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Strategies used by bacteria to grow in macrophages

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INTRODUCTION

Intracellular bacterial pathogens cause a wide range of diseases and significantly contribute to the morbidity and mortality associated with infectious diseases worldwide (1–16) (Table 1). These bacteria use several different strategies to replicate in host cells, and influence host processes such as membrane trafficking, signaling pathways, metabolism, cell death and survival (17–19). Broadly, intracellular bacteria colonize two topologically distinct regions of the host cell, and are divided into cytosolic and intravacuolar bacteria according to their intracellular lifestyle. However, most intracellular bacterial pathogens have unique intracellular life cycles with features strikingly different from one to the other (FIG. 1). It should also be noted that intravacuolar pathogens gain access to the host cytosol to some extent, and that cytosolic bacteria might spend an underestimated part on their intracellular life cycle within membrane-bound compartments (20–22).

Cytosolic bacteria escape from the endocytic pathway and replicate in the host cytosol. The host cytosol indeed constitutes an attractive replicative niche for intracellular bacteria because this subcellular compartment provides an environment rich in nutrients. The cytoplasm also offers the distinct advantage of being separated from the extracellular environment, and thereby, may constitute an ideal hideout where pathogens can evade extracellular immune surveillance and killing.

Alternatively, intracellular bacterial pathogens reside and replicate within the host endomembrane system, which is comprised of an intricate network of membrane-bound organelles and vesicular trafficking intermediates. The replication of intracellular bacteria in these vesicular compartments is accompanied by concomitant vacuolar membrane expansion, which is driven by adaptive strategies from pathogens. Even though the vacuolar intracellular lifestyle requires complex host-pathogen interactions in order to maintain the unique membrane-bound replication niche, bacterial pathogens benefit from this lifestyle that provides protection from the host cytosolic innate immune defenses.

Most intracellular bacteria replicate in myeloid cells, especially in macrophages (2, 23). Macrophages are plastic cells characterized by their phenotypic diversity, and are involved in

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pathogen detection, antigen presentation, cytokine production, tissue reparation, and, more notoriously, microbial killing (24). These cells indeed possess an extensive antimicrobial arsenal, and are endowed with the ability to ingest and destroy microorganisms (25). The observation that most pathogenic bacteria preferentially replicate in macrophages thus constitutes a paradox (2, 17).

This chapter focuses on bacterial pathogens that have the ability to replicate in macrophages, and aims to provide an overview of the strategies deployed by these bacteria to grow intracellularly. A brief description of the defense mechanisms used by macrophages against these intracellular bacteria is provided, and the current knowledge about the pathogenic strategies specifically used by cytosolic and intravacuolar bacteria are reviewed.

DEFENSE MECHANISMS AGAINST INTRACELLULAR BACTERIA

Detection of intracellular bacterial infection by macrophages

Macrophages express a wide range of receptors that trigger innate immune responses and antimicrobial defenses upon bacterial infections (23, 26). These sensors are referred as to pattern recognition receptors (PRRs). PRRs recognized conserved microbial molecules referred to as pathogen-associated molecular patterns (PAMPs) as well as damage-associated molecular patterns (DAMPs) released in response to stress and tissue damage. There are two main classes of PRRs: the membrane-bound receptors (e.g. the Toll-like receptors (TLR)) and the cytosolic receptors (e.g. the NOD-like receptors (NLRs)).

TLRs are localized on the plasma membrane (e.g. TLR4) or on endosomal membrane compartments (e.g. TLR9), and recognize PAMPs such as lipoproteins, lipopolysaccharide (LPS), flagellin, or nucleic acids (27). Upon ligand recognition, TLRs activate signaling pathways and regulate downstream cytokine expression by interacting with adaptor proteins such as MyD88 (myeloid differentiation primary-response protein 88) and TRIF (TIR-domain-containing adaptor protein inducing interferon-β) (26).

In the cytosol, the NLR proteins NOD1 (nucleotide-binding oligomerization domain-containing protein 1) and NOD2 are triggered by the presence of peptidoglycan fragments, and activate NF-κB (28). Interestingly, it was shown that the activation of the NOD1 signaling pathway by peptidoglycan fragments is dependent on the small Rho GTPase RAC1 and, more broadly, that the manipulation of small Rho GTPases by pathogens is a process that can be detected by the host in a NOD1-dependent manner (29). Recognition of PAMPs or DAMPs by other cytosolic NLRs, such as NLRC4 (NLR family, CARD domain-containing 4), NLRP1 (NLR family, PYRIN domain-containing 1) and NLRP3, leads to the assembly of cytosolic multiprotein oligomers termed inflammasomes. In turn, inflammasomes activate caspase-1, induce the extracellular release of IL-1β and IL-18 and trigger a type of inflammatory cell death called pyroptosis (30, 31). The PYHIN member protein AIM2 (absent in melanoma 2) also activates an inflammasome in response to cytosolic DNA (32, 33) released from intracellular bacteria (34–39).

Besides AIM2, there are other PRRs that detect the presence of foreign nucleic acids in the cytosol, and trigger distinct immune responses (26). STING (stimulator of IFN genes) is important in the cytosolic response to nucleic acids (40, 41) such as DNA (42) and cyclic dinucleotides (CDNs) (43), and triggers a type I IFN response upon bacterial infection. STING directly binds to cyclic dinucleotides (CDNs), but not to DNA, and is then both a direct sensor and an adaptor molecule (44). The host cytosolic DNA-sensor cGAMP synthase (cGAS) is able to synthesis cyclic GMP-AMP (cGAMP), which is an endogenous secondary messenger that binds and activates STING (45, 46). Accordingly, cGAS is involved in the secretion of IFN-β following the detection of bacterial DNA in the host cytosol (47–49). It is noteworthy that other cytosolic PRRs also contribute to nucleic acids sensing and induce a type I IFN response including DDX41 (50, 51) and IFI16 (52).

Besides antiviral immune response, the role of type I IFN during bacterial infection is enigmatic. Many intracellular bacteria induce type I IFNs during infection, but, rather than being protective for the host, type I IFNs enhance the host susceptibility to intracellular pathogens such as *Listeria monocytogenes* (53, 54), *Salmonella* Typhimurium (55), *Chlamydia trachomatis* (56, 57) and *Mycobacterium tuberculosis* (58–61). The mechanisms by which type I IFN signaling increases host susceptibility to bacterial infection is an active area of study and is not well defined.

The phagolysosomal pathway is the front line defense against intracellular bacterial pathogens

Macrophages are proficient in the internalization and destruction of bacteria, and in promoting innate immune responses (25). Following phagocytosis, a series of coordinated fusion and fission events with specific compartments of the endocytic pathway ultimately leads to the generation of a phagolysosome. The phagolysosome possesses potent microbicidal features, and has a lumen that constitutes a highly acidic, oxidative, and degradative environment.

The acidification of the phagosome lumen is dependent on proton pumping by the V-ATPase, which is required for the optimal activity of lysosomal hydrolytic enzymes and interferes with bacterial growth by impairing the metabolism of some bacteria (25, 62). The generation of ROS (reactive oxygen species) and RNS (reactive nitrogen species) by the NOX2 NADPH oxidase (63, 64) and the inducible NOS2 nitric oxide synthase (65), respectively, are also important antimicrobial mechanisms of the phagolysosomal pathway. Limitation in the availability of essential nutrients may also impact the growth of bacteria in the phagosome. For instance, NRAMP1 (natural resistance-associated macrophage protein 1) interferes with housekeeping and antioxidative functions of some bacteria by extruding Fe^{2+}, Zn^{2+} and Mn^{2+} from the phagosomal lumen (66). Molecules that directly compromise the integrity of bacteria such as defensins, cathelicidins, lyzozyme, endopeptidases, exopeptidases, as well as hydrolases targeting carbohydrates and lipids are also delivered to the phagosome lumen (25). The sum of these antimicrobial mechanisms defines the harsh environment that culminate in the phagolysosome, an organelle that intracellular bacteria need to avoid or adapt to in order to survive and proliferate.

Bacterial autophagy is a cell-autonomous defense mechanism

Macroautophagy (hereafter autophagy) involves the formation of a double-membrane vesicle (i.e., the autophagosome) that encloses and targets intracellular components for lysosomal degradation. During starvation, autophagy non-selectively targets parts of the cytoplasm in an attempt to maintain cellular homeostasis. Autophagy also selectively targets organelles (e.g. damaged mitochondria) and intracellular microbes. As such, autophagy contributes to innate immunity by restricting the intracellular growth of pathogens (67). Over 30 autophagy-related (ATG) proteins are involved in orchestrating autophagosome formation (68). However, some of these proteins have functions outside of the autophagy pathway and may affect host-pathogen interactions in an autophagy-independent manner (67, 69).

Initiation of autophagy is controlled by the availability of cellular nutrients and energy (70, 71), or by recognition of specific cargo (72). During autophagy, the production of phosphatidylinositol-3-phosphate (PI3P) is involved in the recruitment of ubiquitin-like conjugation systems that catalyze covalent binding of LC3 proteins to the isolation membrane (67, 72, 73). This isolation membrane is then recruited to and engulfs the cytosolic cargo. The resulting autophagosome matures into an autolysosome, in which the intraluminal content is digested (73). In the case of selective autophagy, cargo-specific receptors (74–76) and adaptor proteins containing LC3-interacting region (LIR) recruit the LC3-decorated autophagosome to the cargo (77). Ubiquitylation and ubiquitin-binding protein adaptors such as NBR1, NDP52, Optineurin and p62 are involved in the specific targeting of intracellular bacteria by autophagy (78–83).

Several alternative or non-canonical forms of autophagy do not require some of the core autophagy machinery components (84–86). Alternative autophagy mechanisms also play a role in cell-autonomous defense against intracellular microorganisms as exemplified for *Toxoplasma gondii* (87) and *Staphylococcus aureus* (88). In addition, under some circumstances, the autophagy machinery participates in a degradative process referred as to LC3-associated phagocytosis (LAP) (89–91). In contrast to canonical autophagy, LC3 is conjugated directly to the single-membrane phagosome and the ULK1 complex is dispensable during LAP (90, 91). In addition, the engagement of TLRs (89) and the NOX2-dependent production of ROS are involved in the formation of LC3-associated phagosomes (92). By promoting phagosome acidification and maturation, LAP restricts the intracellular growth of bacterial pathogens such as *S.* Typhimurium (92) and *Burkholderia pseudomallei* (93, 94).

Programmed cell death is a host defense mechanism that destroys the replication niche of intracellular bacteria

Programmed cell death plays an important role in innate immunity and is an effective way to eliminate infected cells and control infection. There are three major cell death pathways: apoptosis, programmed necrosis, and pyroptosis. These cell death pathways all contribute to the host defense against microbial infections (95, 96).

Apoptosis is a non-inflammatory form of cell death triggered by extrinsic (receptor-mediated) and intrinsic (mitochondria-mediated) pathways. Apoptosis is characterized by membrane blebbing, cell shrinkage, DNA fragmentation, increased mitochondrial permeability, and the eventual cell breakdown and release of apoptotic bodies (95, 96). Both external and internal stimuli trigger apoptosis through the activation of cysteine proteases termed caspases, which target intracellular components to induce cell death. Mitochondria are central in modulating host cell death and survival pathways, especially apoptosis (97). As such, mitochondria-dependent cell death pathways are important in host-bacteria interactions (95, 97).

Although necrosis was traditionally regarded as an accidental and uncontrolled cell death, it is now appreciated that some forms of necrosis, referred as to necroptosis, are genetically controlled (96, 98). Necrosis is a caspase-independent cell death pathway characterized by organelle damage, cell swelling, rupture of the plasma membrane, and release of the cellular content. Necrotic cell death is induced upon bacterial infection and physical damage, and is triggered by ROS, lysosomal permeabilization, calpain activation, and depletion of ATP. More specifically, necroptosis is initiated by the activation of RIP1 (receptor-interacting protein 1) and RIP3 kinases that activate downstream targets by phosphorylation and induce cell death, especially when caspase-8 is compromised (99, 100). Many proteins involved in the regulation of apoptosis also regulate necroptosis, highlighting the importance of crosstalk between these cell death pathways (101).

Pyroptosis is a non-apoptotic cell death pathway induced following inflammasome mediated caspase-1 activation. Pyroptosis is characterized by DNA fragmentation, loss of membrane integrity, and release of the cell content. In addition, pyroptosis is associated with the secretion of the pro-inflammatory cytokines IL-1β and IL-18 (30, 31). The induction of pyroptosis decreases the replication of intracellular bacteria within host cells and exposes them to the extracellular immune response (102).

Although some microbes block or delay host cell death to promote their intracellular replication at early times of infection, escape and dissemination may eventually require host cell lysis. In some cases, cell death pathways are co-opted by pathogens as a strategy of pathogenesis (103, 104). Furthermore, by inducing host cell death, bacterial pathogens can eliminate key immune cells and, consequently, evade host defenses (95).

INFECTION OF MACROPHAGES BY INTRAVACUOLAR BACTERIA

Remodeling host pathways through specialized secretion systems

During their intracellular life cycle, intravacuolar bacteria generally reside in a remodeled membrane-bound compartment. In order to successfully survive and replicate in this "sealed" environment, one of the main challenges encountered by intravacuolar pathogens is to exert actions beyond the vacuolar membrane. This is mainly accomplished using systems that secrete effectors that actively modify the host physiology to create an environment permissive to bacterial proliferation.

Protein secretion plays a central role in modulating the interactions of bacteria with their environment. This process is more complex for intravacuolar bacteria because secretion requires translocation across both the bacterial surface (plasma membrane and cell wall) and the host (plasma/vacuole) membrane. Four accessory secretion systems are known to play a role in establishing intravacuolar bacterial replication niches by delivering effector proteins into the host cytosol and by modulating a large variety of host cell functions, including vesicular trafficking and the host immune responses (Table 1).

The type III secretion system (T3SS), also called an injectisome, is found in Gram-negative bacteria that interact with both plant and animal hosts. The T3SS appears to have a common evolutionary origin with the flagellum (105). It is comprised of up to 25 proteins that form a series of rings that span the bacterial inner and outer membranes, and connect the bacterial and the host cytosol with a hollow filament (106). There are two T3SSs present in *Salmonella enterica*, each encoded in distinct genomic islands known as *Salmonella* pathogenicity island 1 (SPI-1) and SPI-2. SPI-1 confers the ability to invade nonphagocytic cells while SPI-2 allows *Salmonella* to survive within mammalian cells and spread to internal organs (105). In contrast, *Chlamydia* species possess one T3SS, which is active both at early and late phase of infection (107). Several putative *Chlamydia* T3SS substrates were identified using *Salmonella* (108), *Shigella* (109, 110), or *Yersinia* (111) as genetically tractable heterologous hosts.

Type IV secretion systems (T4SS) are extensively used by intravacuolar Gram-negative bacteria to colonize host cells. In comparison to other secretion systems, T4SS is unique in its ability to transport nucleic acid in addition to proteins into plant and animal cells, and is evolutionarily related to bacterial conjugation systems (112, 113). The T4SS can be divided into the canonical VirB (type IVA) and Dot/Icm (type IVB) secretion systems.

Both systems are multi-protein complexes that can span the bacterial surface and the host membrane. Essential roles for T4SS in pathogenesis are established in several important intravacuolar bacterial pathogens including *Brucella suis* (VirB) (114), *Legionella pneumophila* (Dot/Icm) (115, 116) and *Coxiella burnetii* (Dot/Icm) (117). In addition, other intravacuolar bacterial pathogens, including *Anaplasma phagocytophilum* and *Ehrlichia chaffeensis*, may also encode functional type IVA secretion systems that have a role in pathogenesis, as their putative T4SS genes were up-regulated during infection and several secreted effectors have been identified (118–120).

Type VI secretion systems (T6SS) are widely distributed in Gram-negative bacteria. The molecular mechanism by which T6SSs translocate effector proteins into target cells is similar to that of bacteriophage-like injection devices. Based on their function, T6SSs can be classified into two categories: eukaryotic cell targeting T6SS and competitor bacterial cell targeting T6SS (121, 122). For intravacuolar bacteria, the function of cell targeting is more relevant to their intracellular life cycle. *Salmonella* spp. harbour five phylogenetically distinct T6SSs, which are differentially distributed among serotypes (123). Two of them, SPI-6 and SPI-19, contribute to the pathogenesis of serotypes *S.* Typhimurium and *S.* Gallinarum in mouse and chicken macrophages, respectively (124, 125).

Mycobacteria are unique among intracellular bacteria due to the composition of their cell wall, which is heavily modified by lipids and termed the mycomembrane. Perhaps as a consequence of this special structure that forms an outer membrane bilayer (126), these species use a family of specialized secretion system named the type VII section systems (T7SS) (127). Putative T7SSs have been identified in some other Gram-positive organisms and are defined by the presence of two conserved elements, a membrane-bound ATPase EccC and a small secretion substrate EsxB (128, 129). *M. tuberculosis* encodes up to five T7SSs (ESX-1 to ESX-5), that do not functionally complement each other. The importance of these secretion systems is highlighted by the fact that loss of the ESX-1 T7SS in *M. tuberculosis* is the most important genetic difference between virulent strains and the attenuated vaccine strain BCG (Bacillus Calmette–Guérin) (130, 131). The detailed structure and function of T7SS are an active area of investigation (132). A current model suggests that the *M. tuberculosis*-containing vacuole is permeabilized by the T7SS effectors ESAT-6 and CFP-10, which allows bacterial products to access the cytosol (61, 127, 133).

Avoidance and adaptation to the phagolysosomal pathway

The majority of intravacuolar bacterial pathogens have mechanisms to avoid being trafficked to the terminal phagolysosome and generate specialized remodelled membrane-bounded compartments. Although the common function of these vacuolar compartments is to support the intracellular replication of these bacteria, the specific features of these compartments are determined in a pathogen-specific fashion. As such, intravacuolar bacteria exploit several strategies to generate their specialized vacuoles, leading to hybrid organelles that are biochemically and morphologically distinct from typical compartments found in uninfected cells (Figure 1).

Some intravacuolar bacteria actively evade the phagolysosomal pathway. Following entry, vacuoles containing *Brucellae*, *Chlamydiae,* and *Legionellae* rapidly diverge from the phagolysosomal pathway and fail to acquire late-phagosomal/lysosomal proteins (134). Both *Legionella*- and *Brucella*-containing vacuoles are ER-like membrane compartments and are associated with ribosomes (135, 136). Bacterial secretion systems are required for this divergent trafficking process (115, 116, 137). For example, *L. pneumophila* type IV effector proteins hijack the early secretory pathway and regulate vesicle traffic between the Golgi and the ER by targeting the host small GTPases Arf1 and Rab1, respectively (138–143). *C. trachomatis* also resides within a vacuole that is segregated from the phagolysosomal pathway. The *Chlamydia*-containing vacuole fuses with Golgi-derived vesicles and traffics to the Golgi area (144). The molecular mechanism of this process is still largely unknown, but the host GTPase Dynamin and Golgin-84 are involved in *Chlamydia*-mediated Golgi fragmentation, which is required for generating the *Chlamydia* replicative vacuoles (145, 146).

Mycobacteria and *Salmonella* exploit another strategy to avoid terminal phagolysosome formation and block phagosome maturation. Mycobacterial vacuoles are decorated with the early-endosomal marker Rab5 and exclude the late-endosomal GTPase Rab7, indicating an early arrest of the vacuole maturation (147). The type VII effector, EsxH, interacts with the host endosomal-sorting complex required for transport (ESCRT) and disrupts phagosome maturation (148). Moreover, the secreted *M. tuberculosis* protein tyrosine phosphatase (PtpA) prevents acidification of Mycobacterial vacuoles by binding and excluding the V-ATPase machinery from the phagosome membrane (149). Interestingly, *Legionella* also targets and inhibits the host V-ATPase using the type IV effector SidK (150). These suggest that targeting the vacuolar proton pump is a common strategy utilized by intravacuolar bacteria for the biogenesis of their specialized vacuoles.

In addition, the active exclusion of PI3P from the *M. tuberculosis*-containing vacuole (151, 152) represents another strategy of maturation arrest by removing the docking site for the Rab5 effectors EEA1 and Hrs (153–155). Conversely, *S.* Typhimurium increases PI3P levels on the vacuole to stimulate biogenesis of a unique compartment with properties of late endosomes, and characterized by transient acquisition of EEA1, gradual lumen acidification and acquisition of some lysosomal glycoproteins (156). However, unlike typical phagolysosomes, *Salmonella*-containing vacuoles are depleted of lysosomal degradation enzymes (157).

C. burnetii is a unique intravacuolar bacterium that does not follow this paradigm. *C. burnetii* resides in a vacuole that resembles a terminal phagolysosome and contains a variety of antimicrobial agents (158). However, instead of passively replicating in its vacuole, *C. burnetii* actively remodels it. For example, *Coxiella* is able to recruit the autophagy marker LC3 to its residing vacuole early after internalization and generate a specialized spacious vacuole in a T4SS-dependent manner (158–160).

Maintenance of vacuole integrity

The specialized vacuolar environment provides an ideal hideout from recognition by cytosolic innate immune sensors. Several studies suggest that damage to the bacterial vacuole membrane exposes the intravacuolar bacteria to the host cytosol, and compromises the ability of these bacteria to colonize host cells (161–166). In macrophages and epithelial cells, cytosolic galectins are able to bind β-galactoside-associated membrane remnants derived from ruptured vacuoles. This leads to the recruitment of autophagy adaptor proteins and targeting by the autophagy machinery (161, 162). In addition, the presence of bacterial components in the host cytosol may activate immune responses and trigger cell death (167). Therefore, maintenance of the integrity of bacteria-containing vacuoles is essential for intravacuolar bacterial pathogens. Accordingly, recent studies suggest that intravacuolar bacteria target multiple host components and signaling pathways to maintain the stability of their residing vacuoles (164, 165).

The formation of specialized bacteria-containing vacuoles largely depends on vesicle trafficking, which relies on the host cytoskeleton network. Thus, it is not surprising that intravacuolar bacterial pathogens exploit effector proteins to remodel the cytoskeleton network. Interestingly, in addition to their role as a physical and structural support for trafficking, the cytoskeleton and cytoskeletal motors also contribute to vacuole stability as exemplified by *Salmonella* and *Chlamydia*-containing vacuoles (168, 169). Interestingly, loss of vacuole integrity is observed during infection with a *Chlamydia* mutant lacking the bacterial protease CPAF, which digests intermediate filament proteins to form a filamentous structure around its containing vacuoles (170). Similarly, during *Salmonella* infection, the type III effector SifA regulates the interaction between cytoskeletal motors and the bacteria-containing vacuole (171, 172), and deletion of SifA leads to loss of vacuole integrity (165, 172, 173).

The formation of specialized bacteria-containing vacuoles is a highly ordered and tightly controlled process that requires the active modifications of the vacuole. The majority of these modifications involve the regulation of small GTPases by secreted bacterial effectors. Remarkably, in addition to their essential role in vacuole development, the timely acquisition of these small GTPases also contributes to vacuole stability (164). For example, one *Legionella* type IV effector, LidA, is involved in recruiting Rab1 and contributes to vacuole integrity (174). Comparatively, during *S.* Typhimurium infection, perturbation of GTPase activity by overexpressing dominant-positive Rab5 or dominant-negative Rab7 causes vacuole rupture and the release of bacteria into the cytosol (175).

A variety of bacterial pathogens affect the stability of the residing vacuoles by subverting their lipid composition for their own benefit. For example, certain phosphatidylinositol phosphates (PIPs) species can be either enriched or excluded from bacteria-containing vacuoles in order to regulate the binding of host signaling molecules and bacterial effectors (176). Nevertheless, instead of just providing a docking site for the recruitment of molecules, some vacuolar membrane lipids may play additional roles in vacuole stability. *C. trachomatis* recruits host sphingomyelin to its vacuole by incorporating lipid-droplets (111). Although the precise role of this molecule in vacuole stability is not yet clear, host sphingomyelin acquisition is absolutely required for vacuole expansion and prevents *Chlamydia* vacuole fragmentation (177, 178). *S.* Typhimurium and *L. pneumophila* are able to reduce cholesterol levels from their specialized vacuoles by secreting phospholipases SseJ and PlaA (179, 180), respectively. This process is thought to be beneficial to these intravacuolar bacteria by increasing membrane fluidity and inhibiting lipid rafts formation. However, exclusion of cholesterol from the vacuolar surface adversely affects its stability. *S.* Typhimurium and *L. pneumophila* use additional secreted effectors, SifA and SdhA, to counter this defect (164, 181, 182).

Autophagy is a double-edged sword for intravacuolar bacteria

Although vacuole integrity is critical for intravacuolar bacteria, interacting with the host cytosol requires some level of vacuole permeabilization. However, membrane-permeabilization leads to targeting by the selective autophagy pathway, as exemplified by *M. tuberculosis* (183). Exposure of *M. tuberculosis* DNA following vacuole permeabilization triggers ubiquitin-mediated autophagy through the cGAS/STING/TANK-binding kinase 1 (TBK1) pathway (49, 183). Similarly, *S.* Typhimurium accidently exposed to the cytosol are targeted by autophagy, as shown by the recruitment of LC3 and other ATG proteins to the bacteria. Adaptor proteins, such as NDP52 and optineurin, as a well as TBK1 have a role in recognizing ubiquitylated *Salmonella* in the cytosol (82, 184).

In order to survive and propagate in the face of host cell-autonomous defense mechanisms, intravacuolar bacteria such as *M. tuberculosis* and *L. pneumophila* antagonize the host autophagy machinery. The *M. tuberculosis* secreted redox regulator Eis inhibits autophagy by indirectly blocking JUN N-terminal kinase (JNK) activation (185). The suppression of the PI3-kinase VPS34 (151) and exclusion of PI3P from *M. tuberculosis*-containing vacuoles (152) could be another active strategy to avoid autophagy, as PI3P is required for autophagy initiation. In addition, a recent study suggests that *M. tuberculosis* inhibits autophagy flux in a T7SS-dependent manner, which suggests the existence of an uncharacterized autophagy modulator (186). Remarkably, the *L. pneumophila* effector RavZ directly and irreversibly uncouples ATG8 proteins attached to phosphatidylethanolamine on pre-autophagosomal structures (187).

In contrast, some bacteria, such as *C. burnetii* (159, 188, 189), *B. abortus* (190), and *A. phagocytophilum* (191, 192), subvert the autophagic pathway for the formation of their specialized vacuole. For example, pharmacological inhibition of autophagy flux arrests the biogenesis of *Coxiella* and *Anaplasma*-containing vacuoles, and impairs their growth in macrophages (189, 192). Both *C. burnetii* and *A. phagocytophilum* vacuoles are decorated with the autophagic protein beclin-1 (BECN1) (191, 193), a protein that forms a complex with VPS34 and is required for the initial steps of the autophagy pathway. Interestingly, *C. burnetii* infection also induces recruitment of the anti-apoptotic protein B cell lymphoma 2 (BCL-2) to its vacuole surface, and the interaction between BECN1 and BCL-2 inhibits host apoptosis (193). Therefore, some intravacuolar bacteria use the autophagy pathway to promote intracellular replication and to block apoptosis (194). It is also possible that the autophagy pathway plays an important role in providing nutrients to the specialized vacuoles of these pathogens (67, 194). One *C. burnetii* T4SS effector protein, Cig2, is involved in recruiting autophagosomes to *Coxiella* vacuoles (159). However, the detailed mechanism of how autophagosomes are recruited and incorporated into vacuoles still needs to be elucidated, although bacterial secretion systems and effectors are likely to play a pivotal role in these processes (67).

Manipulation of host cell death by intravacuolar bacteria

Even though intravacuolar bacteria reside in an environment that prevents host cytosolic sensing, the subversion of the host processes required to generate their specialized vacuoles can pose stresses to eukaryotic cells and trigger host cell death. Hence, intravacuolar bacterial pathogens have developed a myriad of strategies to modulate host cell death pathways (96, 195).

Mitochondria play a central role in modulating host cell death and survival. As such, many intravacuolar bacteria subvert mitochondria-dependent cell death pathways. *L. pneumophila* and *C. burnetii* secrete the type IV effector proteins SidF and AnkG into the host cytosol and target the proapoptotic factors BNIP3 and P32, respectively, thereby inhibiting mitochondria-mediated apoptotic signaling (196, 197). Similarly, *C. trachomatis* inhibits apoptosis by secreting the protease CPAF which degrades pro-apoptotic BH3-only proteins, including BIM, PUMA, and BAD (198, 199).

In addition to dampening pro-apoptotic pathways, activation of pro-survival pathways is another mechanism utilized by intravacuolar bacteria to prevent host cell death. The master regulators of the innate immune response NF-κB promotes cell survival, and the subversion of the NF-κB pathway also represents a common bacterial strategy to modulate host cell death. For example, the *L. pneumophila* effector LegK1 phosphorylates and inactivates the

NF-κB inhibitor IκBα and p100, thus enhancing the activation of the NF-κB pro-survival pathway (200). Some intravacuolar bacteria alter the activity of host kinases that are linked to NF-κB activation. One *S.* Typhimurium effector, AvrA, has acetyltransferase activity that targets MAPKKs and strongly inhibits the JNK signaling pathway (201), which helps dampen inflammatory and cell death responses. Moreover, although the molecular mechanisms are still largely unknown, Akt activation and enhancement of cell survival have been observed during *C. trachomatis* (202), *C. burnetii* (203), *and S.* Typhimurium (204) infections.

Although intravacuolar bacteria are separated from most cytosolic immune sensing mechanisms, their specialized secretion systems poke holes in host membranes and can be detected by the host cell. It has been shown that the basal body rod component of the T3SS SPI-1 apparatus of *S.* Typhimurium (PrgJ) is detected by NLRC4 and triggers inflammasome activation. However, SsaI, the equivalent basal body rod component of the T3SS SPI-2, is not detected by NLRC4. This constitutes an evasion strategy required for *S.* Typhimurium virulence (205).

INFECTION OF MACROPHAGES BY CYTOSOLIC BACTERIA

General strategies deployed by cytosolic bacteria to infect macrophages

During their intracellular life cycle, *B. pseudomallei*, *Francisella tularensis,* and *L. monocytogenes* escape from a phagosome, replicate in the macrophage cytosol, and manipulate host immune responses (18). In contrast to *F. tularensis*, both *B. pseudomallei* and *L. monocytogenes* exploit the host actin polymerization machinery to move within the cytosol and spread from cell-to-cell (206). The intracellular life cycles of *B. pseudomallei* and *F. tularensis* also have unique features such as the formation of multinucleated giant cells (MNGC) (207, 208) and the translocation to a membrane-bound compartment subsequent to replication in the host cytosol (22), respectively. It is noteworthy that other cytosolic bacterial pathogens such as *Mycobacterium marinum*, *Rickettsia* spp. and *Shigella flexneri* are also known to infect macrophages (18). However, macrophages are not the primary cells targeted during Rickettsial infections (209), and *Shigella* spp. induce macrophage cell death instead of replicating intracellularly as a strategy of pathogenesis (210–212).

Cytosolic bacteria secrete virulence factors within the host cells in order to establish infection. In *L. monocytogenes*, the virulence factors involved in phagosomal escape and cell-to-cell spread are well characterized, and are mostly encoded on the *Listeria* pathogenicity island-1 (LIPI-1) (213, 214). *L. monocytogenes* mainly relies on the canonical Sec translocation system to secrete virulence factors (215, 216) while the translocation of effectors in *B. pseudomallei* and *F. tularensis* depends on specialized secretion systems. In *B. pseudomallei*, a genetic locus with similarity to the *Salmonella* and *Shigella* type III secretion system (T3SS) is required for replication in host cells (217, 218). A type VI secretion system (T6SS) also contributes to the cell-to-cell spread ability and the virulence of *B. pseudomallei*, but the effectors secreted by this system are not well characterized (219, 220). Interestingly, it was recently shown that the T6SS effector VrgG5 mediates host cell fusion (221, 222), which is suggested to be required for intercellular spread (223). Similarly, several virulence determinants involved in the intracellular life cycle of *F. tularensis* are encoded on the *Francisella* pathogenicity island (FPI) (224, 225). A number of the genes within this island share sequence homology to the Type VI secretion system gene clusters found in *Vibrio cholerae* and *Pseudomonas aeruginosa* (226, 227), and the products of at least two of these genes are translocated in the host cytosol during infection (228).

Mechanisms used by cytosolic bacteria to escape phagosomes

The ability to escape from vacuoles is crucial to cytosolic pathogens. Following internalization, cytosolic bacteria escape from a phagosomal compartment in as early as 5 minutes, which may reflect a need to escape the vacuole before fusion with the lysosome (18). Cytosolic bacteria that exploit actin-based motility to spread from cell-to-cell also encounter a double-membrane vesicle referred as to the secondary vacuole. Escape from the secondary vacuole seems to process in a similar manner to the escape from the primary vacuole, at least for *L. monocytogenes* and *S. flexneri* (18).

L. monocytogenes avoids fusion with lysosomes, and escapes an acidified compartment with features of a late endosome (229, 230). More specifically, *L. monocytogenes* escapes the phagosome using the cholesterol-dependent cytolysin (CDC) Listeriolysin O (LLO) (231), which forms pores in vacuoles, blocks the trafficking of the bacteria within the endosomal pathway (229, 230), and ultimately promotes membrane disruption (232–234). LLO activity is compartmentalized by its acidic pH optimun, which prevents damage to the host cell (235). In addition, the reduction of LLO by GILT (γ-Interferon-inducible lysosomal thiol reductase) (236) and the CFTR (Cystic fibrosis transmembrane conductance regulator)-dependent increase in chloride concentration (237) potentiate the activity of LLO within the phagosome. The escape of *L. monocytogenes* from vacuoles is also facilitated by a phosphatidylinositol-specific phospholipase C (PlcA) and a broad-range phospholipase C (PlcB) (238). Whereas Goldfine and colleagues showed that PlcA promotes phagosomal escape by activating the host PKCβ (239, 240), the involvement of PlcB in this process is attributed to a wide-spectrum activity again phospholipids and to an ability to mediate membrane fusion (241, 242). The interplay between LLO and the phospholipases C (PLCs) is not completely understood, but it is possible that these Listerial factors synergistically destabilize the membrane to promote optimal escape from the phagosome. It has also been suggested that PLCs translocate into the host cytosol through LLO pores (243).

The detailed molecular mechanisms involved in phagosomal escape are mostly uncharacterized in most other cytosolic bacterial pathogens. *B. pseudomallei* mutants lacking components of the T3SS and, more specifically, the T3SS effector BopA have a defect in the escape from the phagosome (93, 217). In *F. tularensis*, the secretion system encoded by the FPI is required for phagosomal escape, but the specific set of virulence factors involved is not well characterized (225, 228, 244–246). Interestingly, the *Shigella* effectors IpaB and IpaC, like LLO, form a pore complex that binds cholesterol and inserts into cell membranes (247, 248), and *Rickettsia* spp. produces a haemolysin and phospholipases that may play a role in escape from vacuoles (249–252).

Sensing of the vacuole to cytosol transition by cytosolic bacteria

Cytosolic bacteria are likely to exploit distinct sets of virulence factors at different stages of their intracellular life cycle. The sensing of the environmental changes encountered throughout their passage within the host cell is likely to be critical for the tight regulation of their virulence factors. However, although it is established that cytosolic bacteria induce the expression of specific sets of genes during intracellular infections (253–256), less is known about their spatial and temporal regulation within the intracellular niche. The intricacies of virulence factors regulation is exemplified by the on/off expression of the *S. flexneri* T3SS apparatus during cell infection, which is specifically activated during bacterial entry (257).

The Crp-family member transcription factor PrfA up-regulates virulence factors expression once *L. monocytogenes* reaches the host cytosol (258, 259). The activity of PrfA integrates both environmental and bacterial signals, and is regulated by temperature (260), a bacterial auto-repressor (261), and the availability of specific nutrients (262–268). Reniere *et al.* (269) recently identified that bacterial and host derived glutathione allosterically binds and activates PrfA in the host cytosol. Given that enhanced PrfA activation is dispensable for vacuole escape (270), the authors suggested a two-step activation mechanism based on the oxidation-reduction states of PrfA thiols and glutathione in the vacuole (oxidizing/repressing environment) and in the cytosol (reducing/activating environment). Although it is suggested that *S. flexneri* also takes advantage of an intracellular signal to activate the MxiE transcription factor during infection (271), the environmental cues and the bacterial regulatory machinery used to regulate virulence factors expression are still largely unknown in other cytosolic bacteria.

Manipulation of host sensing pathways by cytosolic bacteria

Although cytosolic bacteria are protected from the extracellular immune responses and escape the microbicidal lysosomal environment, their detection by cytosolic PRRs activates defense mechanisms including autophagy, host cell death pathways, and secretion of cytokines. However, under some instances, it should be noted that the detection by the host cell and the subsequent consequences might be advantageous for the pathogen. For example, as discussed above, there is increasing evidence that some intracellular bacteria benefit from IFN-β signaling (272).

The notion that cytosolic pathogens employ active mechanisms to delay or redirect the immune response is attractive, but is supported by few studies. *L. monocytogenes* might dampen the host immune response by secreting the virulence factor InlC and by interfering with the activation of NF-κB during intracellular infection (273), but it is unclear whether this has an impact on virulence (274). It is also speculated that the active modulation of the immune response by *F. tularensis* contributes to pathogenesis, although the mechanisms remain poorly understood (224, 275). The ability of *L. monocytogenes* to grow in host cells without inducing inflammasomes activation is quite striking, and might involved regulatory mechanisms that limit flagellin expression during infection in order to avoid detection by NAIP5/NLRC4 (30, 276, 277). It is also plausible that *L. monocytogenes* avoids autolysis in host cells to minimize detection by the AIM2 inflammasome. Sauer et al. (37) demonstrated that while *L. monocytogenes* mutants with cell wall defects lyse within host cells and induce the AIM2 inflammasome, wild-type bacteria showed very little intracellular bacteriolysis and induced low levels of pyroptosis. Conversely, the activation of the AIM2/ASC inflammasome plays a pivotal role in the innate immune response to *F. tularensis*, and leads to the control of bacterial replication both in macrophages and *in vivo* (34–36, 278). However, the *F. tularensis* protein encoded by FTL-0325 delays inflammasome activation during infection (279), possibly by contributing to the structural integrity of the bacterial surface rather than by actively repressing host inflammasome activation (280). Interestingly, it was recently shown that the *S. flexneri* T3SS effector OspC3 inhibits the induction of a non-canonical inflammasome by directly targeting caspase-4, the human ortholog of mouse caspase-11 (281). Nevertheless, it is not known whether this mechanism is active in myeloid cells or if other cytosolic pathogens use a similar strategy to inhibit inflammasome activation.

Interaction of cytosolic bacteria with the autophagy pathway

Infection of host cells by cytosolic pathogens such as *L. monocytogenes* and *S. flexneri* leads to induction of autophagy (282–284), and not surprisingly, mechanisms of autophagy evasion are described for most cytosolic bacterial pathogens. *L. monocytogenes* uses ActA and InlK to recruit host proteins (Arp2/3 and Ena/VASP, and the Major Vault Protein) to the bacterial surface and interfere with autophagic recognition (78, 285). *L. monocytogenes* also causes stalling of pre-autophagosomal structures by reducing the autophagic flux and PI3P levels in a PLCs-dependent manner (283). Accordingly, it was recently demonstrated that the inactivation of both ActA and PlcA drastically impairs the ability of *L. monocytogenes* to avoid autophagy and to grow within macrophages (286). *S. flexneri* also interferes with autophagic recognition by secreting IcsB, which competes with ATG5 for binding to the bacterial protein VirG/IcsA (287). Interestingly, the *F. tularensis* polysaccharidic O-antigen contributes to autophagy evasion by preventing ubiquitylation and the recruitment of the autophagy adaptor p62 (288). Overall, these studies clearly demonstrate that interference with autophagy is common among cytosolic bacteria.

Instead of being targeted by macroautophagy, *B. pseudomallei* is targeted by LAP (93). *B. pseudomallei* mutants lacking the T3SS effector BopA showed a defect in escaping single-membrane vacuoles and an increased colocalization with LC3 (93). Although the *B. pseudomallei* proteins BimA and BopA have high homology with the *S. flexneri* proteins IcsA and IcsB, it was suggested that they interfere with autophagy through different mechanisms (289, 290). However, recent data suggests that IcsB not only interferes with autophagic recognition of the bacterial surface, but also inhibits LAP or LC3 recruitment to vacuolar remnants early during *S. flexneri* infection (291). In addition, *L. monocytogenes* is also targeted by LAP early during macrophage infection (292). Overall, these studies suggest that the autophagy machinery can target cytosolic bacteria at the entry stage of their intracellular life cycle.

In most cases, it is assumed that the activity of the autophagy pathway is detrimental for cytosolic bacteria although it might not always be the case. For example, a proportion of *F. tularensis* re-enters a membrane-bound compartment with autolysosomal features subsequent to replication in the cytosol (22), but it is not clear whether this represents a mechanism of dissemination or a host cellular defense mechanism. Evidence also suggests that autophagy provides *F. tularensis* with the nutrients required for intracellular replication (293). It is thus plausible that cytosolic bacteria benefit from an increase in nutrient availability resulting from autophagy activation.

Manipulation of host cell death pathways by cytosolic bacteria

Although cytosolic bacteria may protect the intracellular replication niche by suppressing host cell death pathways, death of the infected host cell is inevitable because the host cell has multilayered mechanisms to induce cell death pathways in response to bacterial infections. For example, infection of macrophages with *B. pseudomallei* induces an early NLRC4-dependent pyroptosis, and late caspase-1-dependent and -independent cell death pathways (294). More specifically, induction of apoptosis was observed in caspase-1/11-deficicent macrophages infected with *B. pseudomallei* (294). Similarly, caspase-1-deficient macrophages infected with *F. tularensis* undergo AIM2/ASC-dependent caspase-8-mediated apoptosis (295). As already mentioned, *F. tularensis* delays inflammasome activation during the early stages of infection (279), but also uses TolC-secreted effectors to delay the activation of the intrinsic apoptotic pathway in order to preserve the host cell (296). This suggests that cytosolic bacteria have to interact with several host pathways in order to inhibit, or at least delay, cell death. Nevertheless, our understanding of the mechanisms used by cytosolic bacteria to inhibit host cell death pathways remains mostly superficial.

Another strategy used by cytosolic bacteria to maintain their replication niche is to induce host pro-survival pathways. For example, *S. flexneri* and *R. rickettsii* activate pro-survival pathways to dampen cell death signals in nonphagocytic cells (297–299), but it is unknown whether these pathways also promote macrophage survival during infection. Cytosolic bacteria such as *L. monocytogenes* damage host membranes during their intracellular life cycle. However, in the case of *L. monocytogenes*, LLO has several mechanisms to minimize damage to the host cell membranes (231). In addition, adaptive mechanisms are likely to be deployed by the host cell to withstand and repair the lesions, and thus promote survival (300). The induction of autophagy is a common host response to several bacterial toxins, and might constitute a host protection or detoxification mechanism (300, 301). Interestingly, a tight relationship exists between autophagy and apoptosis, and it is possible that bacterial pathogens target host components shared by both pathways to promote survival of the host cell (301).

Some cytosolic bacteria may benefit from inducing a specific host cell death pathway during infection. For example, it was suggested that the accumulation of apoptotic cell debris leads to alternative macrophage activation and decreased bacterial clearance during *F. tularensis* infection (302). It thus seems conceivable that *F. tularensis* prevents early cell death induction to allow intracellular bacterial replication (279, 296), but then specifically induces apoptosis to promote an anti-inflammatory immune response (302, 303). Interestingly, *B. pseudomallei* deliver a cycle-inhibiting factor homolog (CHBP) that triggers macrophage-specific apoptosis (304), but whether this leads to an anti-inflammatory immune response remains to be determined. Although macrophages infected with *L. monocytogenes* do not undergo apoptotic cell death (305), *L. monocytogenes* exploits the process of apoptotic cell death clearance by macrophages (i.e., efferocytosis) to facilitate cell-to-cell spread and dissemination (306). Overall, these studies suggest that cytosolic bacteria manipulate the host apoptotic pathway to their own advantage during intracellular infection.

CONCLUDING REMARKS

Macrophages, as major immune sentinel cells, play an essential role in sensing and destroying invading microorganisms, and release cytokines that shape the immune response of other cells. In comparison to extracellular bacterial pathogens, which are fully expose to all immune cells and components, intracellular bacteria hide and replicate within host cells. Paradoxically, many intracellular bacterial pathogens preferentially replicate within macrophages. Following long-term evolutionary selection, these highly adapted intracellular bacteria have selected a specific replication niche inside these host cells and have evolved sophisticated mechanisms that facilitate their intracellular replication (Fig. 1). The study of these bacterial pathogens has led to numerous mechanistic insights on basic host processes including endocytosis, vesicle trafficking and innate immune responses.

As reviewed here, intracellular bacteria have to deal with a variety of host defense mechanisms after being phagocytosed by macrophages (Table 2). In order to facilitate a comparative analysis of bacterial counter strategies, we exemplify some pathogen-host interactions that illustrate how these organisms use their effector arsenal to face the challenges encountered during an intracellular infection (Table 3). A delicate balance exists between the ability of the host cell to control invading microbes and the ability of pathogens to hide from or subvert these defenses. Due to the length of this review, we are not able to cover all aspects of host-pathogen interaction, but we highlight some of the most important and current topics in the field. Importantly, several interesting questions are still not fully understood. For example, both *M. tuberculosis* and *M. marinum* encode ESX-1 type VII secretion systems, however, they replicate in a vacuolar compartment and in the cytosol, respectively. It is puzzling why these genetically related bacteria that share common secretion systems replicate in distinct intracellular niches. Also, the function of type I interferon induction during intracellular bacterial infection is still elusive. Elucidating the molecular mechanisms underlying pathogen subversion strategies and how they specifically affect innate immune responses is critical to a comprehensive understanding of bacterial pathogenesis and the host response. In addition, the study of host-intracellular bacteria interactions may provide clues for advancing the development of novel therapeutics against pathogens and inflammatory diseases.

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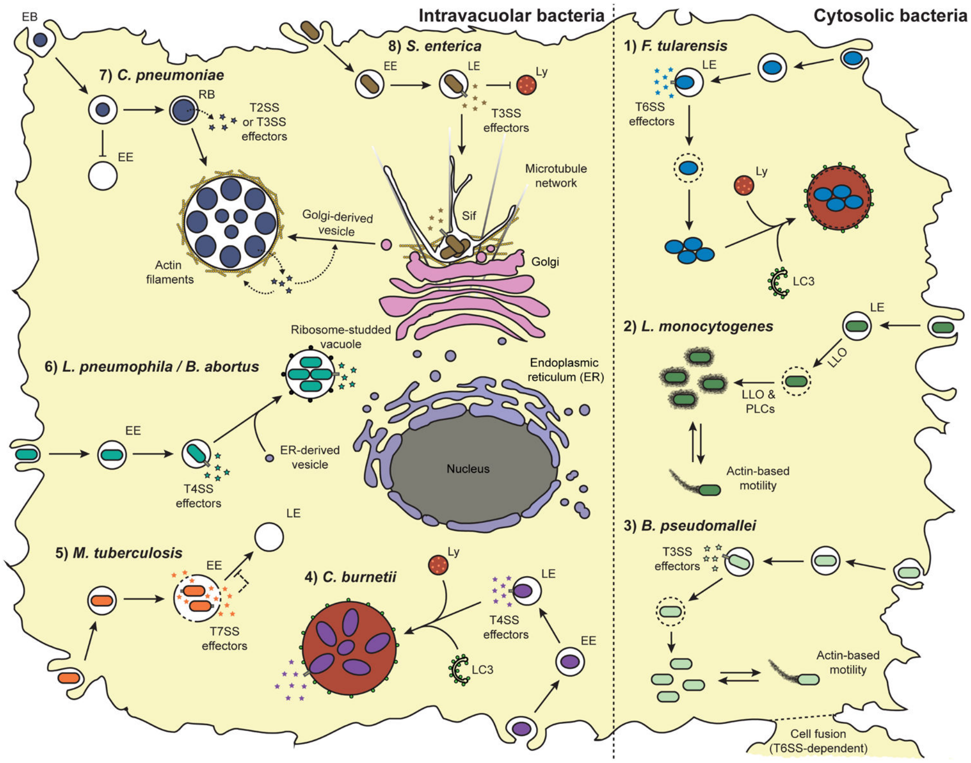

Figure 1.

Lifestyles of intracellular bacterial pathogens. (1) *F. tularensis* escapes a late endosome (LE)-like vacuole in a T6SS-dependent manner. Following replication in the cytosol, *F. tularensis* may retranslocate to a membrane-bound compartment resembling an autolysosome. (2) *L. monocytogenes* escapes the phagolysosomal pathway using the T2SS (Sec) effectors LLO and PLCs. *L. monocytogenes* replicates rapidly in the cytosol and hijacks the host actin polymerization machinery to move within and between cells. (3) *B. pseudomallei* escapes into the cytosol in a T3SS-dependent manner. *B. pseudomallei* performs actin-based motility and promotes host cell fusion. (4) *C. burnetii* is adapted to the phagolysosomal pathway and resides in a spacious phagolysosomal-like compartment. The Dot/Icm system (T4SS) is required for recruiting the autophagosomal marker LC3 and for vacuole biogenesis. (5) *M. tuberculosis* arrests phagosome maturation at the early endosome (EE) stage in a T7SS-dependent manner. (6) *L. pneumophila* and *B. abortus* segregate from the endocytic route at the EE stage, recruit endoplasmic reticulum (ER)-derived vesicles, and form ribosome-studded specialized vacuoles in a T4SS-dependent manner. (7) *C. pneumoniae* segregates from the endocytic route and form a unique inclusion vacuole by recruiting Golgi-derived vesicles. *C. pneumoniae* effectors promote Golgi fragmentation and generate actin filaments around the inclusion. *Chlamydia* is found in two different forms: the non-replicating infectious elementary body (EB) and the intracytoplasmic replicative reticulate body (RB). T2SS and T3SS effectors are thought to be involved in the intracellular life cycle of *Chlamydia*. (8) *S. enterica* replicates in a late endosome (LE)-like compartment that excludes lysosomal degradation enzymes. The *S. enterica* containing vacuole migrates to the microtubule-organizing centre (MTOC) and forms *Salmonella*-induced filaments (Sif) along microtubules in a T3SS-dependent manner.

Table 1

Characteristics and diseases associated with intracellular pathogens that infect human myeloid cells

Pathogen	Targeted myeloid cells	Secretion system[a]	Replication niche	Disease	Reference
Anaplasma phagocytophilum	Neutrophil	T4SS	Vacuole	Human granulocytic anaplasmosis	(3)
Brucella spp.[b]	Macrophage	T4SS	Vacuole	Brucellosis	(4)
Burkholderia pseudomallei	Macrophage	T3SS & T6SS	Cytosol	Melioidosis	(5)
Chlamydia pneumoniae	Macrophage	T3SS	Vacuole	Pneumonia and bronchitis	(6)
Citrobacter koseri	Macrophage	T3SS (putative)	Vacuole	Meningitis	(7)
Coxiella burnetii	Macrophage	T4SS	Vacuole	Q fever	(8)
Ehrlichia chaffeensis	Macrophage	T4SS	Vacuole	Ehrlichiosis	(9)
Francisella tularensis	Macrophage	T6SS	Cytosol	Tularemia	(10)
Legionella pneumophila	Macrophage	T4SS	Vacuole	Legionaire's disease	(11)
Listeria monocytogenes	Macrophage	T2SS (Sec)	Cytosol	Listeriosis	(12)
Mycobacterium tuberculosis	Macrophage	T7SS	Vacuole	Tuberculosis	(13)
Rhodococcus equi	Macrophage	T2SS (Sec)	Vacuole	Pneumonia	(14)
Rickettsia rickettsii	Macrophage	T4SS (putative)	Cytosol	Rocky Mountain spotted fever	(15)
Salmonella enterica	Macrophage	T3SS & T6SS	Vacuole	Salmonellosis	(16)

[a] Bacterial secretion systems involved in pathogenesis.

[b] *Brucella abortus, B. canis, B. suis,* and *B. melitensis*.

Table 2

General strategies used by intravacuolar and cytosolic bacteria to deal with challenges encountered within host cells

Challenge	Strategies utilized by intracellular bacteria	
	Intravacuolar	**Cytosolic**
Phagolysosomal pathway	Avoidance, blockage and adaptation	Escape into the cytosol
Access to nutrients	Hijack host vesicle trafficking to promote vacuole biogenesis and nutrient acquisition	Direct access to cytosolic nutrients
Microenvironment	Intravacuolar bacteria create and enlarge their replication space using a variety of effectors	The cytosol constitutes a spacious and favorable environments
Innate recognition	The vacuolar compartment provides a hideout from cytosolic innate sensing[a]	Cytosolic bacteria are directly exposed to the cytosolic sensing machinery, and need to down- regulate PAMP expression to delay recognition
Autophagy	Maintenance of vacuole integrity Inhibition of autophagy initiation Inhibition of autophagy flux	Inhibition of autophagy initiation Inhibition of autophagy flux Prevention of autophagic recognition
Host cell death	Dampening of pro-apoptotic pathways Activation of pro-survival pathways	Interference with inflammasone activation Dampening of pro-apoptotic pathways Activation of pro-survival pathways

[a]Some components of intravacuolar bacterial pathogens can be recognized by host cytosolic sensing mechanisms due to damage to the pathogen-containing vacuoles or effector translocation by specialized secretion systems. For example, DNA from *M. tuberculosis*, PrgJ from *S. enterica* and the flagellum of *L. pneumophila* are recognized by the host cytosolic sensing machinery.

Table 3

Examples of factors used by intracellular bacteria to counteract host defense mechanisms

Subverted host defense mechanism	Strategies	Pathogen	Example of bacterial factors
Immune detection	Actin-based cell-to-cell spread	*B. pseudomallei*	BimA
		L. monocytogenes	ActA
	Modification of the immune response	*F. tularensis*	T6SS effectors
		L. monocytogenes	InlC
	Low flagellin expression	*L. monocytogenes*	MogR
	Maintenance of vacuole integrity	*C. pneumoniae*	CPAF
		L. pneumophila	LidA, PlaA, SdhA
		S. Typhimurium	SseJ, SifA
Phagolysosomal pathway	Blockage of phagosome maturation	*L. pneumophila*	SidK
		M. tuberculosis	EsxH, PtpA
		S. Typhimurium	Unknown
	Escape from the vacuole	*B. pseudomallei*	BopA
		F. tularensis	T6SS effector(s)
		L. monocytogenes	LLO, PLCs
	Evasion of the endocytic pathway	*Brucella* spp.	VirB(T4SS) effectors
		C. trachomatis	Unknown
		L. pneumophila	Dot/Icm(T4SS) effectors
	Phagosome permeabilization	*M. tuberculosis*	ESAT-6, CFP-10
	Survival in a lysosome-like structure	*C. burnetii*	Dot/Icm(T4SS) effectors
Autophagy	Escape from LAP	*B. pseudomallei*	BopA
	Formation of a replication niche	*A. phagocytophilum*	Ats-1
		Brucella spp.	VirB(T4SS) effectors
		C. burnetii	Dot/Icm(T4SS) effectors
	Interference with recognition	*F. tularensis*	O-antigen
		L. monocytogenes	ActA, InlK
	Inhibition of autophagy initiation/flux	*L. monocytogenes*	PLCs
		L. pneumophila	RavZ
		M. tuberculosis	Eis
	Nutrients acquisition	*F. tularensis*	Unknown
Cell death	Inhibition/delay of apoptosis	*C. burnetii*	AnkG
		C. trachomatis	CPAF
		F. tularensis	TolC-dependent
		L. pneumophila	SidF
		L. pneumophila	LegK1
		S. Typhimurium	AvrA
	Induction of apoptosis	*B. pseudomallei*	CHBP
	Hijacking of efferocytosis	*L. monocytogenes*	LLO, ActA
	Evasion of inflammasome	S. Typhimurium	SsaI