracellular pathogens[139]. Several *L. pneumophila* effectors inhibit host autophagy, likely to subvert the lysosomal fusion with the LCV. RavZ disrupts autophagy thorugh irreversibly deconjugation of phosphatidylethanolamine from the autophagy-related ubiquitin like protein, LC3, which prevent LCV localization to autophagosomes[140]. Interestingly, RavZ-mediated

LC3 delipidation is sufficient to impair autophagic targeting of other intracellular pathogens, including *Coxiella burnetii* and *Listeria monocytogenes* [141,142]. Autophagy is also impaired through the action of a *L. pneumophila* effectors S1P-lyase (LpSPL; LegS2) and Lpg1137, via modulation of sphingolipid metabolism and cleavage of the autophagy-associated SNARE syntaxin-17, respectively [143,144]. Paradoxically, the *L. pneumophila* effector LegA9, an ankyrin-containing protein, upregulates autophagy and contributes to macrophage restriction of *L. pneumophila* through an unknown mechanism[145]. Thus, autophagy is central to *L. pneumophila* intracellular replication and further investigation will likely reveal additional sophisticated mechanisms employed by *L. pneumophila* to subvert host autophagy.

Inhibition of host protein synthesis

Seven *L. pneumophila* effectors, Lgt1-3, SidI, SidL, LegK4 and RavX, are capable of inhibiting eukaryotic protein synthesis (reviewed in [146]). Lgt1-3 are glycosyltransferases that modify eukaryotic GTPase elongation factor 1A (eEF1A), an essential component of the eukaryotic protein translation elongation complex. Lgt1-3 glycosylate eEF1A at Ser-53 through covalent addition of a glucose moiety. Ser-53 is conserved in eukaryotes and located in the GTPase domain of eEF1A. Ectopic expression of Lgt1 in yeast is cytotoxic due to inactivation of eEF1A [147–150]. Moreover, Lgt1-3 cooperate with the SidE family of effectors to facilitate acquisition of essential host-derived amino acids (see below)[151].

The effector SidI is a predicted glycosyltransferase capable of hydrolyzing GDP-mannose. SidI interacts with eEF1A and eEF1Bγ. However, interaction with eEF1A is not sufficient for SidI-mediated translation inhibition. eEF1A additionally upregulates the eukaryotic heat shock response through interaction with heat shock regulatory protein 1 (HSF1) [152], and SidI is sufficient for induction of the host stress response[153]. However, translation inhibition alone is insufficient to induce the heat shock response[153]. Since SidI-mediated protein translation inhibition is suppressed by its metaeffector, MesI, translation inhibition may not be the bona fide function of SidI within host cells [154]. Although SidI is not individually required for *L. pneumophila*, its enzymatic activity is uniquely deleterious to *L. pneumophila* intracellular replication in the absence of MesI [153,155]. The mechanism by which SidI prevents L. pneumophila intracellular replication is currently unknown.

The effector kinase, LegK4 impairs host translation through phosphorylation of cytosolic Hsp70. Phosphorylated Hsp70 associates with translating polysomes but has attenuated ATPase activity, which impairs its refolding capacity and, consequently, protein translation[156]. Detailed molecular mechanisms by which SidI, SidL and RavX inhibit host protein translation are currently unknown.

Inhibition of host translation is hypothesized to facilitate acquisition of essential nutrients from host cells[151]. However, inhibition of protein translation also contributes to restriction of *L. pneumophila* via effector-triggered immunity infection models (see below) [157–160]. Thus, subversion of host translation is central to *L. pneumophila* pathogenicity but also facilitates pathogen detection and restriction by macrophages.

Modulation of host ubiquitination pathways

L. pneumophila intracellular replication hinges on effector-mediated modulation of cellular ubiquitin pathways. Subversion of ubiquitin pathways involves the concerted action of several effectors, most of which are indispensable for intracellular replication. Comprehensive and detailed reviews are available on subversion of ubiquitination pathways by *L. pneumophila* effectors [161,162]. Ubiquitination is regulated by *L. pneumophila* effectors through novel enzymatic activity and molecular mimicry of eukaryotic ubiquitin ligases and deubiquitinases [163–170]. Of note is the recent discovery of novel effector-mediated ubiquitin modulation mechanisms. The SidE family of effectors (SidE/SdeABC) and their metaeffector, SidJ, cooperate to facilitate LCV biogenesis. The SidE family of effectors are mono-ADP-ribosyltransferases that ligate ubiquitin to ER associated Rab GTPases independently of host E1 and E2 enzymes [166,171]. The SidE family ubiquitinate ER-associated Rab GTPases, Rag GTPases and host reticulon 4 to regulate tubular ER dynamics for biogenesis of the LCV and activity of the mechanistic target of rapamycin complex 1 (mTORC1; see below) [151,166,171]. SidJ utilizes host calmodulin as a co-factor to polyglutamylate and inactivate SidE effectors [165,172,173]. SidJ-mediated regulation of the SidE family is critical for intracellular replication in natural and accidental hosts [174,175].

The effector deamidases MavC and MvcA are functional antagonists that temporally regulate ubiquitination and activity of the host E2 enzyme Ube2N. MavC catalyzes E1-independent monoubiquitylation and inhibition of Ube2N [176]. However, Ube2N activity is restored through the action of MvcA, which

deubiquitinates Ube2N-Ub [177]. In mammalian cells, Ube2N inactivation impairs ubiquitination and proteasomal degradation of the host inhibitor of κB03B1 (IκBα), which restricts NF-κB-mediated gene expression (see below)[167]. MavC and MvcA are both regulated by a metaeffector, Lpg2149, which binds and inhibits the deamidase activity of both effectors [176]. Increased expression of *mavC* in transmissive phase bacteria overcomes Lpg2149-mediated restriction and facilitates temporal regulation of Ube2N activity [176]. MavC and MvcA are individually dispensable for *L. pneumophila* intracellular replication, which is likely a consequence of functional redundancy with other effectors in the host systems tested.

Effector-mediated nutrient acquisition and modulation of host metabolism

L. pneumophila is reliant on host-derived amino acids for intracellular replication [178–180]. *L. pneumophila* is auxotrophic for valine, threonine, serine, leucine, methionine, arginine, isoleucine and cysteine, which is a preferred source of carbon during intracellular replication [181,182]. The effector AnkB facilitates acquisition of host-derived amino acids through recruitment of host polyubiquitinated (polyUb) proteins to the surface of the LCV. AnkB contains a CaaX motif, which is farnesylated and anchors the effector to the LCV surface, ankyrin repeats and an F-box domain. The ankyrin and F-box domains facilitate attachment of host polyUb proteins to the LCV membrane, which are subsequently proteolyzed by host 26S proteasome [170,183]. Both genetic deletion of *ankB* and chemical inhibition of 26S proteasomes impair *L. pneumophila* intracellular replication and supplementation of cell culture with free amino acids rescues these growth defects[184]. Thus, effector-mediated subversion of the ubiquitin-proteasome system facilitates *L. pneumophila* nutrient acquisition from host cells[186].

L. pneumophila relies on host-derived amino acids, but free amino acid levels are tightly regulated in eukaryotic cells. mTORC1 is a conserved complex composed of mTOR kinase and several regulatory enzymes that is regulated in part by availability of amino acids and other nutrients (Figure 2)[185]. Upon activation, mTORC1 controls several cellular processes including repression of autophagy, translation initiation and lysosome biosynthesis[186]. The Lgt and SidE effector families (see above) act antagonistically toward mTORC1. Lgts-mediated translation inhibition results in mTORC1 activation whereas the SidE family of effectors negatively regulate mTORC1 by ubiquitination and inhibition Rag GTPases, which contribute to amino-acid sensing by mTORC1 and liberation of host amino acids for bacterial intake[151].

Intracellular *L. pneumophila* acquire iron from the host cell via the function of the effector MavN/IroT. MavN/IroT spans the LCV membrane and functions to transport iron into the LCV [189]. *L. pneumophila* strains lacking *mavN/iroT* are attenuated for intracellular growth and exhibit characteristics of iron starvation [187,188]. MavN/IroT is one of very few *L. pneumophila* effectors universally required for intracellular replication and its activity provides insight into mechanisms of iron acquisition by intravacuolar pathogens[189].

L. pneumophila effectors also directly modulate host cell metabolism. The effector LamA subverts glucose metabolism to regulate encystation of amoebae, which occurs as a result of environmental stress. Amoebal cysts are hypothesized to enhance environmental stability of intracellular *L. pneumophila*. However, although retained in a viable state, *L. pneumophila* is unable to replicate within amoebal cysts [189–191]. Price *et al.* recently reported that LamA, an effector amylase, induces a "hyper-glucose" state in host cells via degradation of host glycogen. Consequently, amoebae are unable to synthesize the cellulose-rich cyst wall[192]. In human macrophages (hMDMs), LamA triggers a pro-inflammatory response that moderately restricts bacterial replication. LamA mediated high glucose levels in hMDMs shift the metabolism to aerobic glycolysis which directly triggers a rapid M1-like pro-inflammatory polarization and pro-inflammatory cytokine production. Moreover, LamA augments IFN-γ-mediated IDO1 activity, which depletes cellular tryptophan. Although *L. pneumophila* is not auxotrophic for tryptophan, host-derived tryptophan is important for *L. pneumophila* replication within macrophages [192,193].

A recent study also revealed that *L. pneumophila* encodes an effector ADP-ribosyltransferase that modifies a class of host NAD+-dependent glutamate dehydrogenases (GDH). The effector *Legionella* ADP-ribosyltransferase 1 (Lart1; Lpg0181) ADP-ribosylates GDH on a conserved arginine within the nucleotide-binding pocket, which renders GDH inactive[194]. However, the role of Lart1-mediated ADP-ribosylation of GDH during *L. pneumophila* infection has not been fully elucidated. A *L. pneumophila* Δ*lart1* mutant is not impaired for replication within *A. castellanii*, suggesting that within this host, Lart1 functions redundantly with other effectors. This study has uncovered an additional mechanism by which *L. pneumophila* may subvert host cell metabolism.

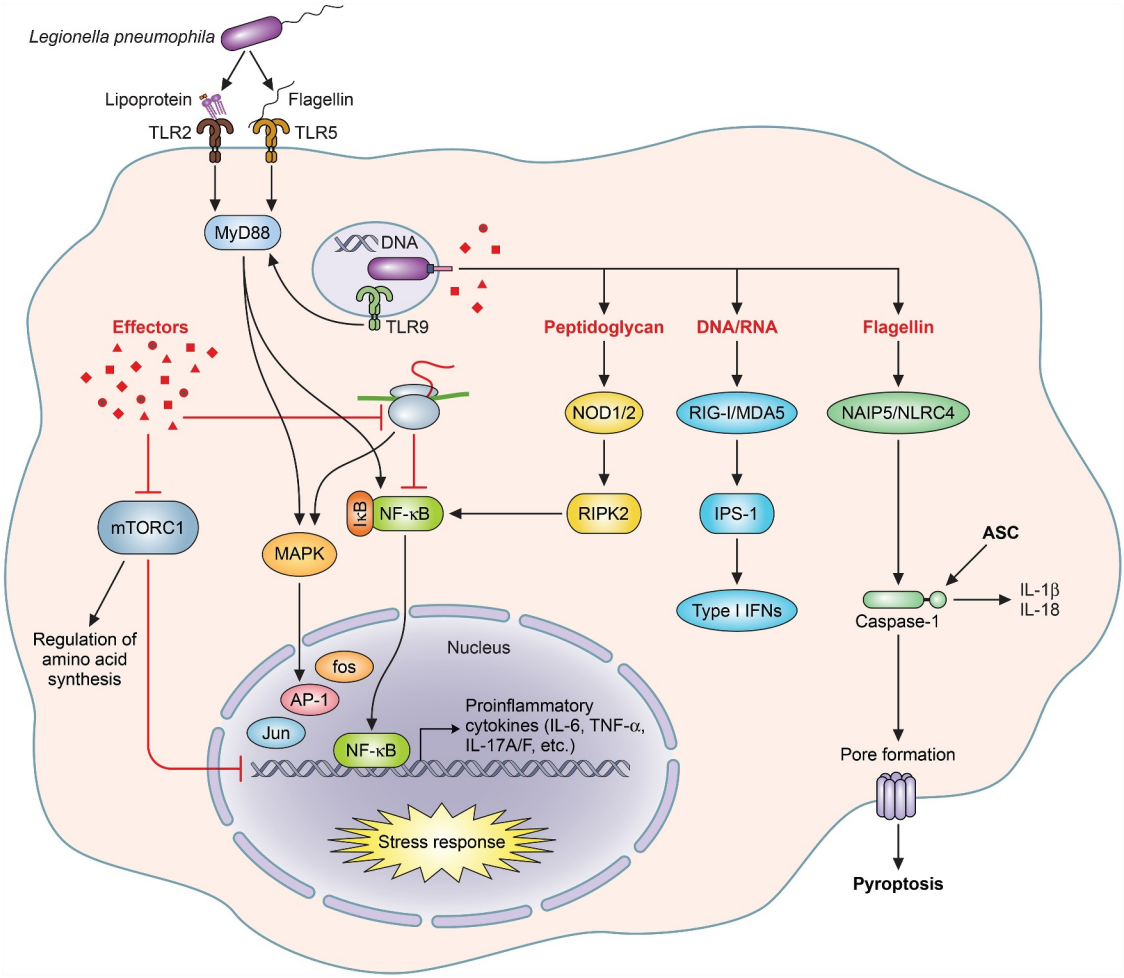

Figure 2. Innate immune signaling initiated by *L. pneumophila* within macrophages. The schematic represents the activation of multiple pathways upon mammalian phagocyte infection with *L. pneumophila*. *Legionella*-associated molecular patterns are recognized via pattern recognition receptors (PRRs) of the phagocyte. Activation of PRRs and cytosolic sensors triggers downstream molecules and processes that eventually lead to restriction of *L. pneumophila* replication. Specifically, TLR2, TLR5 and TLR9 discern bacterial lipoprotein, flagellin and dsDNA, respectively, which activate downstream NF-kB mediated proinflammatory cytokine response. Effector-dependent translation inhibition activates NF-κB and MAPK signaling to initiates a proinflammatory transcriptional response. *Legionella* effectors also inhibit the mTORC1 complex which negatively affects the amino acid synthesis and proinflammatory cytokine production. *Legionella* flagellin is recognized by the NAIP5/NLRC4 inflammasome and downstream activation of caspase-1 leads to pyroptosis and IL-1β/IL-18 cytokine release. NOD1/2 recognizes degradative products of bacterial peptidoglycan, eliciting RIPK2-dependent NF-κB activation and proinflammatory cytokine production. Bacterial DNA/RNA is sensed in the host cell cytosol by RIG-I and MDA5 which leads to type I interferon production.

Thus, *L. pneumophila* has evolved an extensive repertoire of effectors that regulate diverse host cell processes. This work has collectively shed light on mechanism of *Legionella* virulence and broad themes in host–pathogen interactions.

The *Legionella* type II secretion system

L. pneumophila encodes a type II secretion system (T2SS), also called the *Legionella* secretion pathway (Lsp), which is important for virulence and persistence in the environment [195]. T2SSs are highly conserved, evolutionarily related to bacterial type IV pili, and broadly distributed among members of the phylum [196–199]. The T2SS plays a crucial role in bacterial pathogenicity by exporting various virulence factors, toxins, lipases, proteases, chitinases and novel proteins outside the bacterial cell [198]. Prior to secretion via T2SS, unfolded or folded protein substrates enter the periplasm through either the Sec translocon or the twin-arginine translocon (Tat), respectively. The T2SS machinery is comprised broadly of four subcomplexes composed of 12 core proteins: T2S C, D, E, F, G, H, I, J, K, L, M and O [200,201]. The first subcomplex is a "secretin," which facilitates substrate translocation across the outer membrane and is composed of T2S

D oligomers. These T2S D oligomers are associated with the second subcomplex, an inner membrane heterooligomer comprised of T2S F, L, and M multimers, which creates a periplasmic channel. The inner and outer membrane complexes are coupled by a "clamp protein," T2S C. The third subcomplex, a periplasmic "pseudopilus," consists of T2G G, H, I, J and K components, which may function as a "piston" to propel the substrates through the outer membrane subcomplex. The fourth subcomplex is an ATPase and hexamer of T2S E protein. Finally, T2S O, an inner membrane prepilin peptidase allows pseudopilin maturation [201,202].

The *Legionella* T2SS is important for both intra and extracellular survival of *L. pneumophila*. It plays a crucial role in biofilm formation, intracellular replication in amoeba and macrophages, suppression of cytokine response from infected cells, growth and persistence in the murine lungs [195,199,203–208]. Over 25 T2SS substrates have been identified in *L. pneumophila* [199,202]. These substrates include ProA, PlaA, Map, PlaC (acetyltransferase), PlcA (phospholipase C), SrnA (ribonuclease), ChiA (chitinase), CelA (cellulase), LapA, LapB (aminopeptidases), and NttA,B,C,D,E,G [204,209–212]. NttA,C,D,E are required for *L. pneumophila* replication within multiple species of amoebae[212]. The T2SS substrates LegP and Map contain eukaryotic-like protease motifs [204,213]. Interestingly, LegP is a confirmed substrate of the Dot/Icm T4SS [68], but the mechanism by which a single protein could serve as a substrate for both secretions systems is unknown. The T2SS substrates ProA, PlaC and SrnA are necessary for optimal infection in *H. vermiformis* and *N. lovaniensis* whereas NttA is required to replicate in *A. castallanii* [209,210,214]. These observations suggest that T2SS substrates may shape the host range of *L. pneumophila*.

The T2SS contributes to *L. pneumophila* virulence in cultured mammalian cells and mouse models of LD [203,215]. Loss-of-function mutation of the T2SS genes *lspF, lspDE, lspG, lspK*, and *lspO* impairs *L. pneumophila* infection of human macrophage cell lines, mouse macrophage cell lines, primary BMDMs and alveolar epithelial cells [203,205,215–218]. The T2SS substrate, ChiA, is important for survival in the mouse lung [204,209]. ChiA is a chitinase that possesses additional peptidase activity and degrades mucin *in vitro* [208]. Degradation of mucin *in vivo* may enhance *L. pneumophila* motility in airways and enhance access to alveolar macrophages. Another substrate, ProA, causes lung tissue damage and transferring degradation that can enhance iron acquisition [204,219–221]. The contribution of the full range of T2S substrates to *L. pneumophila* virulence has yet to be elucidated, but current data support an essential role for this secretion system and its substrates in infection.

The T2SS additionally attenuates the mammalian innate immune response. T2SS function decreases cytokine and chemokine levels in the supernatants of macrophages and epithelial cells and within the mouse lung during *L. pneumophila* infection [205,215]. The T2SS decreases cytokine abundance via transcriptional and post-transcriptional mechanisms. The metalloprotease ProA dampens cytokine production at the post-transcriptional level; however, substrates responsible for attenuation of gene expression have yet to be identified[215]. Moreover, a role for the T2SS in TNF-α -mediated macrophage defense against *L. pneumophila* was demonstrated by restoration of T2SS mutant growth upon antibody neutralization of TNF[215]. This suggests that T2SS promotes *L. pneumophila* growth in macrophages and epithelia by dampening cytokine response in addition to some unidentified mechanisms. The evolutionary basis T2SS-mediated attenuation of inflammation is unclear since amoebae lack pro-inflammatory genes and signaling cascades. Whether the attenuated inflammatory response is due to serendipitous function of T2SS substrates or macrophage response to infection is unclear.

Truchan *et al.* recently observed localization of the T2SS substrates ProA and ChiA to the cytoplasmic face of the LCV membrane (LCVM)[222]. The authors hypothesize that translocation of ProA and ChiA to the LCVM is results from LCV permeability, as observed by galectin-3 accumulation around the LCV. Interestingly, this phenomenon was observed in human but not mouse macrophages. ProA and ChiA localized to the LCVM in U937 cells and differentiated polymorphonuclear cells (PBMCs) but not in BMDMs from permissive mouse strains. Mechanisms by which the LCV becomes semipermeable and the observed differences between mouse and human macrophages are unclear.

Taken together, T2SS contributes to *L. pneumophila* virulence via environmental survival and persistence in the host. Future studies are required to completely understand the role of T2SS substrates in disease progression and to define the underlying mechanisms of action.

Host immune responses to *Legionella* infection

Macrophage detection of *L. pneumophila* infection

L. pneumophila activates an orchestrated and robust inflammatory response during infection of healthy

hosts. Inbred mouse models of *L. pneumophila* infection and LD have been pivotal defining mechanisms of host defense against intracellular pathogens. *L. pneumophila* has therefore emerged as an invaluable model pathogen to study host defense mechanism including innate immunity, inflammasome activation and acute lung inflammation.

Innate immune detection of *L. pneumophila* involves synergistic recognition of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs) including toll-like receptors (TLRs), nucleotide-binding oligomerization domain-like receptor (NLRs), and Rig-like helicases (RLH) that results in the activation of immune responses and pathogen clearance (Figure 2) [223,224]. Pro-inflammatory gene expression through NF-κB and AP-1 (Jun/Fos) downstream of TLRs is mediated through signal cascades involving the adaptors MyD88/TRIF and mitogen activated kinases (MAPKs). MyD88 triggers a signaling cascade that results in upregulation of pro-inflammatory gene expression [225,226]. MyD88-deficient mice are highly susceptible to *L. pneumophila* infection and due to severe defects in leukocyte recruitment, proinflammatory cytokine and chemokine secretion [227–230]. Intracellular pathogen detection is also facilitated through inflammasome activation and effector-mediated immunity. Detection of *L. pneumophila* results in upregulation of a complex and highly orchestrated inflammatory response *in vivo* that involves both myeloid and somatic cells.

Role of PAMP recognition in *L. pneumophila* replication

TLR2, TLR4, TLR5 and TLR9 contribute to innate immunity against *L. pneumophila* through activation of signal transduction cascades culminating in NF-κB and AP-1-mediated pro-inflammatory gene expression [228,230–233] (Figure 2). Interestingly, TLR4, which recognizes lipopolysaccharide (LPS), does not play a major role in *L. pneumophila* recognition [231,234,235]. TLR2-deficient mice have delayed production of proinflammatory cytokines and neutrophil recruitment during *L. pneumophila* infection[228]. TLR5 detects bacterial flagellin and enhances neutrophil recruitment to the *L. pneumophila*-infected lung at early time points post-infection [230,232]. TLR9 signaling additionally contributes to *L. pneumophila* but likely functions redundantly with TLR5. Interestingly, TLR9 signaling is more important for restriction of *L. pneumophila* in Balb/c mice than in A/J mice, likely owing to differences in the genetic backgrounds of these hosts [230,236]. TLR signaling synergizes with intracellular PAMP detection for optimal induction of inflammation and restriction of *L. pneumophila* intracellular replication.

Nucleotide-binding and oligomerization domain protein 1 (NOD1) and NOD2 consist of a N-terminal interaction domain, a nucleotide binding central domain and a leucine-rich repeat (LRR) C-terminal variable domain[235]. NOD1 and NOD2 detect cytosolic peptidoglycan and initiate signaling through activation of receptor interacting protein kinase 2 (RIP2), which culminates in activation of NF-κB (Figure 2) [226,235]. Loss of RIP2-mediated signaling impairs Dot/Icm-dependent immune responses but has only modest effects on pro-inflammatory cytokine production by BMDMs[237]. Loss of RIP2-mediated signaling enhances *L. pneumophila* bacterial burden in the mouse lung, likely through impaired neutrophil recruitment[238].

Retinoic-acid inducible gene-I (RIG-I)-like helicases (RLHs) melanoma-differentiation-associated gene-5 (MDA5) and RIG-I also contribute to macrophage detection of *L. pneumophila* through detection of cytosolic nucleic acids. RIG-I/MDA5 activation results in signal transduction involving multiple adaptor proteins, including interferon-β promoter stimulator-1 (IPS-1) (Figure 2) [235,239]. This in turn activates NF-κB and interferon regulatory factors (IRFs), which enhance the production of proinflammatory cytokines and type-I interferons (IFN-I), respectively [235,241]. *L. pneumophila* activates this pathway via RNA translocation into the host cell[240]. Interestingly, despite increased IFN-I during *L. pneumophila* infection, impaired IFN-I receptor (IFNAR) signaling does not affect *L. pneumophila* replication in the lungs [240,241].

Role of inflammasomes in *L. pneumophila* infection

Inflammasomes are large intracellular protein complexes that recognize pathogens and cellular stressors. Generally, pathogen sensing by inflammasomes results in activation of effector caspases, caspase-1 and caspase-8, the consequence of which is pyroptosis and secretion of IL-1β and IL-18[242]. The NAIP5/NLRC4 inflammasome detects bacterial flagellin and is responsible for potent restriction of *L. pneumophila* by wild-type C57BL/6 macrophages (Figure 2) [243,244]. *L. pneumophila* has been instrumental in delineating structural and functional insights into flagellin detection by NAIP5/NLRC4 and consequent pathogen restriction [245–247]. A comprehensive review detailing mechanisms of inflammasome activation by *L. pneumophila* is available[247]. However, a critical role for pyroptosis in host restriction *of L. pneumophila* was recently described. Pyroptosis

occurs through caspase-1 cleavage of Gasdermin D (GSDMD). Cleaved GSDMD forms pores in the plasma membrane and culminates in release of cytosolic components, cytokines and eventual cell lysis[248]. NLRC4-mediated restriction of *L. pneumophila* is facilitated through activation of caspase-7 and GSDMD-mediated pyroptosis [249,250]. The mechanism by which caspase-7 contributes to restriction of *L. pneumophila* has yet to be fully elucidated, but its role in cell death further suggests an essential role for inflammatory host cell death in NAIP5/NLRC4-mediated restriction of *L. pneumophila*.

Cytokine responses during *L. pneumophila* infection and bystander responses

Pro-inflammatory cytokines are pivotal for host defense against *L. pneumophila*. Of these cytokines, IFN-γ and TNFα are primarily responsible for immune clearance of *L. pneumophila*. High concentrations of IFN-γ are generated in the mouse lung in response to *L. pneumophila* infection, the majority of which is made by natural killer (NK) cells in a MyD88-dependent manner[229]. NK cells make IFN-γ in response to IL-12 and IL-18; however, loss of IL-18 only has a modest effect on IFN-γ production and bacterial burden in the lung [230,251]. Conversely, monocyte generated IL-12 is crucial for IFN-γ production by NK cells in the *Legionella*-infected lung [230,252]. IFN-γ-deficient mice are unable to clear pulmonary *L. pneumophila* and, in contrast to wild-type strains, will succumb to infection [230,253]. IFN-γ facilitates cell-autonomous restriction of *L. pneumophila* through upregulation of interferon-stimulated genes, oxidative stress, nutritional remodeling and xenophagy[254]. Interferon-stimulated immune responsive gene 1 (IRG1) additionally restricts *L. pneumophila* through the production of itaconic acid [255]. Thus, IFN-γ production by non-infected cells drives cell-autonomous macrophage restriction of *L. pneumophila*.

In addition to IFN-γ, high levels of TNFα are generated in response to *L. pneumophila* infection in the lung[256]. Loss of TNF-mediated signaling impairs pulmonary bacterial clearance and survival of *L. pneumophila*-infected mice [256,257]. In the lung, TNF is produced primarily by uninfected inflammatory monocytes and neutrophils but activate antimicrobial defenses in infected macrophages[252]. TNFα signals through TNFR1 and TNFR2; however, TNFR1-mediated signaling plays a more central role in host defense against *L. pneumophila*[258]. Signaling through TNFR1 enhances macrophage antimicrobial activity through enhanced lysosomal fusion with LCVs. TNFα synergizes with IFN-γ and IFN-I to restrict *L. pneumophila*[258].

Several *L. pneumophila* effectors (Lgt1-3, SidI, SidL, LegK4 and RavX; see above) inhibit host protein synthesis, which impairs cytokine production during infection. However, a robust pro-inflammatory response in generated. In the *L. pneumophila*-infected lung, infected and uninfected cells produce different sets of cytokines. Despite potent effector-mediated translation inhibition, *L. pneumophila*-infected cells secrete IL-1 through mRNA superinduction[160]. IL-1 receptor (IL-1 R)-mediated signaling is required for the production of proinflammatory cytokines (IL-12, TNFα, and IL-6) by bystander monocytes and neutrophils[159]. Recent work from Shin and colleagues revealed how IL-1 promotes bystander pro-inflammatory cytokine production. IL-1 R signaling stimulates the production of granulocyte colony-stimulating factor (GM-CSF) by the alveolar epithelium. GM-CSF signaling synergizes with TLR-mediated signaling to enhance aerobic glycolysis, which enhances pro-inflammatory cytokine production by bystander cells and consequent activation of infected alveolar macrophages[259]. Thus, the inflammatory response to *L. pneumophila* in vivo is highly orchestrated and involves myeloid and somatic cells.

Interestingly, the *L. pneumophila* effector LegC4, which is important for replication within *A. castellanii*, paradoxically attenuates *L. pneumophila* fitness in the mouse lung. This is likely through exacerbation of cytokine-mediated restriction in macrophages, but the mechanism by which this occurs is unknown [155,260].

The adaptive immune response to *L. pneumophila*

Adaptive immunity additionally contributes to host defense against *L. pneumophila*. CD4+ and CD8 + T cells contribute to host defense against *L. pneumophila* [261]. Following mediastinal lymph node priming with *L. pneumophila*, differentiated Th17 and Th1 T cells infiltrate lungs and produce IL-17 and IFN-γ, respectively [262]. Furthermore, Th17-mediated restriction of *L. pneumophila* depends on NLRC4 inflammasome and MyD88, whereas the Th1 response is initiated in the absence of MyD88[262]. CD8 + T cells are additionally a source of IFN-γ and promote M1 macrophage skewing[263].

Mice mount a humoral response to *L. pneumophila* introduced either via intranasal inoculation or intravenous injection[264]. After primary exposure to *L. pneumophila*, mice generate IgA and IgG responses in the bronchoalveolar lavage fluid and serum,

respectively, followed by the establishment of memory B cells in the lung[264]. Thirty novel *L. pneumophila*-specific B cell antigens have been identified and include components of bacterial membrane[265]. Immunization of guinea pigs with *L. pneumophila* Hsp60 or OmpS results in partial protection from *L. pneumophila* infection [265,266]. However, the adaptive immune response to *Legionella* infection is considerably feeble and does not provide prolonged immunity against the pathogen since reinfection has been reported [267]. Altogether, these studies demonstrate the importance of innate immunity in host restriction of *L. pneumophila* and elaborate challenges associated with the development of lasting immunity in susceptible individuals.

Concluding remarks and future directions

Past four decades of research on *Legionella* have brought important insights into the virulence strategies and mechanisms employed by *Legionella* to replicate within environmental and mammalian phagocytes and cause disease in humans. To date, the genus *Legionella* has the largest and most diverse effector repertoire amongst intracellular pathogens. Despite the diversity among *Legionella* species, there are common themes in their virulence strategies including Dot/Icm effector translocation and acquisition of eukaryotic-like effectors that have resulted from extensive co-evolution with protozoa. *Legionella* has also emerged as an invaluable model pathogen to understand innate immunity and major breakthroughs have been made using mouse models of LD. Despite these major advances, many questions remain open. The study of *Legionella* bacteria is intriguing in many ways and future studies will teach us not only about bacterial pathogenicity but also eukaryotic cell biology and mammalian antimicrobial immune defenses. We are excited for what the next four decades will reveal about *Legionella* virulence.

Abbreviations

Legionella; Legionnaires' Disease; pathogenicity; virulence; Dot/Icm secretion system; effector proteins; host–pathogen interactions; host response; innate immunity; effector-mediated immunity

Acknowledgments

We thank Fern Ness and Patrick Lane for assistance with figure preparation and apologize to the authors whose work we could not discuss due to space constraints. Research in the Shames Lab is funded by a NIH COBRE Research Project Award (to S.R.S.) and a NIH Kansas-INBRE postdoctoral fellowship (to D.C.).

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the National Institute of General Medical Sciences [P20GM103418]; National Institute of General Medical Sciences [P20GM130448].

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References

[1] Cunha BA, Burillo A, Bouza E. Legionnaires' disease. Lancet. 2016;387(10016):376–385.
[2] Cordes LG, Fraser DW. Legionellosis: legionnaires' disease; Pontiac fever. Med Clin N Am. 1980;64(3):395–416.
[3] Brenner DJ. Classification of the Legionnaires' Disease Bacterium: legionella pneumophila, genus novum, species nova, of the Family Legionellaceae, familia nova. Ann Intern Med. 1979;90(4):656.
[4] Stout JE, Yu VL. Legionellosis. New Engl J Medicine. 1997;337(10):682–687.
[5] Von Baum H, Ewig S, Marre R, et al., Group CN for CAPS. Community-acquired legionella pneumonia: new insights from the German competence network for community acquired pneumonia. Clin Infect Dis. 2008;46(9):1356–1364.
[6] Gomez-Valero L, Buchrieser C. Intracellular parasitism, the driving force of evolution of Legionella pneumophila and the genus Legionella. Genes Immun. 2019;20(5):394–402.
[7] Mondino S, Schmidt S, Buchrieser BC. Molecular mimicry: a paradigm of host-microbe coevolution illustrated by Legionella. Mbio. 2020;11(5). DOI:10.1128/mBio.01201-20
[8] Fitzhenry R, Weiss D, Cimini D, et al. Legionnaires' disease outbreaks and Cooling Towers, New York City, New York, USA. Emerg Infect Dis. 2017;23(11):1769–1776.
[9] Hamilton KA, Prussin AJ, Ahmed W, et al. Outbreaks of legionnaires' disease and Pontiac fever 2006–2017. Curr Environ Heal Reports. 2018;5(2):263–271.
[10] Borges V, Nunes A, Sampaio DA, et al. Legionella pneumophila strain associated with the first evidence of person-to-person transmission of Legionnaires' disease: a unique mosaic genetic backbone. Sci Rep-uk. 2016;6:26261.

[11] Muder RR, Victor LY. Infection due to legionella species other Than L. pneumophila. Clin Infect Dis. 2002;35(8):990–998.
[12] Yu VL, Plouffe JF, Pastoris MC, et al. Distribution of legionella species and serogroups isolated by culture in patients with sporadic community-acquired legionellosis: an international collaborative survey. J Infect Dis. 2002;186(1):127–128.
[13] Mondino S, Schmidt S, Rolando M, et al. Legionnaires' disease: state of the art knowledge of pathogenesis mechanisms of Legionella. Annu Rev Pathol Mech Dis. 2020;15(1):439–466.
[14] Best MG, Stout JE, Yu VL, et al. Tatlockia micdadei (Pittsburgh pneumonia agent) growth kinetics may explain its infrequent isolation from water and the low prevalence of Pittsburgh pneumonia. Appl Environ Microb. 1985;49(6):1521–1522.
[15] Rowbotham TJ. Preliminary report on the pathogenicity of Legionella pneumophila for freshwater and soil amoebae. J Clin Pathol. 1980;33(12):1179.
[16] Fliermans CB, Cherry WB, Orrison LH, et al. Ecological distribution of Legionella pneumophila. Appl Environ Microb. 1981;41(1):9–16.
[17] Hoffmann C, Harrison CF, Hilbi H. The natural alternative: protozoa as cellular models for Legionella infection. Cell Microbiol. 2013;16(1):15–26.
[18] Anand CM, Skinner AR, Malic A, et al. Interaction of L. pneumophila and a free living amoeba (Acanthamoeba palestinensis). J Hyg. 1983;91 (2):167–178.
[19] Barker J, Brown MR, Collier PJ, et al. Relationship between Legionella pneumophila and Acanthamoeba polyphaga: physiological status and susceptibility to chemical inactivation. Appl Environ Microb. 1992;58 (8):2420–2425.
[20] Cirillo JD, Cirillo SLG, Yan L, et al. Intracellular Growth in Acanthamoeba castellanii Affects Monocyte Entry Mechanisms and Enhances Virulence of Legionella pneumophila. Infect Immun. 1999;67 (9):4427–4434.
[21] King CH, Shotts EB, Wooley RE, et al. Survival of coliforms and bacterial pathogens within protozoa during chlorination. Appl Environ Microb. 1988;54 (12):3023–3033.
[22] Murga R, Forster TS, Brown E, et al. Role of biofilms in the survival of Legionella pneumophila in a model potable-water system. Microbiology+. 2001;147(Pt 11):3121–3126.
[23] Rogers J, Dowsett AB, Dennis PJ, et al. Influence of temperature and plumbing material selection on biofilm formation and growth of Legionella pneumophila in a model potable water system containing complex microbial flora. Appl Environ Microb. 1994;60 (5):1585–1592.
[24] Personnic N, Striednig B, Hilbi H. Quorum sensing controls persistence, resuscitation, and virulence of Legionella subpopulations in biofilms. Isme J. 2020;-(1):196–210.
[25] Burstein D, Amaro F, Zusman T, et al. Genomic analysis of 38 Legionella species identifies large and diverse effector repertoires. Nat Genet. 2016;48(2):167–175.
[26] Gomez-Valero L, Rusniok C, Carson D, et al. More than 18,000 effectors in the Legionella genus genome provide multiple, independent combinations for replication in human cells. Proc Natl Acad Sci. 2019;116:201808016.
[27] Isberg RR, O'Connor TJ, Heidtman M. The Legionella pneumophila replication vacuole: making a cosy niche inside host cells. Nat Rev Microbiol. 2009;7(1):13–24.
[28] Cirillo SLG, Lum J, Cirillo JD. Identification of novel loci involved in entry by Legionella pneumophila. Microbiology+. 2000;146 (Pt 6):1345–1359.
[29] Stone BJ, Kwaik YA. Expression of multiple Pili by Legionella pneumophila: identification and characterization of a Type IV Pilin gene and its role in adherence to mammalian and protozoan cells. Infect Immun. 1998;66(4):1768–1775.
[30] Liu M, Conover GM, Isberg RR. Legionella pneumophila EnhC is required for efficient replication in tumour necrosis factor α-stimulated macrophages. Cell Microbiol. 2008;10(9):1906–1923.
[31] Newton HJ, Sansom FM, Dao J, et al. Significant role for ladC in initiation of Legionella pneumophila Infectionword▿. Infect Immun. 2008;76:3075–3085.
[32] Bellinger-Kawahara C, Horwitz MA. Complement component C3 fixes selectively to the major outer membrane protein (MOMP) of Legionella pneumophila and mediates phagocytosis of liposome-MOMP complexes by human monocytes. J Exp Med. 1990;172 (4):1201–1210.
[33] Krinos C, High AS, Rodgers FG. Role of the 25 kDa major outer membrane protein of Legionella pneumophila in attachment to U-937 cells and its potential as a virulence factor for chick embryos. J Appl Microbiol. 1999;86(2):237–244.
[34] Vandersmissen L, Buck ED, Saels V, et al. A Legionella pneumophila collagen-like protein encoded by a gene with a variable number of tandem repeats is involved in the adherence and invasion of host cells. FEMS Microbiol Lett. 2010;306(2):168–176.
[35] Fields BS. The molecular ecology of legionellae. Trends Microbiol. 1996;4(7):286–290.
[36] Husmann LK, Johnson W. Adherence of Legionella pneumophila to guinea pig peritoneal macrophages, J774 mouse macrophages, and undifferentiated U937 human monocytes: role of Fc and complement receptors. Infect Immun. 1992;60(12):5212–5218.
[37] Lau HY, Ashbolt NJ. The role of biofilms and protozoa in Legionella pathogenesis: implications for drinking water. J Appl Microbiol. 2009;107(2):368–378.
[38] Payne NR, Horwitz MA. Phagocytosis of Legionella pneumophila is mediated by human monocyte complement receptors. J Exp Med. 1987;166(5):1377–1389.
[39] Elliott JA, Winn WC. Treatment of alveolar macrophages with cytochalasin D inhibits uptake and subsequent growth of Legionella pneumophila. Infect Immun. 1986;51(1):31–36.
[40] Gibson III FC, Tzianabos AO, Rodgers FG. Adherence of Legionella pneumophila to U-937 cells, guinea-pig alveolar macrophages, and MRC-5 cells by a novel, complement-independent binding mechanism. Can J Microbiol. 1994;40(10):865–872.

[41] Rodgers FG, Gibson III FC. Opsonin-independent adherence and intracellular development of Legionella pneumophila within U-937 cells. Can J Microbiol. 1993;39(7):718–722.
[42] Cao Z, Jefferson DM, Panjwani N. Role of carbohydrate-mediated adherence in cytopathogenic mechanisms of acanthamoeba. J Biol Chem. 1998;273 (25):15838–15845.
[43] Horwitz MA. Phagocytosis of the legionnaires' disease bacterium (legionella pneumophila) occurs by a novel mechanism: engulfment within a Pseudopod coil. Cell. 1984;36(1):27–33.
[44] Bozue JA, Johnson W. Interaction of Legionella pneumophila with Acanthamoeba castellanii: uptake by coiling phagocytosis and inhibition of phagosome-lysosome fusion. Infect Immun. 1996;64 (2):668–673.
[45] Rittig MG, Burmester G-R G-R, Krause A. Coiling phagocytosis: when the zipper jams, the cup is deformed. Trends Microbiol. 1998;6(10):384–388.
[46] Hilbi H, Segal G, Shuman HA. Icm/Dot-dependent upregulation of phagocytosis by Legionella pneumophila. Mol Microbiol. 2001;42(3):603–617.
[47] Flanagan MD, Lin S. Cytochalasins block actin filament elongation by binding to high affinity sites associated with F-actin. J Biological Chem. 1980;255(3):835–838.
[48] King CH, Fields BS, Shotts EB, et al. Effects of cytochalasin D and methylamine on intracellular growth of Legionella pneumophila in amoebae and human monocyte-like cells. Infect Immun. 1991;59 (3):758–763.
[49] Prashar A, Bhatia S, Tabatabaeiyazdi Z, et al. Mechanism of invasion of lung epithelial cells by filamentous L egionella pneumophila. Cell Microbiol. 2012;14(10):1632–1655.
[50] Hayashi T, Miyake M, Fukui T, et al. Exclusion of Actin-Binding Protein p57/Coronin-1 from Bacteria-Containing Phagosomes in Macrophages Infected with Legionella. Biol Pharm Bull. 2008;31 (5):861–865.
[51] Chang B, Kura F, Amemura-Maekawa J, et al. Identification of a Novel Adhesion Molecule Involved in the Virulence of Legionella pneumophila. Infect Immun. 2005;73(7):4272–4280.
[52] Gruenberg J. The endocytic pathway: a mosaic of domains. Nat Rev Mol Cell Bio. 2001;2(10):721–730.
[53] Kornfeld S, Mellman I. The Biogenesis of Lysosomes. Annu Rev Cell Dev Bi. 1989;5(1):483–525.
[54] Derré I, Isberg RR. Legionella pneumophila replication vacuole formation involves rapid recruitment of proteins of the early secretory system. Infect Immun. 2004;72(5):3048–3053.
[55] Horwitz MA. Formation of a novel phagosome by the Legionnaires' disease bacterium (Legionella pneumophila) in human monocytes. J Exp Med. 1983;158 (4):1319–1331.
[56] Sturgill-Koszycki S, Swanson MS. Legionella pneumophila Replication vacuoles mature into acidic, endocytic organelles. J Exp Med. 2000;192(9):1261–1272.
[57] Xu L, Shen X, Bryan A, et al. Inhibition of host vacuolar H+-ATPase activity by a Legionella pneumophila effector. PLoS Pathogens. 2010;6(3):e1000822.
[58] Prevost MS, Pinotsis N, Dumoux M, et al. The Legionella effector WipB is a translocated Ser/Thr phosphatase that targets the host lysosomal nutrient sensing machinery. Sci Rep. 2017;7(1):9450.
[59] Tilney LG, Harb OS, Connelly PS, et al. How the parasitic bacterium Legionella pneumophila modifies its phagosome and transforms it into rough ER: implications for conversion of plasma membrane to the ER membrane. J Cell Sci. 2001;114(Pt 24):4637–4650.
[60] Horwitz MA. Formation of a novel phagosome by the Legionnaires' disease bacterium (Legionella pneumophila) in human monocytes. J Exp Med. 1983;158 (4):1319–1331.
[61] Escoll P, Song O-R, Viana F, et al. Legionella pneumophila modulates mitochondrial dynamics to trigger metabolic repurposing of infected macrophages. Cell Host Microbe. 2017;22(302–316.e7):302–316.e7.
[62] Berger KH, Merriam JJ, Isberg RR. Altered intracellular targeting properties associated with mutations in the Legionella pneumophila dotA gene. Mol Microbiol. 1994;14(4):809–822.
[63] Chong A, Lima CA, Allan DS, et al. The purified and recombinant Legionella pneumophila Chaperonin alters mitochondrial trafficking and microfilament Organization. Infect Immun. 2009;77(11):4724–4739.
[64] Escoll P, Buchrieser C. Metabolic reprogramming of host cells upon bacterial infection: why shift to a Warburg-like metabolism? Febs J. 2018;285 (12):2146–2160.
[65] Conover GM, Derré I, Vogel JP, et al. The Legionella pneumophila LidA protein: a translocated substrate of the Dot/Icm system associated with maintenance of bacterial integrity. Mol Microbiol. 2003;48(2):305–321.
[66] Shohdy N, Efe JA, Emr SD, et al. Pathogen effector protein screening in yeast identifies Legionella factors that interfere with membrane trafficking. Proceedings of the National Academy of Sciences 2005; 102 (13):4866–4871.
[67] Dorer MS, Kirton D, Bader JS, et al. RNA interference analysis of Legionella in Drosophila Cells: exploitation of early secretory apparatus dynamics. PLoS Pathog. 2006;2(4):e34.
[68] De Felipe KS, Glover RT, Charpentier X, et al. Legionella Eukaryotic-like Type IV substrates interfere with Organelle trafficking. PLoS Pathog. 2008;4(4): e1000117.
[69] Heidtman M, Chen EJ, Moy M, et al. Large-scale identification of Legionella pneumophila Dot/Icm substrates that modulate host cell vesicle trafficking pathways. Cell Microbiol. 2009;11(2):230–248.
[70] Ninio S, Celli J, Roy CRA. A Legionella pneumophila effector protein encoded in a region of genomic plasticity binds to Dot/Icm-modified vacuoles. PLoS Pathog. 2009;5(1):e1000278.
[71] Pan X, Lührmann A, Satoh A, et al. Ankyrin repeat proteins comprise a diverse family of bacterial Type IV effectors. Science. 2008;320:1651–1654.
[72] Campodonico EM, Chesnel L, Roy CR. A yeast genetic system for the identification and characterization of substrate proteins transferred into host cells by the Legionella pneumophila Dot/Icm system. Mol Microbiol. 2005;56(4):918–933.

[73] Swart AL, Hilbi H. Phosphoinositides and the fate of Legionella in Phagocytes. Front Immunol. 2020;11:25.
[74] Hardiman CA, McDonough JA, Newton HJ, et al. The role of Rab GTPases in the transport of vacuoles containing Legionella pneumophila and Coxiella burnetii. Biochem Soc Trans. 2012;40(6):1353–1359.
[75] Sherwood RK, Roy CR. A Rab-Centric perspective of bacterial pathogen-occupied vacuoles. Cell Host Microbe. 2013;14(3):256–268.
[76] Finsel I, Ragaz C, Hoffmann C, et al. The Legionella Effector RidL Inhibits Retrograde Trafficking to Promote Intracellular Replication. Cell Host Microbe. 2013;14(1):38–50.
[77] Elwell C, Engel J. Emerging role of retromer in modulating pathogen growth. Trends Microbiol. 2018;26 (9):769–780.
[78] Horwitz MA. The Legionnaires' disease bacterium (Legionella pneumophila) inhibits phagosome-lysosome fusion in human monocytes. . J Exp Med. 1983;158(6):2108–2126.
[79] Kwaik YA. The phagosome containing Legionella pneumophila within the protozoan Hartmannella vermiformis is surrounded by the rough endoplasmic reticulum. Appl Environ Microb. 1996;62 (6):2022–2028.
[80] Newsome AL, Baker RL, Miller RD, et al. Interactions between Naegleria fowleri and Legionella pneumophila. Infect Immun. 1985;50(2):449–452.
[81] Cazalet C, Gomez-Valero L, Rusniok C, et al. Analysis of the Legionella longbeachae Genome and Transcriptome Uncovers Unique Strategies to Cause Legionnaires' Disease. PLoS Genet. 2010;6(2):e1000851.
[82] Wood RE, Newton P, Latomanski EA, et al. Dot/Icm effector translocation by legionella longbeachae creates a replicative vacuole similar to that of legionella pneumophila despite translocation of distinct effector repertoires. Infect Immun. 2015;83(10):4081–4092.
[83] Brumell JH, Scidmore MA. Manipulation of Rab GTPase function by intracellular bacterial pathogens. Microbiol Mol Biol R. 2007;71(4):636–652.
[84] Spanò S, Galán JE. Taking control: hijacking of Rab GTPases by intracellular bacterial pathogens. Small Gtpases. 2017;9(1–2):182–191.
[85] Steiner B, Weber S, Hilbi H. Formation of the Legionella-containing vacuole: phosphoinositide conversion, GTPase modulation and ER dynamics. Int J Med Microbiol. 2018;308(1):49–57.
[86] Kagan JC, Stein M-P, Pypaert M, et al. Legionella subvert the functions of Rab1 and Sec22b to create a replicative Organelle. J Exp Med. 2004;199 (9):1201–1211.
[87] Machner MP, Isberg RR. Targeting of host Rab GTPase function by the intravacuolar pathogen Legionella pneumophila. Dev Cell. 2006;11(1):47–56.
[88] Murata T, Delprato A, Ingmundson A, et al. The Legionella pneumophila effector protein DrrA is a Rab1 guanine nucleotide-exchange factor. Nat Cell Biol. 2006;8(9):971–977.
[89] Gaspar AH, Machner MP. VipD is a Rab5-activated phospholipase A1 that protects Legionella pneumophila from endosomal fusion. Proc Natl Acad Sci. 2014;111(12):4560–4565.
[90] Rink J, Ghigo E, Kalaidzidis Y, et al. Rab conversion as a mechanism of progression from early to late endosomes. Cell. 2005;122(5):735–749.
[91] Rojas R, Van Vlijmen T, Mardones GA, et al. Regulation of retromer recruitment to endosomes by sequential action of Rab5 and Rab7. J Cell Biol. 2008;183(3):513–526.
[92] Romano-Moreno M, Rojas AL, Williamson CD, et al. Molecular mechanism for the subversion of the retromer coat by the Legionella effector RidL. Proc Natl Acad Sci. 2017;114(52):E11151–60.
[93] Bärlocher K, Welin A, Hilbi H. Formation of the Legionella replicative compartment at the crossroads of retrograde trafficking. Front Cell Infect Mi. 2017;7:482.
[94] Byrne B, Swanson MS. Expression of Legionella pneumophilaVirulence Traits in Response to Growth Conditions. Infect Immun. 1998;66(7):3029–3034.
[95] Brüggemann H, Hagman A, Jules M, et al. Virulence strategies for infecting phagocytes deduced from the in vivo transcriptional program of Legionella pneumophila. Cell Microbiol. 2006;8(8):1228–1240.
[96] Hammer BK, Tateda ES, Swanson MS. A two-component regulator induces the transmission phenotype of stationary-phase Legionella pneumophila. Mol Microbiol. 2002;44(1):107–118.
[97] Dalebroux ZD, Edwards RL, Swanson MS. SpoT governs Legionella pneumophila differentiation in host macrophages. Mol Microbiol. 2009;71(3):640–658.
[98] Bachman MA, Swanson MS. RpoS co-operates with other factors to induce Legionella pneumophila virulence in the stationary phase. Mol Microbiol. 2001;40 (5):1201–1214.
[99] Hammer BK, Swanson MS. Co-ordination of Legionella pneumophila virulence with entry into stationary phase by ppGpp. Mol Microbiol. 1999;33 (4):721–731.
[100] Zusman T, Gal-Mor O, Segal G. Characterization of a Legionella pneumophila relA Insertion Mutant and Roles of RelA and RpoS in Virulence Gene Expression. J Bacteriol. 2002;184(1):67–75.
[101] Molofsky AB, Swanson MS. Legionella pneumophila CsrA is a pivotal repressor of transmission traits and activator of replication. Mol Microbiol. 2003;50 (2):445–461.
[102] Sahr T, Brüggemann H, Jules M, et al. Two small ncRNAs jointly govern virulence and transmission in Legionella pneumophila. Mol Microbiol. 2009;72 (3):741–762.
[103] Rasis M, Segal SG. The LetA-RsmYZ-CsrA regulatory cascade, together with RpoS and PmrA, post-transcriptionally regulates stationary phase activation of Legionella pneumophila Icm/Dot effectors. Mol Microbiol. 2009;72(4):995–1010.
[104] Alli OAT, Gao L-Y, Pedersen LL, et al. Temporal pore formation-mediated egress from macrophages and alveolar epithelial cells by Legionella pneumophila. Infect Immun. 2000;68(11):6431–6440.
[105] Gao L, Kwaik YA. The mechanism of killing and exiting the protozoan host Acanthamoeba polyphaga by Legionella pneumophila. Environ Microbiol. 2000;68 (1):79–90.

[106] Molmeret M, Alli OAT, Zink S, et al. icmT is essential for pore formation-mediated Egress of Legionella pneumophila from Mammalian and Protozoan Cells. Infect Immun. 2002;70(1):69–78.
[107] Chen J, De Felipe KS, Clarke M, et al. Legionella effectors that promote nonlytic release from Protozoa. Science. 2004;303(5662):1358–1361.
[108] Segal G, Shuman HA. Legionella pneumophila utilizes the same genes to multiply within Acanthamoeba castellanii and Human Macrophages. Infect Immun. 1999;67(5):2117–2124.
[109] Harb OS, Gao L, Kwaik YA. From protozoa to mammalian cells: a new paradigm in the life cycle of intracellular bacterial pathogens. Minireview. Environ Microbiol. 2000;2(3):251–265.
[110] Molmeret M, Horn M, Wagner M, et al. Amoebae as Training Grounds for Intracellular Bacterial Pathogens. Appl Environ Microb. 2005;71(1):20–28.
[111] Park JM, Ghosh S, O'Connor TJ. Combinatorial selection in amoebal hosts drives the evolution of the human pathogen Legionella pneumophila. Nat Microbiol. 2020;5(4):599–609.
[112] Rolando M, Sanulli S, Rusniok C, et al. Legionella pneumophila effector RomA uniquely modifies host Chromatin to repress gene expression and promote intracellular bacterial replication. Cell Host Microbe. 2013;13(4):395–405.
[113] Cazalet C, Rusniok C, Brüggemann H, et al. Evidence in the Legionella pneumophila genome for exploitation of host cell functions and high genome plasticity. Nat Genet. 2004;36(11):1165–1173.
[114] Li W, Bandyopadhyay J, Hwaang HS, et al. Two thioredoxin reductases, trxr-1 and trxr-2, have differential physiological roles in Caenorhabditis elegans. Mol Cells. 2012;34(2):209–218.
[115] Conover GM, Martinez-Morales F, Heidtman MI, et al. Phosphatidylcholine synthesis is required for optimal function of Legionella pneumophila virulence determinants. Cell Microbiol. 2008;10(2):514–528.
[116] Marra A, Blander SJ, Horwitz MA, et al. Identification of a Legionella pneumophila locus required for intracellular multiplication in human macrophages. Proc Natl Acad Sci. 1992;89(20):9607–9611.
[117] Berger KH, Isberg RR. Two distinct defects in intracellular growth complemented by a single genetic locus in Legionella pneumophila. Mol Microbiol. 1993;7:7–19.
[118] Brand BC, Sadosky AB, Shuman HA. The Legionella pneumophila icm locus: a set of genes required for intracellular multiplication in human macrophages. Mol Microbiol. 1994;14(4):797–808.
[119] Segal G, Purcell M, Shuman HA. Host cell killing and bacterial conjugation require overlapping sets of genes within a 22-kb region of the Legionella pneumophila genome. Proc Natl Acad Sci. 1998;95(4):1669–1674.
[120] Vogel JP, Andrews HL, Wong SK, et al. Conjugative transfer by the virulence system of Legionella pneumophila. Science. 1998;279(5352):873–876.
[121] Christie PJ, Vogel JP. Bacterial type IV secretion: conjugation systems adapted to deliver effector molecules to host cells. Trends Microbiol. 2000;8(8):354–360.
[122] Hamilton CM, Lee H, Li P-L, et al. TraG from RP4 and TraG and VirD4 from Ti Plasmids Confer Relaxosome Specificity to the Conjugal Transfer System of pTiC58. J Bacteriol. 2000;182(6):1541–1548.
[123] Vincent CD, Friedman JR, Jeong KC, et al. Identification of the DotL coupling protein subcomplex of the Legionella Dot/Icm type IV secretion system. Mol Microbiol. 2012;85(2):378–391.
[124] Trokter M, Waksman G. Translocation through the conjugative Type IV secretion system requires unfolding of its protein substrate. J Bacteriol. 2018;200(6): e00615–17.
[125] Meir A, Macé K, Lukoyanova N, et al. Mechanism of effector capture and delivery by the type IV secretion system from Legionella pneumophila. Nat Commun. 2020;11(1):2864.
[126] Kwak M-J, Kim JD, Kim H, et al. Architecture of the type IV coupling protein complex of Legionella pneumophila. Nat Microbiol. 2017;2(9):17114.
[127] Kim H, Kubori T, Yamazaki K, et al. Structural basis for effector protein recognition by the Dot/Icm Type IVB coupling protein complex. Nat Commun. 2020;11 (1):2623.
[128] Vincent CD, Vogel JP. The Legionella pneumophila IcmS-LvgA protein complex is important for Dot/Icm-dependent intracellular growth. Mol Microbiol. 2006;61(3):596–613.
[129] Sutherland MC, Nguyen TL, Tseng V, et al. The Legionella IcmSW complex directly interacts with DotL to mediate translocation of adaptor-dependent substrates. PLoS Pathog. 2012;8(9):e1002910.
[130] Ghosal D, Jeong KC, Chang Y-W, et al. Molecular architecture, polar targeting and biogenesis of the Legionella Dot/Icm T4SS. Nat Microbiol. 2019;4 (7):1173–1182.
[131] Cambronne ED, Roy CR. The Legionella pneumophila IcmSW complex interacts with multiple Dot/Icm effectors to facilitate Type IV translocation. PLoS Pathog. 2007;3(12):e188.
[132] Coers J, Kagan JC, Matthews M, et al. Identification of Icm protein complexes that play distinct roles in the biogenesis of an organelle permissive for Legionella pneumophila intracellular growth. Mol Microbiol. 2000;38(4):719–736.
[133] Meir A, Chetrit D, Liu L, et al. Legionella DotM structure reveals a role in effector recruiting to the Type 4B secretion system. Nat Commun. 2018;9(1):507.
[134] Lifshitz Z, Burstein D, Peeri M, et al. Computational modeling and experimental validation of the Legionella and Coxiella virulence-related type-IVB secretion signal. Proc Natl Acad Sci. 2013;110:E707–15.
[135] Jeong KC, Sutherland MC, Vogel JP. Novel export control of a L egionella Dot/Icm substrate is mediated by dual, independent signal sequences. Mol Microbiol. 2015;96(1):175–188.
[136] Bardill JP, Miller JL, Vogel JP. IcmS-dependent translocation of SdeA into macrophages by the Legionella pneumophila type IV secretion system. Mol Microbiol. 2005;56(1):90–103.
[137] Luo Z-Q, Isberg RR Multiple substrates of the Legionella pneumophila Dot/Icm system identified by interbacterial protein transfer. Proceedings of the National Academy of Sciences 2004; 101(3):841–846.

[138] Nagai H, Kagan JC, Zhu X, et al. A bacterial guanine nucleotide exchange factor activates ARF on Legionella Phagosomes. Science. 2002;295(5555):679–682.
[139] Mizushima N. Autophagy in infection and immunity. Curr Top Microbiol. 2009;335:71–84.
[140] Choy A, Dancourt J, Mugo B, et al. The *Legionella* effector RavZ inhibits host autophagy through irreversible Atg8 deconjugation. Science. 2012;338 (6110):1072–1076.
[141] Omotade TO, Roy CR. Legionella pneumophila excludes autophagy adaptors from the Ubiquitin-labeled vacuole in which it resides. Infect Immun. 2020;88(8):88.
[142] Newton HJ, Kohler LJ, McDonough JA, et al. A screen of Coxiella burnetii mutants reveals important roles for Dot/Icm effectors and host autophagy in vacuole biogenesis. PLoS Pathog. 2014;10(7):e1004286.
[143] Rolando M, Escoll P, Nora T, et al. Legionella pneumophila S1P-lyase targets host sphingolipid metabolism and restrains autophagy. Proc Natl Acad Sci. 2016;113(7):1901–1906.
[144] Arasaki K, Mikami Y, Shames SR, et al. Legionella effector Lpg1137 shuts down ER-mitochondria communication through cleavage of syntaxin 17. Nat Commun. 2017;8(1):15406.
[145] Khweek AA, Caution K, Akhter A, et al. A bacterial protein promotes the recognition of the L egionella pneumophila vacuole by autophagy. Eur J Immunol. 2013;43(5):1333–1344.
[146] Belyi Y. Targeting Eukaryotic mRNA translation by Legionella pneumophila. Frontiers Mol Biosci. 2020;7:80.
[147] Belyi Y, Niggeweg R, Opitz B, et al. Legionella pneumophila glucosyltransferase inhibits host elongation factor 1A. Proc Natl Acad Sci. 2006;103 (45):16953–16958.
[148] Belyi Y, Tabakova I, Stahl M, et al. Lgt: a Family of Cytotoxic Glucosyltransferases produced by Legionella pneumophila▿ †. J Bacteriol. 2008;190(8):3026–3035.
[149] Belyi Y, Tartakovskaya D, Tais A, et al. Elongation Factor 1A Is the Target of Growth Inhibition in Yeast Caused by Legionella pneumophila Glucosyltransferase Lgt1. J Biol Chem. 2012;287(31):26029–26037.
[150] Tzivelekidis T, Jank T, Pohl C, et al. Aminoacyl-tRNA-Charged Eukaryotic Elongation Factor 1A Is the Bona Fide Substrate for Legionella pneumophila Effector Glucosyltransferases. PLoS One. 2011;6(12):e29525.
[151] Leon JAD, Qiu J, Nicolai CJ, et al. Positive and negative regulation of the master metabolic regulator mTORC1 by two families of Legionella pneumophila effectors. Cell Rep. 2017;21(8):2031–2038.
[152] Vera M, Pani B, Griffiths LA, et al. The translation elongation factor eEF1A1 couples transcription to translation during heat shock response. Elife. 2014;3: e03164.
[153] Shen X, Banga S, Liu Y, et al. Targeting eEF1A by a Legionella pneumophila effector leads to inhibition of protein synthesis and induction of host stress response. Cell Microbiol. 2009;11(6):911–926.
[154] Joseph AM, Pohl AE, Ball TJ, et al. The Legionella pneumophila Metaeffector Lpg2505 (MesI) Regulates SidI-Mediated Translation Inhibition and Novel Glycosyl Hydrolase Activity. Infect Immun. 2020;88 (5). DOI:10.1128/IAI.00853-19
[155] Shames SR, Liu L, Havey JC, et al. Multiple Legionella pneumophila effector virulence phenotypes revealed through high-throughput analysis of targeted mutant libraries. Proc Natl Acad Sci. 2017;114(48):E10446–54.
[156] Moss SM, Taylor IR, Ruggero D, et al. Legionella pneumophila Kinase Phosphorylates the Hsp70 Chaperone Family to Inhibit Eukaryotic Protein Synthesis. Cell Host Microbe. 2019;25(3):454–462.e6.
[157] Barry KC, Fontana MF, Portman JL, et al. IL-1α Signaling Initiates the Inflammatory Response to Virulent Legionella pneumophila In Vivo. J Immunol. 2013;190(12):6329–6339.
[158] Fontana MF, Banga S, Barry KC, et al. Secreted bacterial effectors that inhibit host protein synthesis are critical for induction of the innate immune response to virulent Legionella pneumophila. PLoS Pathog. 2011;7 (2):e1001289.
[159] Copenhaver AM, Casson CN, Nguyen HT, et al. IL-1R signaling enables bystander cells to overcome bacterial blockade of host protein synthesis. Proc Natl Acad Sci. 2015;112(24):7557–7562.
[160] Barry KC, Ingolia NT, Vance RE. Global analysis of gene expression reveals mRNA superinduction is required for the inducible immune response to a bacterial pathogen. Elife. 2017;6:e1004229.
[161] Qiu J, Luo Z-Q. Hijacking of the Host Ubiquitin Network by Legionella pneumophila. Front Cell Infect Mi. 2017;7:487.
[162] Kitao T, Nagai H, Kubori T. Divergence of Legionella Effectors Reversing Conventional and Unconventional Ubiquitination. Front Cell Infect Mi. 2020;10:448.
[163] Qiu J, Yu K, Fei X, et al. A unique deubiquitinase that deconjugates phosphoribosyl-linked protein ubiquitination. Cell Res. 2017;27(7):865–881.
[164] Sulpizio AG, Minelli ME, Mao Y. Glutamylation of Bacterial Ubiquitin Ligases by a Legionella Pseudokinase. Trends Microbiol. 2019;27(12):967–969.
[165] Black MH, Osinski A, Gradowski M, et al. Bacterial pseudokinase catalyzes protein polyglutamylation to inhibit the SidE-family ubiquitin ligases. Science. 2019;364(6442):787–792.
[166] Qiu J, Sheedlo MJ, Yu K, et al. Ubiquitination independent of E1 and E2 enzymes by bacterial effectors. Nature. 2016;533(7601):120–124.
[167] Gan N, Nakayasu ES, Hollenbeck PJ, et al. Legionella pneumophila inhibits immune signalling via MavC-mediated transglutaminase-induced ubiquitination of UBE2N. Nat Microbiol. 2019;4(1):134–143.
[168] Puvar K, Iyer S, Fu J, et al. Legionella effector MavC targets the Ube2N~Ub conjugate for noncanonical ubiquitination. Nat Commun. 2020;11:2365.
[169] Lin Y-H, Lucas M, Evans TR, et al. RavN is a member of a previously unrecognized group of Legionella pneumophila E3 ubiquitin ligases. PLOS Pathog. 2018;14(2): e1006897.
[170] Lomma M, Dervins-Ravault D, Rolando M, et al. The Legionella pneumophila F-box protein Lpp2082 (AnkB) modulates ubiquitination of the host protein parvin B and promotes intracellular replication. Cell Microbiol. 2010;12(9):1272–1291.

[171] Kotewicz KM, Ramabhadran V, Sjoblom N, et al. A Single Legionella Effector Catalyzes a Multistep Ubiquitination Pathway to Rearrange Tubular Endoplasmic Reticulum for Replication. Cell Host Microbe. 2017;21(2):169–181.
[172] Sulpizio A, Minelli ME, Wan M, et al. Protein polyglutamylation catalyzed by the bacterial calmodulin-dependent pseudokinase SidJ. Elife. 2019;8:e51162.
[173] Bhogaraju S, Bonn F, Mukherjee R, et al. Inhibition of bacterial ubiquitin ligases by SidJ–calmodulin catalysed glutamylation. Nature. 2019;572(7769):382–386.
[174] Liu Y, Luo Z-Q. The Legionella pneumophila Effector SidJ Is Required for Efficient Recruitment of Endoplasmic Reticulum Proteins to the Bacterial Phagosome▿. Infect Immun. 2007;75(2):592–603.
[175] Jeong KC, Sexton JA, Vogel JP. Spatiotemporal regulation of a Legionella pneumophila T4SS substrate by the metaeffector SidJ. PLOS Pathog. 2015;11(3):1–22.
[176] Valleau D, Quaile AT, Cui H, et al. Discovery of Ubiquitin Deamidases in the Pathogenic Arsenal of Legionella pneumophila. Cell Rep. 2018;23(2):568–583.
[177] Gan N, Guan H, Huang Y, et al. Legionella pneumophila regulates the activity of UBE 2N by deamidase-mediated deubiquitination. Embo J. 2020;39(4):e102806.
[178] Sauer J-D, Bachman MA, Swanson MS The phagosomal transporter A couples threonine acquisition to differentiation and replication of Legionella pneumophila in macrophages. Proceedings of the National Academy of Sciences 2005; 102(28):9924–9929.
[179] Eylert E, Herrmann V, Jules M, et al. Isotopologue Profiling of Legionella pneumophila ROLE OF SERINE AND GLUCOSE AS CARBON SUBSTRATES. J Biol Chem. 2010;285 (29):22232–22243.
[180] Price CTD, Richards AM, Dwingelo JEV, et al. Amoeba host-L egionella synchronization of amino acid auxotrophy and its role in bacterial adaptation and pathogenic evolution. Environ Microbiol. 2014;16 (2):350–358.
[181] Tesh MJ, Miller RD. Amino acid requirements for Legionella pneumophila growth. J Clin Microbiol. 1981;13(5):865–869.
[182] George JR, Pine L, Reeves MW, et al. Amino acid requirements of Legionella pneumophila. J Clin Microbiol. 1980;11(3):286–291.
[183] Price CTD, Al-Khodor S, Al-Quadan T, et al. Indispensable Role for the Eukaryotic-Like Ankyrin Domains of the Ankyrin B Effector of Legionella pneumophila within Macrophages and Amoebae▿. Infect Immun. 2010;78(5):2079–2088.
[184] Price CTD, Al-Quadan T, Santic M, et al. Host Proteasomal Degradation Generates Amino Acids Essential for Intracellular Bacterial Growth. Science. 2011;334(6062):1553–1557.
[185] Efeyan A, Zoncu R, Sabatini DM. Amino acids and mTORC1: from lysosomes to disease. Trends Mol Med. 2012;18(9):524–533.
[186] Mohr I, Sonenberg N. Host Translation at the Nexus of Infection and Immunity. Cell Host Microbe. 2012;12 (4):470–483.
[187] Isaac DT, Laguna RK, Valtz N, et al. MavN is a Legionella pneumophila vacuole-associated protein required for efficient iron acquisition during intracellular growth. Proc Natl Acad Sci. 2015;112(37): E520817.
[188] Portier E, Zheng H, Sahr T, et al. IroT/mavN, a new iron-regulated gene involved in L egionella pneumophila virulence against amoebae and macrophages. Environ Microbiol. 2015;17(4):1338–1350.
[189] Schaap P, Schilde C. Encystation: the most prevalent and underinvestigated differentiation pathway of eukaryotes. Microbiology. 2018;164(5):727–739.
[190] Bouyer S, Imbert C, Rodier M, et al. Long-term survival of Legionella pneumophila associated with Acanthamoeba castellanii vesicles. Environ Microbiol. 2007;9(5):1341–1344.
[191] Kilvington S, Price J. Survival of Legionella pneumophila within cysts of Acanthamoeba polyphaga following chlorine exposure. J Appl Microbiol. 1990;68:519–525.
[192] Price C, Jones S, Mihelcic M, et al. Paradoxical Pro-inflammatory Responses by Human Macrophages to an Amoebae Host-Adapted Legionella Effector. Cell Host Microbe. 2020;27(4):571–584.e7.
[193] Jones SC, Price CTD, Santic M, et al. Selective requirement of the shikimate pathway of legionella pneumophila for intravacuolar growth within human macrophages but not within Acanthamoeba. Infect Immun. 2015;83(6):2487–2495.
[194] Black MH, Osinski A, Park GJ, et al. A Legionella effector ADP-ribosyltransferase inactivates glutamate dehydrogenase. J Biol Chem. 2021;296:100301.
[195] Söderberg MA, Dao J, Starkenburg SR, et al. Importance of Type II Secretion for Survival of Legionella pneumophila in Tap Water and in Amoebae at Low Temperatures▿. Appl Environ Microb. 2008;74(17):5583–5588.
[196] Peabody CR, Chung YJ, Yen M-R, et al. Type II protein secretion and its relationship to bacterial type IV pili and archaeal flagella. Microbiology. 2003;149(Pt 11):3051–3072.
[197] Hospenthal MK, Costa TRD, Waksman G. A comprehensive guide to pilus biogenesis in Gram-negative bacteria. Nat Rev Microbiol. 2017;15 (6):365–379.
[198] Cianciotto NP. Type II secretion: a protein secretion system for all seasons. Trends Microbiol. 2005;13 (12):581–588.
[199] White RC, Cianciotto NP. Assessing the impact, genomics and evolution of type II secretion across a large, medically important genus: the Legionella type II secretion paradigm. Microb Genom. 2019;5(6). DOI:10.1099/mgen.0.000273
[200] Cianciotto NP, White RC, Maurelli AT. Expanding Role of Type II Secretion in Bacterial Pathogenesis and Beyond. Infect Immun. 2017;85(5):e00014–17.
[201] Ghosal D, Kim KW, Zheng H, et al. In vivo structure of the Legionella type II secretion system by electron cryotomography. Nat Microbiol. 2019;4 (12):2101–2108.
[202] Thomassin J, Moreno JS, Guilvout I, et al. The trans-envelope architecture and function of the type 2

secretion system: new insights raising new questions. Mol Microbiol. 2017;105(2):211–226.
[203] Rossier O, Starkenburg SR, Cianciotto NP. Legionella pneumophila Type II Protein Secretion Promotes Virulence in the A/J Mouse Model of Legionnaires' Disease Pneumonia. Infect Immun. 2004;72 (1):310–321.
[204] DebRoy S, Dao J, Söderberg M, et al. Legionella pneumophila type II secretome reveals unique exoproteins and a chitinase that promotes bacterial persistence in the lung. Proc Natl Acad Sci. 2006;103 (50):19146–19151.
[205] Mallama CA, McCoy-Simandle K, Cianciotto NP. The Type II Secretion System of Legionella pneumophila Dampens the MyD88 and Toll-Like Receptor 2 Signaling Pathway in Infected Human Macrophages. Infect Immun. 2017;85(4):e00897–16.
[206] White RC, Gunderson FF, Tyson JY, et al. Type II Secretion-Dependent Aminopeptidase LapA and Acyltransferase PlaC Are Redundant for Nutrient Acquisition during Legionella pneumophila Intracellular Infection of Amoebas. Mbio. 2018;9(2): e00528–18.
[207] Hales LM, Shuman HA. The Legionella pneumophila rpoS Gene Is Required for Growth within Acanthamoeba castellanii. J Bacteriol. 1999;181 (16):4879–4889.
[208] Rehman S, Grigoryeva LS, Richardson KH, et al. Structure and functional analysis of the Legionella pneumophila chitinase ChiA reveals a novel mechanism of metal-dependent mucin degradation. PLOS Pathog. 2020;16(5):e1008342.
[209] Rossier O, Dao J, Cianciotto NP. The Type II Secretion System of Legionella pneumophila Elaborates Two Aminopeptidases, as Well as a Metalloprotease That Contributes to Differential Infection among Protozoan Hosts▿. Appl Environ Microb. 2008;74 (3):753–761.
[210] Rossier O, Dao J, Cianciotto NP. A type II secreted RNase of Legionella pneumophila facilitates optimal intracellular infection of Hartmannella vermiformis. Microbiology+. 2009;155:882–890.
[211] Pearce MM, Cianciotto NP. Legionella pneumophila secretes an endoglucanase that belongs to the family-5 of glycosyl hydrolases and is dependent upon type II secretion. FEMS Microbiol Lett. 2009;300(2):256–264.
[212] Portlock TJ, Tyson JY, Dantu SC, et al. Structure, dynamics and cellular insight into novel substrates of the Legionella pneumophila Type II secretion system. Frontiers Mol Biosci. 2020;7:112.
[213] DebRoy S, Aragon V, Kurtz S, et al. Legionella pneumophila Mip, a Surface-Exposed Peptidylproline cis-trans-Isomerase, Promotes the Presence of Phospholipase C-Like Activity in Culture Supernatants. Infect Immun. 2006;74:5152–5160.
[214] Tyson JY, Pearce MM, Vargas P, et al. Multiple Legionella pneumophila Type II Secretion Substrates, Including a Novel Protein, Contribute to Differential Infection of the Amoebae Acanthamoeba castellanii, Hartmannella vermiformis, and Naegleria lovaniensis. Infect Immun. 2013;81:1399–1410.
[215] McCoy-Simandle K, Stewart CR, Dao J, et al. Legionella pneumophila Type II Secretion Dampens the Cytokine Response of Infected Macrophages and Epithelia. Infect Immun. 2011;79:1984–1997.
[216] Liles MR, Edelstein PH, Cianciotto NP. The prepilin peptidase is required for protein secretion by and the virulence of the intracellular pathogen Legionella pneumophila. Mol Microbiol. 1999;31:959–970.
[217] Rossier O, Cianciotto NP. Type II protein secretion is a subset of the PilD-dependent processes that facilitate intracellular infection byLegionella pneumophila. Infect Immun. 2001;69(4):2092–2098.
[218] Polesky AH, Ross JTD, Falkow S, et al. Identification of Legionella pneumophilaGenes Important for Infection of Amoebas by Signature-Tagged Mutagenesis. Infect Immun. 2001;69(2):977–987.
[219] Baskerville A, Conlan JW, Ashworth LA, et al. Pulmonary damage caused by a protease from Legionella pneumophila. Brit J Exp Pathol. 1986;67:527–536.
[220] Conlan JW, Baskerville A, Ashworth LAE. Separation of Legionella pneumophila Proteases and Purification of a Protease Which Produces Lesions Like Those of Legionnaires" Disease in Guinea Pig Lung. Microbiology+. 1986;132:1565–1574.
[221] Williams A, Baskerville A, Dowsett AB, et al. Immunocytochemical demonstration of the association betweenLegionella pneumophila, its tissue-destructive protease, and pulmonary lesions in experimental Legionnaires' disease. J Pathol. 1987;153(3):257–264.
[222] Truchan HK, Christman HD, White RC, et al. Type II Secretion Substrates of Legionella pneumophila Translocate Out of the Pathogen-Occupied Vacuole via a Semipermeable Membrane. Mbio. 2017;8(3): e00870–17.
[223] Massis LM, Zamboni DS. Innate Immunity to Legionella Pneumophila. Front Microbiol. 2011;2:109.
[224] Schuelein R, Ang DKY, Van Driel IR, et al. Immune Control of Legionella Infection: an in vivo Perspective. Front Microbiol. 2011;2:126.
[225] Medzhitov R, Preston-Hurlburt P, Kopp E, et al. MyD88 is an adaptor protein in the hToll/IL-1 receptor family signaling pathways. Mol Cell. 1998;2 (2):253–258.
[226] Kumagai Y, Takeuchi O, Akira S. Pathogen recognition by innate receptors. J Infect Chemother. 2008;14 (2):86–92.
[227] Archer KA, Roy CR. MyD88-dependent responses involving Toll-Like Receptor 2 are important for protection and clearance of Legionella pneumophila in a mouse model of Legionnaires' disease. Infect Immun. 2006;74(6):3325–3333.
[228] Hawn TR, Smith KD, Aderem A, et al. Myeloid Differentiation Primary Response Gene (88)– and Toll-Like Receptor 2–Deficient Mice Are Susceptible to Infection with Aerosolized Legionella pneumophila. J Infect Dis. 2006;193(12):1693–1702.
[229] Spörri R, Joller N, Albers U, et al. MyD88-dependent IFN-γ production by NK cells is key for control of Legionella pneumophila Infection. J Immunol. 2006;176(10):6162–6171.

[230] Archer KA, Alexopoulou L, Flavell RA, et al. Multiple MyD88-dependent responses contribute to pulmonary clearance of Legionella pneumophila. Cell Microbiol. 2009;11:21–36.
[231] Girard R, Pedron T, Uematsu S, et al. Lipopolysaccharides from Legionella and Rhizobium stimulate mouse bone marrow granulocytes via Toll-like receptor 2. J Cell Sci. 2003;116(2):293–302.
[232] Hawn TR, Berrington WR, Smith IA, et al. Altered Inflammatory Responses in TLR5-Deficient Mice Infected with Legionella pneumophila. J Immunol. 2007;179(10):6981–6987.
[233] Newton CA, Perkins I, Widen RH, et al. Role of Toll-Like Receptor 9 in Legionella pneumophila-Induced Interleukin-12 p40 production in Bone Marrow-Derived Dendritic Cells and Macrophages from Permissive and Nonpermissive Mice. Infect Immun. 2007;75(1):146–151.
[234] Lettinga KD, Florquin S, Speelman P, et al. Toll-Like Receptor 4 Is Not Involved in Host Defense against Pulmonary Legionella pneumophila Infection in a Mouse Model. J Infect Dis. 2002;186(4):570–573.
[235] Kawai T, Akira S. The roles of TLRs, RLRs and NLRs in pathogen recognition. Int Immunol. 2009;21 (4):317–337.
[236] Bhan U, Trujillo G, Lyn-Kew K, et al. Toll-Like Receptor 9 Regulates the Lung Macrophage Phenotype and Host Immunity in Murine Pneumonia Caused by Legionella pneumophila. Infect Immun. 2008;76(7):2895–2904.
[237] Shin S, Case CL, Archer KA, et al. Type IV secretion-dependent activation of host MAP Kinases induces an increased proinflammatory Cytokine response to Legionella pneumophila. PLoS Pathog. 2008;4(11):e1000220.
[238] Frutuoso MS, Hori JI, Pereira MSF, et al. The pattern recognition receptors Nod1 and Nod2 account for neutrophil recruitment to the lungs of mice infected with Legionella pneumophila. Microbes Infect. 2010;12 (11):819–827.
[239] Akira S, Uematsu S, Takeuchi O. Pathogen Recognition and Innate Immunity. Cell. 2006;124 (4):783–801.
[240] Monroe KM, McWhirter SM, Vance RE. Identification of Host Cytosolic Sensors and Bacterial Factors Regulating the Type I Interferon Response to Legionella pneumophila. PLoS Pathog. 2009;5(11): e1000665.
[241] Ang DKY, Oates CVL, Schuelein R, et al. Cutting Edge: pulmonary Legionella pneumophila Is Controlled by Plasmacytoid Dendritic Cells but Not Type I IFN. J Immunol. 2010;184(10):5429–5433.
[242] Von Moltke J, Ayres JS, Kofoed EM, et al. Recognition of bacteria by inflammasomes. Annu Rev Immunol. 2013;31(1):73–106.
[243] Molofsky AB, Byrne BG, Whitfield NN, et al. Cytosolic recognition of flagellin by mouse macrophages restricts Legionella pneumophila infection. J Exp Med. 2006;203 (4):1093–1104.
[244] Ren T, Zamboni DS, Roy CR, et al. Flagellin-Deficient Legionella Mutants Evade Caspase-1- and Naip5-Mediated Macrophage Immunity. PLoS Pathog. 2006;2(3):e18.
[245] Tenthorey JL, Haloupek N, López-Blanco JR, et al. The structural basis of flagellin detection by NAIP5: a strategy to limit pathogen immune evasion. Science. 2017;358(6365):888–893.
[246] Zamboni DS, Kobayashi KS, Kohlsdorf T, et al. The Birc1e cytosolic pattern-recognition receptor contributes to the detection and control of Legionella pneumophila infection. Nat Immunol. 2006;7(3):318–325.
[247] Mascarenhas DPA, Zamboni DS. Inflammasome biology taught by Legionella pneumophila. J Leukocyte Biol. 2017;101(4):841–849.
[248] Kovacs SB, Miao EA. Gasdermins: effectors of Pyroptosis. Trends Cell Biol. 2017;27(9):673–684.
[249] Gonçalves AV, Margolis SR, Quirino GFS, et al. Gasdermin-D and Caspase-7 are the key Caspase-1/8 substrates downstream of the NAIP5/NLRC4 inflammasome required for restriction of Legionella pneumophila. PLOS Pathog. 2019;15(6):e1007886.
[250] Akhter A, Gavrilin MA, Frantz L, et al. Caspase-7 Activation by the Nlrc4/Ipaf Inflammasome Restricts Legionella pneumophila Infection. PLoS Pathog. 2009;5 (4):e1000361.
[251] Brieland JK, Jackson C, Hurst S, et al. Immunomodulatory Role of Endogenous Interleukin-18 in Gamma Interferon-Mediated Resolution of Replicative Legionella pneumophila Lung Infection. Infect Immun. 2000;68(12):6567–6573.
[252] Casson CN, Doerner JL, Copenhaver AM, et al. Neutrophils and Ly6Chi monocytes collaborate in generating an optimal cytokine response that protects against pulmonary Legionella pneumophila infection. PLOS Pathog. 2017;13(4):e1006309.
[253] Brieland J, Freeman P, Kunkel R, et al. Replicative Legionella pneumophila lung infection in intratracheally inoculated A/J mice. A murine model of human Legionnaires' disease. Am J Pathology. 1994;145:1537–1546.
[254] Price JV, Russo D, Ji DX, et al. IRG1 and Inducible Nitric Oxide Synthase Act Redundantly with Other Interferon-Gamma-Induced Factors To Restrict Intracellular Replication of Legionella pneumophila. Mbio. 2019;10(6). DOI:10.1128/mBio.02629-19
[255] Naujoks J, Tabeling C, Dill BD, et al. IFNs Modify the Proteome of Legionella-Containing Vacuoles and Restrict Infection Via IRG1-Derived Itaconic Acid. PLoS Pathog. 2016;12:e1005408.
[256] Brieland JK, Remick DG, Freeman PT, et al. In vivo regulation of replicative Legionella pneumophila lung infection by endogenous tumor necrosis factor alpha and nitric oxide. Infect Immun. 1995;63(9):3253–3258.
[257] Fujita M, Ikegame S, Harada E, et al. TNF receptor 1 and 2 contribute in different ways to resistance to Legionella pneumophila-induced mortality in mice. Cytokine. 2008;44(2):298–303.
[258] Ziltener P, Reinheckel T, Oxenius OA. Neutrophil and Alveolar Macrophage-Mediated Innate Immune Control of Legionella pneumophila Lung Infection via TNF and ROS. PLOS Pathog. 2016;12(4):e1005591.

[259] Liu X, Boyer MA, Holmgren AM, et al. Legionella-Infected Macrophages Engage the Alveolar Epithelium to Metabolically Reprogram Myeloid Cells and Promote Antibacterial Inflammation. Cell Host Microbe. 2020;28(5):683–698.e6.
[260] Ngwaga T, Hydock AJ, Ganesan S, et al. Potentiation of Cytokine-mediated restriction of Legionella intracellular replication by a Dot/Icm-translocated effector. J Bacteriol. 2019;201(14). DOI:10.1128/JB.00755-18
[261] Susa M, Ticac B, Rukavina T, et al. Legionella pneumophila infection in intratracheally inoculated T cell-depleted or -nondepleted A/J mice. J Immunol Baltim Md. 1998;160:316–321.
[262] Trunk G, Oxenius A. Innate Instruction of CD4 + T Cell Immunity in Respiratory Bacterial Infection. J Immunol. 2012;189(2):616–628.
[263] Kusaka Y, Kajiwara C, Shimada S, et al. Potential Role of Gr-1+ CD8+ T Lymphocytes as a Source of Interferon-γ and M1/M2 Polarization during the Acute Phase of Murine Legionella pneumophila Pneumonia. J Innate Immun. 2018;10(4):1–11.
[264] Joller N, Spörri R, Hilbi H, et al. Induction and protective role of antibodies in Legionella pneumophila infection. Eur J Immunol. 2007;37:3414–3423.
[265] Weber SS, Joller N, Küntzel AB, et al. Identification of Protective B Cell Antigens of Legionella pneumophila. J Immunol. 2012;189(2):841–849.
[266] Weeratna R, Stamler DA, Edelstein PH, et al. Human and guinea pig immune responses to Legionella pneumophila protein antigens OmpS and Hsp60. Infect Immun. 1994;62(8):3454–3462.
[267] Buchholz U, Reber F, Lehfeld A-S, et al. Probable reinfection with Legionella pneumophila – a case report. Int J Hyg Environ Health. 2019;222(2):315–318.