Bacterial manipulation of innate immunity to promote infection

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Abstract | The mammalian innate immune response provides a barrier against invading pathogens. Innate immune mechanisms are used by the host to respond to a range of bacterial pathogens in an acute and conserved fashion. Host cells express pattern recognition receptors that sense pathogen-associated molecular patterns. After detection, an arsenal of antimicrobial mechanisms is deployed to kill bacteria in infected cells. Innate immunity also stimulates antigen-specific responses mediated by the adaptive immune system. In response, pathogens manipulate host defence mechanisms to survive and eventually replicate. This Review focuses on the control of host innate immune responses by pathogenic intracellular bacteria.

Pattern recognition receptor
A receptor of the innate immune system that recognizes and responds to conserved microorganism-associated molecular patterns.

NOD-like receptor
An intracellular sensor that detects cytosolic microbial components, cell injury and 'danger' signals (such as ATP and toxins).

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Host cells express a range of receptors that act as microbial sensors. These receptors sense microorganisms and transduce signals that activate immune responses. Host cells use several strategies to recognize specific pathogen-associated molecular patterns (PAMPs) and to alert the immune system. The aim is to activate innate and adaptive immune responses, eliminate the encountered pathogens and establish long-lasting protective immunity against them. PAMPs are sensed by pattern recognition receptors (PRRs), the best-characterized of which are Toll-like receptors (TLRs). There are 10 TLRs in humans, 13 in mice[1] and 222 in sea urchins[2], which have evolved to recognize PAMPs from fungi, bacteria, viruses and parasites. TLR3, TLR7, TLR8 and TLR9 detect microbial nucleic acids, whereas TLR2, TLR4 and TLR5 recognize lipoproteins, lipopolysaccharide (LPS) and flagellin, respectively. The picture of TLR activation is complex, as TLR adaptor molecules display pleiotropic functions. TLR adaptor proteins include myeloid differentiation primary response protein 88 (MYD88), TIR-domain-containing adaptor protein (TIRAP), TRIF-related adaptor molecule (TRAM) and TIR-domain-containing adaptor protein inducing *IFNb* (TRIF), which modulate innate immune responses such as cytokine signalling and secretion, phagosome maturation, NOD-like receptor (NLR) activation, inflammasome activation and autophagy. In addition, TLR accessory molecules at the cell surface (such as MD2 (also known as LY96) and RP105 (also known as CD180)), in the endoplasmic reticulum (ER; such as UNC93B, PRAT4A and gp96) and accessory molecules that directly interact with TLR ligands (such as CD14, CD36, high-mobility group box 1 protein (HMGB1) and LL37) have roles in tuning the innate immune response against pathogens. These molecules can act as either positive regulators (for example, MD2, HMGB1 and RP105 in B cells) or negative regulators (for example, RP105 in dendritic cells (DCs) and macrophages)[3].

Another family of PRRs are the NLRs, which are C-type lectin receptors that detect bacterial and viral molecules in the cytoplasm, leading to the secretion of interleukin-1β (IL-1β). Members of this family include NOD1 and NOD2, NACHT-, LRR- and pyrin-domain-containing protein 1 (NALP1)–NALP14, NLR family CARD domain-containing protein 4 (NLRC4; also known as IPAF), neuronal apoptosis inhibitory protein (NAIP; also known as BIRC1), class II transactivator, NOD3, NOD9 and NOD27. NOD1 and NOD2 detect peptidoglycan-derived meso-diaminopimelic acid and muramyl dipeptide (MDP), respectively. NOD1 has been shown to recognize *Shigella flexneri*, enteropathogenic *Escherichia coli*, *Helicobacter pylori*, *Pseudomonas aeruginosa, Chlamydia spp.*, *Campylobacter jejuni* and *Haemophilus influenza*. NOD2 is specialized in recognizing *Salmonella enterica*, *Listeria monocytogenes* and *Streptococcus pneumoniae*. NODs trigger the secretion of inflammatory cytokines. In addition, NOD action synergizes with TLR activation.

After internalization by the host cell, several bacterial pathogens reside in intracellular membrane-bound compartments. Bacteria-containing vacuoles provide an enclosed space in which the host cell can direct high local concentrations of reactive oxygen species (ROS), reactive nitrogen intermediates (RNI) and antimicrobial peptides to eliminate intracellular pathogens. Bacteria that escape

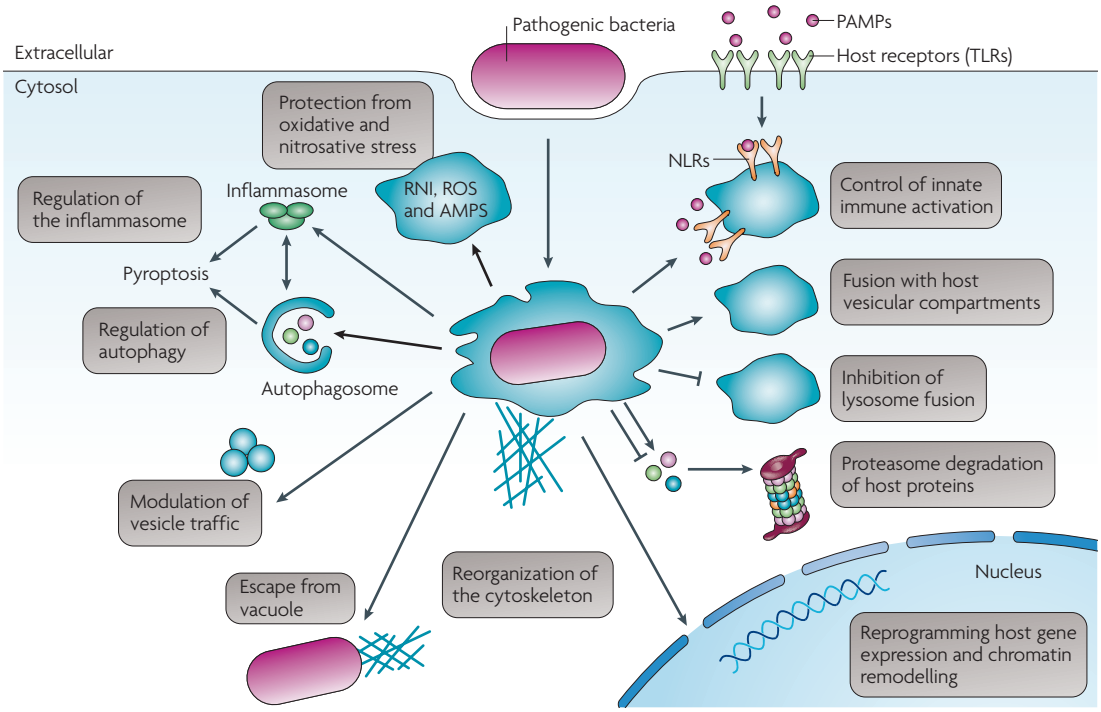

Figure 1 | **Manipulation of host innate immunity by intracellular pathogenic bacteria.** Once pathogens are recognized by extracellular or endosomal receptors, the host cell uses bacterial compartmentalization, oxidative and nutrient stress, antimicrobial peptides, lysosome-mediate degradation, autophagy, inflammasome activation and pyroptosis to kill the pathogen. Some intracellular pathogens can control the signalling pathways activated by host receptors, interact with endocytic pathway, escape from the phagosome, inhibit fusion with lysosomes, manipulate vesicular trafficking and avoid autophagosome degradation and inflammasome activation. AMPS, antimicrobial peptides; NLRs, Nod-like receptors; RNI, reactive nitrogen intermediates; ROS, reactive oxygen species; PAMPs, pathogen-associated molecular patterns; TLRs, Toll-like receptors.

such membrane-bound compartments can encounter another mechanism of resistance such as ubiquitylation and proteasome degradation or autophagy[4] (FIG. 1). Furthermore, channel-forming toxins, virulence factors and other PAMPs derived from pathogens can trigger the activation of the inflammasome. The inflammasome is involved in the activation of caspase 1, which in turn promotes the maturation of several ILs, the recruitment of inflammatory cells to sites of infection and the activation of a specialized host cell death pathway known as pyroptosis (FIG. 1).

However, many pathogens have learnt how to subvert host defence mechanisms. In this Review, we describe how intracellular bacterial pathogens can manipulate host cell receptors that mediate internalization and signalling, membrane trafficking, autophagy and inflammasome activation to their benefit and how they counterattack oxidative burst.

Host cell-invading bacterial pathogens

At the single cell level, interactions with receptors directly influence the intracellular fate of bacterial pathogens (FIG. 2). For example, mycobacteria interact with a large range of receptors such as macrophage mannose receptor (MMR), DC-specific ICAM3-grabbing non-integrin (DC-SIGN)[5,6], dectin 1 (also known as CLEC7A), TLR2 (REF. 7), mannose-binding lectin, surfactant-associated protein A (SPA) and SPD[5,6]. In addition, class A scavenger receptors and CD14, as well as complement receptor 1 (CR1) and CR3 are involved in the attachment and uptake of *Mycobacterium tuberculosis* by professional phagocytes[5,6,8,9]. Although binding of pathogens to most receptors induces a strong pro-inflammatory response, DC-SIGN and MMR have also been reported to induce the secretion of IL-10, which dampens pro-inflammatory responses mediated by T helper 1 cells. This is advantageous for *M. tuberculosis*, as it has been shown that the presence of IL-10 favours *M. tuberculosis* replication[8,10].

In the case of *L. monocytogenes*, the invasion protein internalin A (InlA) is recognized by the human adhesion molecule E-cadherin. A second invasion protein, InlB, targets the hepatocyte growth factor receptor MET and participates with InlA during host cell invasion by promoting adhesion and internalization through a zipper mechanism[11]. *Bartonella bacilliformis*, the agent of Carrion's disease, interacts with multiple surface-exposed membrane proteins of human erythrocytes, including glycophorin A and glycophorin B. As erythrocytes do not have an active cytoskeleton, which could be subverted to promote invasion, *B. bacilliformis* and *Bartonella henselae* enter these cells by an active process that depends on deformin, which is responsible for the formation of deep invaginations in the erythrocyte membrane[12].

Salmonella spp. and *Shigella* spp. use a trigger mechanism for invading non-professional phagocytes, such as epithelial cells. Both *Salmonella* spp.

Inflammasome
A molecular complex of several proteins that on assembly cleaves pro-interleukin-1 (IL-1), thereby producing active IL-1. Based on the pyrin-domain containing Nod-like receptors (NLR) NALP1 and NALP3, which require ASC to bridge the NLRs to pro-caspase 1.

Muramyl dipeptide
A peptidoglycan constituent of both Gram-positive and Gram-negative bacteria. It is composed of *N*-acetylmuramic acid linked by its lactic acid moiety to the amino terminus of an L-alanine D-isoglutamine dipeptide.

Reactive oxygen species
These include superoxide (O_2^-), hydroxyl radicals (OH^-) and hydrogen peroxide (H_2O_2). They are generated as products of normal respiration from the electron transport chain that is present in the mitochondria or in the endoplasmic reticulum, or they can be catalysed by a wide array of enzymes (NADPH oxidase, xanthine oxidase, peroxidases and NADPH oxidase isoform).

Reactive nitrogen intermediates
These include nitric oxide ($NO^\cdot$) and its derivates such as NONOates, *S*-nitrosothiols, peroxynitrite, nitrite and nitrous acid. NO is a highly reactive and diffusible free radical, soluble in both lipids and water, and capable of reacting with oxygen and reactive oxygen species to form reactive nitrogen intermediates, $NO_2^\cdot$, NO_2^-, NO_3^-, N_2O_3, and the highly mycobactericidal $ONOO^-$.

Proteasome
A giant multicatalytic protease that resides in the cytosol and nucleus.

Caspase 1
A cysteine protease that contains a cysteine residue in the active site and that cleaves its substrate after an aspartic acid residue. It was previously known as interleukin-1 β-converting enzyme.

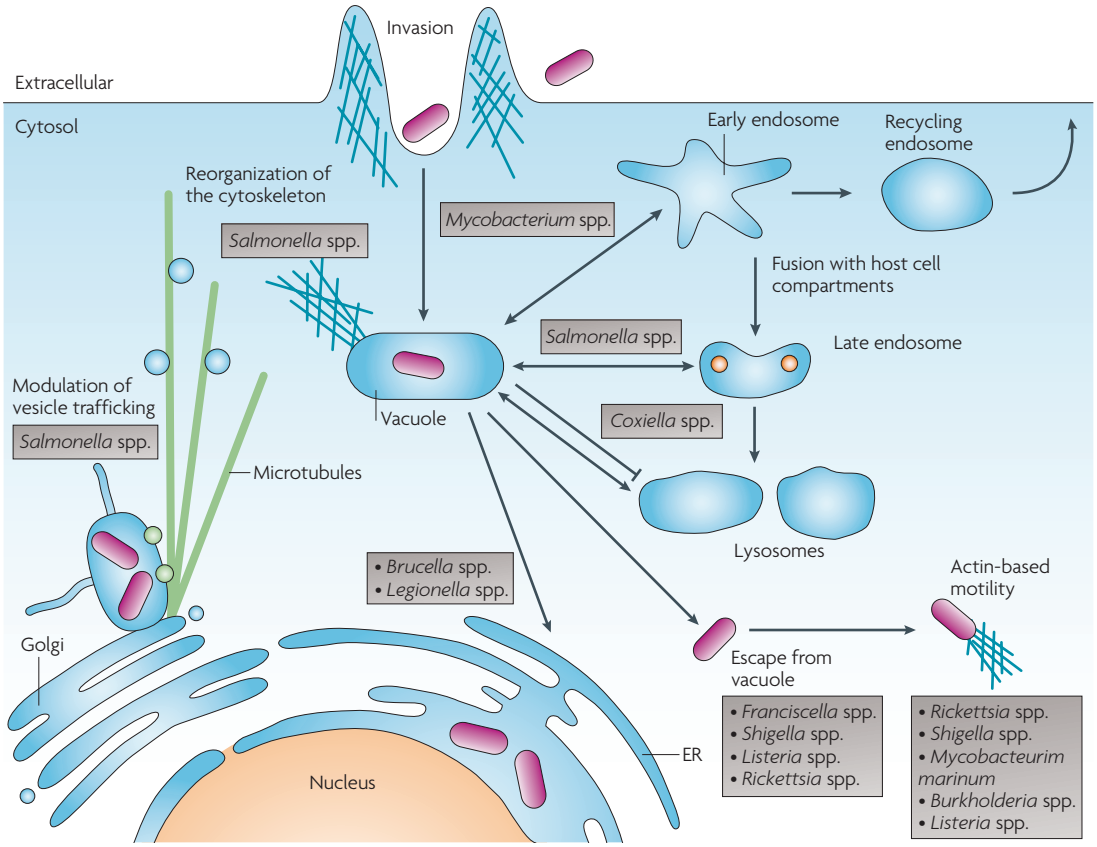

Figure 2 | **Intracellular pathogens manipulate host membrane trafficking to resist innate immunity and promote survival and replication.** Bacteria are internalized by host cells. However, once inside the cell different pathogens follow alternative pathways of intracellular trafficking to establish a replication niche. Some bacteria reside in specialized vesicles that are termed vacuoles or phagosomes and can manipulate host cell molecular motors to induce vacuolar and vesicular movement along microtubules. In non-polarized cells, microtubules are organized with minus ends gathered at the microtubule-organizing centre and plus ends extending towards the cell periphery. Vacuolar bacteria can also interact with different host cell compartments of the endocytic pathway (such as endosomes and lysosomes) and can replicate in host cell compartments such as the endoplasmic reticulum (ER). Other bacteria escape from vacuoles to remain into the cytosol and then promote actin-mediated motility. Fusion with lysosomes results in bacterial killing by lytic enzymes and acidic pH. To successfully establish a replicative niche, almost all intracellular pathogens inhibit or delay lysosome fusion.

Pyroptosis
An inflammatory process of cellular self-destruction that causes cell lysis and the secretion of interleukin-1β (IL-1β) and IL-18, in a caspase 1-dependent way.

Pathogenicity island
A large region of genomic DNA that encodes genes associated with virulence. A pathogenicity island is typically transferred horizontally between bacterial strains and is often inserted into tRNA genes in the genome.

ARP2/3 complex
A complex that consists of seven subunits comprising two Arp proteins and five highly conserved protein subunits: p16, p20, p21, p34 and p40e. The ARP2 and ARP3 subunits are structurally related to actin.

and *Shigella* spp. use type III secretion systems (T3SS), which are needle-like complexes spanning bacterial inner and outer membranes[13], to inject effector proteins into host cells. In *Salmonella* spp. the chromosomal genes encoding this secretion system are clustered in the *Salmonella* pathogenicity island 1 (SPI1), and their products are absolutely necessary for the orchestration of these events in non-phagocytic cells. The T3SS secreted effectors, SopE, SopE2 and SopB, initiate actin cytoskeleton rearrangement by activating Rho GTPases (for example, cell-division cycle 42 (CDC42) and Rac) and associated proteins (RhoGEFs, the ARP2/3 complex, Wiskott–Aldrich syndrome protein (WASP) and SCAR)[14–16]. SopB alters cellular phosphoinositide phosphate (PtdInsP) and inositol phosphate (InsP) metabolism. Other secreted effectors include SipA and SipC, which are actin-binding proteins that modulate actin dynamics[14–16]. The stimulation of CDC42 by SopE, SopE2 and SopB also triggers several mitogen-activated protein kinase (MAPK) pathways, including the extracellular signal-regulated kinase (ERK), Jun N-terminal kinase (JNK) and p38 pathways, which results in the activation of the transcription factors activator protein 1 (AP1) and nuclear factor-κB (NF-κB)[17]. These transcription factors then direct the production of pro-inflammatory cytokines, such as IL-8, and stimulate the inflammatory response. However, actin remodeling initiated by *Salmonella* spp. is a transient process that is reversed 2–3 hours following entry into the host cell by the activity of the SPI1 T3SS effector protein SptP. The amino terminus of SptP folds to mimic a eukaryotic RhoGTPase-activating protein (GAP), and its carboxyl terminus encodes a tyrosine phosphatase[18]. The GAP activity of SptP inactivates the RhoGTPase switch, leading to cytoskeletal recovery[19], and its tyrosine phosphatase activity has a role in the downregulation of ERK signalling and IL-8 secretion mediated by *S. enterica* subsp. *enterica* serovar Typhimurium following invasion[20,21]. After invasion of intestinal epithelial cells, *S.* Typhimurium can also

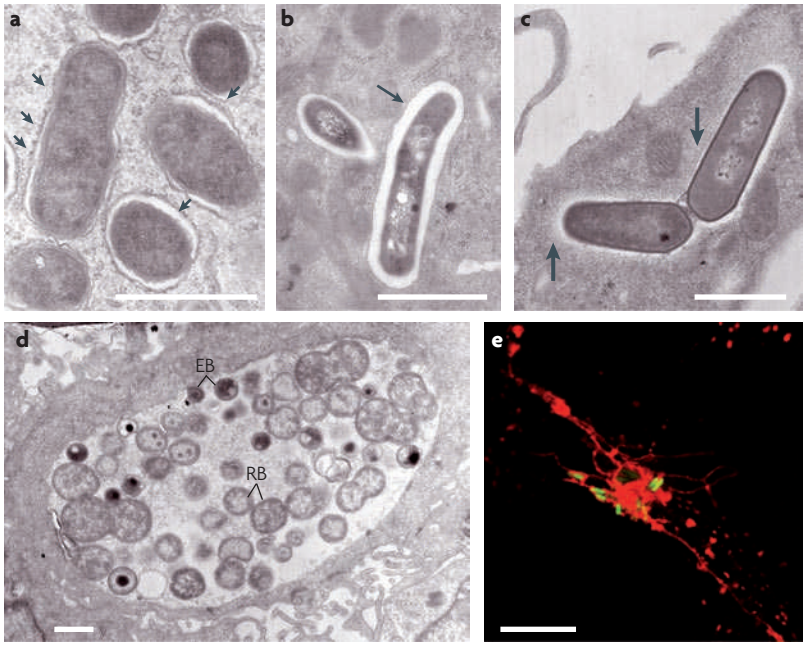

Figure 3 | **Survival niches of pathogenic bacteria. a** | *Brucella* spp. is an intracellular pathogen that can persist for long periods of time in the host and establish a chronic disease. Once internalized, *Brucella* spp. are localized in a *Brucella*-containing vacuole that transiently interacts with early endosomes. However, *Brucella*-containing vacuoles do not fuse with lysosomes and instead fuse with membrane from the endoplasmic reticulun (ER) to establish a vacuole suited for replication. The micrograph shows infection of bone marrow-derived mouse macrophages with *Brucella abortus* at 30 hours post infection. Each vacuole contains a single bacterium. The vacuolar membrane is studded with ribosomes (indicated by arrowheads). **b** | Bone marrow-derived mouse macrophages infected with *Mycobacterium avium* at 1 day post infection. The bacterium resides in an immature phagosome. The phagosome membrane (indicated by the arrow) is closely apposed to the bacterial surface. **c** | Bone marrow-derived mouse macrophages infected with *Listeria monocytogenes* at 4 hours post infection. This micrograph shows a dividing bacterium free in the cytosol. A network of actin filaments surrounds the bacterium (indicated by arrows). **d** | Bone marrow-derived mouse macrophages infected with *Chlamydia pneumoniae* at 30 hours post infection. The inclusion contains many bacteria. Both the EB (elementary bodies) and RB (reticulate bodies) forms can be found, in the same inclusion. **e** | *Salmonella* spp. reside in a vacuole. In HeLa cells up to 10 hours post invasion, wild-type *Salmonella spp*. use type III secretion system effectors to induce the formation of tubular membranous extensions from the vacuole, which are named *Salmonella*-induced filaments. The immunofluorescence image shows *Salmonella* (green) and characteristic *Salmonella*-induced, filaments extending from the vacuole, visualized using lysosomal-associated membrane protein 1 (red). Scale bar in electron microscopy images is 10μM; scale bar in immunofluorescence image is 1μM. Images courtesy of Chantal de Chastellier and Stéphane Méresse, Centre d'Immunologie de Marseille-Luminy, Paris, France.

downregulate IL-8 production through the SspH1 effector. SspH1 localizes to the host cell nucleus and inhibits NF-κB-dependent gene expression through its ubiquitin ligase activity[22]. SspH1 can also interfere with pro-inflammatory signalling by binding to and activating host serine/threonine protein kinase 1 (PKN1). The SPI1 T3SS effector AvrA has also been reported to inhibit NF-κB activity and pro-inflammatory cytokine secretion[23].

In *Shigella* spp. several effector proteins encoded by the *ipa* operon (IpaA, IpaB, IpaC and IpaD) are required for entry[24]. IpaB interacts with the cell surface hyaluronan receptor, which localizes to cholesterol-rich lipid rafts, suggesting that lipid rafts are important for *Shigella* spp. binding and entry into the host cell[25]. Lipids also play a part during the active entry of bacterial pathogens. PtdIns5P accumulates in epithelial cells infected with *S. flexneri*. The translocated bacterial virulence factor IpgD specifically dephosphorylates phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2) into PtdIns5P, which probably facilitates both membrane ruffling and bacterial invasion[26].

Subversion of innate immunity during invasion

Recently, a new family of bacterial molecules has been identified because its members have sequence similarity with the mammalian Toll/IL-1 receptor (TIR) protein family. One such protein is *Brucella* TIR protein 1 (Btp1); *Brucella abortus* Btp1 mutants were shown to be unable to control DC maturation[27]. Btp1 is highly homologous to the TcpB protein of *Brucella melitensis* and to TcpC and TlpA from *E. coli* and *S. enterica* serovar Enteritidis, respectively. Infection of mice with *S. enterica* serouvar Enteritidis TlpA mutants resulted in reduced lethality compared with infection with wild-type bacteria[28]. Furthermore, mice infected with *E. coli* TcpC mutants showed a reduced bacterial burden and tissue damage in the kidneys[29]. All of these proteins interfere with TLR signalling[27–29]. Specifically, Btp1 and TcpB interfere with the activation of downstream responses by acting on the TLR2–MYD88-dependent signalling pathway[27,30], and a recent study found that the interaction of Btp1 or TcpB and the adaptor TIRAP blocks NF-κB activation[32].

In addition to such blocking mechanisms, bacteria such as *Brucella* spp. or *Ochrobactrum* spp. have evolved to hide from TLR recognition by expressing non-classical LPS, which has the propensity to poorly activate TLR pathways[31,32]. *B. abortus* lipid A has a diaminoglucose backbone (instead of a glucosamine one) and its acyl groups are longer (C18, C19 and C28 rather than C12 and C14) and are linked to the core only by amide bonds (and not ester and amide bonds). This feature is also observed in *Ochrobactrum* spp., the LPS of which is different from enterobacterial LPSs. Interestingly, *Yersinia pestis* grown at 37°C expresses a tetraacetylated LPS that is also poorly recognized by TLR4. In the case of *M. tuberculosis*, ESAT6, CFP10 and the ESAT6–CFP10 complex interfere with the signalling pathway activated by LPS, which shows that these secreted mycobacterial proteins are important for the control of innate immune responses[33,34].

Establishing a niche post invasion

Escape from the early vacuole. One way to avoid the harsh environment resulting from fusion with degradative enzyme-rich endocytic compartments (FIG. 2) is to rapidly escape the nascent vacuole, invade the cytoplasm and use the actin cytoskeleton to facilitate intracellular movement. This strategy is used by *Burkholderia pseudomallei*, the causative agent of the melioidois, *L. monocytogenes* (FIG. 3), *Rickettsia rickettsii, Mycobacterium marinum* and *S. flexneri*. *S. flexneri* expresses IpaB, which, in addition to its role in host cell entry, has a lytic activity that is used to breach the vacuolar membrane[35]. Similarly, *L. monocytogenes* uses a pore forming toxin, listeriolysin O,

and to a lesser extent the bacterial phosphatidyl inositol phospholipase C[36] to disrupt the vacuolar membrane[37].

To facilitate intracellular movements following vacuolar lysis, intracellular bacteria can either trigger actin nucleation-promoting factors (NPFs) or express NPF mimics such as ActA to activate the ARP2/3 complex and initiate host actin polymerization in the cytosol[38]. *L. monocytogenes* that is associated with actin-rich 'comet tails' inside infected cells do not colocalize with ubiquitylated proteins[39]. However, *L. monocytogenes* mutants that express ActA mutations (which therefore have a limited ability to initiate actin-based motility) colocalized with ubiquitylated proteins[39]. These findings suggest that *L. monocytogenes* avoids recognition by the ubiquitin system through rapid intercellular movement.

Interactions with the early endocytic pathway. The interaction of the nascent vacuole with early endosomes occurs 5–20 minutes after invasion and is transient. However, vacuoles containing *M. tuberculosis* and *Mycobacterium avium* show the characteristics of early endosomal organelles that do not mature into late endosomal and lysosomal compartments[40]. Specifically, vacuoles containing mycobacteria retain the early endosomal GTPase RAB5 but selectively exclude the late endosomal GTPase RAB7 and the early endosome antigen 1 (EEA1), both of which are regulators of vesicular trafficking and phagosome maturation[41,42] (FIG. 2). Indeed, mycobacteria express a large molecular arsenal, which is involved in maintaining a block on vacuole maturation. Mycobacterial mannose-capped lipoarabinomannan (ManLAM) can transiently disrupt PtdIns3P accumulation in the vacuolar membrane or its interaction with EEA1 by altering calcium signalling. This may be reinforced by the activity of SapM, a secreted PtdIns3P phosphatase[43]. The glycosylphosphatidylinositol anchor of ManLAM also alters phagosome maturation by directly inserting itself into lipid rafts on the phagosomal membrane[44,45]. Phenolic glycolipid[46] or trehalose dimycolate[47] could also slow down phagosome maturation. In addition, *M. tuberculosis* interferes with the conversion of PtdIns to PtdIns3P by a class III phosphatidylinositide 3-kinase (PI3K) and the association of EEA1 with the phagosome membrane through RAB5 (REFS 41,48). This apparent redundancy can be explained by the fact that each different molecular player may contribute to slowing down the maturation process, but that no individual factor by itself can block it completely. In addition, it has been shown recently that maturation arrest requires a close apposition between the *M. avium* surface and the vacuole membrane[40].

Interactions with the late endocytic pathway. Following internalization and interaction with early endosomes, *Salmonella*-containing vacuoles avoid fusion with lysosomes and remain in a late endosomal-like compartment[49,50]. *Salmonella* spp. use two distinct T3SS encoded by SPI1 and SPI2 to inject bacterial effectors into host cells. SPI1-encoded effectors have been shown to be essential for bacterial invasion of epithelial cells[15,51], but it is likely that in macrophages SopE is the only SPI1 effector with a role in the maturation of *Salmonella*-containing vacuoles[52]. The SPI2 secretion system is required for bacterial proliferation in macrophages[53,54], and the SPI2 effector SpiC was shown to inhibit homotypic endosome fusion[55]. SpiC interacts with the host protein TassC, a member of the NIPSNAP protein family[56]. The *Caenorhabditis elegans* NIPSNAP-1 protein is expressed in an operon encoding SNAP-25-like proteins (vesicle-associated membrane proteins), suggesting that NIPSNAPs could be a new class of proteins that control membrane trafficking[56].

Concomitant with the onset of bacterial replication in epithelial cells is the appearance of membrane tubules termed *Salmonella*-induced filaments, or SIFS[57] (FIG. 3). The SIF membrane contains large amounts of lysosomal glycoproteins[57,58], and SIF biogenesis is under the control of SifA, a T3SS effector encoded outside SPI2 that is essential for the formation of SIFS in epithelial cells[59]. SifA and PipB2 effectors regulate trafficking of vesicles onto microtubules by interacting with the molecular motor kinesin 1 (FIG. 2). These events are important for the maintenance of the membrane integrity of *Salmonella*-containing vacuoles[60] and replication in infected cells[60–62].

Interactions with the exocytic pathway. Successful infection by *Brucella* spp. (FIG. 3) requires the bacterium to interact with and replicate in the ER of phagocytes. To reach the ER, the *Brucella*-containing vacuole needs to be modified at early time points of infection. This is mediated by the *Brucella* spp. β-cyclic glucan, which can modulate the lipid and protein composition of the *Brucella*-containing vacuole to facilitate its fusion with the ER membranes[63]. *Brucella* spp. also require the presence of the *virB* T4SS and the host factors glyceraldehyde 3-phosphate dehydrogenase and RAB2 to ensure its replication[64,65]. *Brucella* spp. *virB* and *Legionella* spp. *dot/icm* (defect in organelle trafficking/intracellular multiplication) both encode specialized T4SSs that can transfer effectors from the bacterial cytosol into the host cytoplasm. Biogenesis of the *Legionella pneumophila* replication compartment depends on a rapid interception of COP1-dependent vesicular trafficking from ER exit sites by secreted type IV effector proteins[66].

Chlamydia trachomatis has a marked requirement for sphingolipids and cholesterol produced in the ER and the Golgi apparatus[67] (FIG. 3). In human epithelial cells, *C. trachomatis* induces a fragmentation of the Golgi apparatus that is triggered by the successive cleavage of golgin 84 (REF. 68). Thus, *C. trachomatis* morphologically and functionally manipulates a host organelle, generating Golgi ministacks surrounding the bacterial inclusion to enhance lipid acquisition and secure its replication and development.

Subversion of innate immune effectors

In response to inflammatory stimuli generated by internalized pathogens, macrophages and neutrophils produce chemically reactive molecules such as antimicrobial peptides and a rapid burst of ROS followed by a prolonged production of RNI[69] (BOX 1).

T4SS
(Type IV secretion system). A well-characterized secretion apparatus, encoded by the *virB* operon from *Agrobacterium* spp., which can transport DNA and proteins into host cells. *Helicobacter pylori*, *Bordetella pertussis*, *Brucella* spp. and *Legionella pneumophila* have homologous operons that are used to secrete proteins and toxins into the eukaryotic cytoplasm.

NADPH oxidase
An enzyme composed of five core polypeptides, p22phox, gp91phox p47phox, p67phox and p40phox, and two low-molecular-mass guanine nucleotide-binding proteins, Rac (p21rac) and RAP1A.

Nitric oxide synthase
(NOS). An enzyme that uses electrons derived from NADPH to convert (oxidize) L-arginine to L-citrulline and NO·. Three NOS isoforms are known, NOS1, NOS2 and NOS3. NOS2 has also been termed iNOS, for inducible and independent (of increased intracellular Ca^{2+}) and was first cloned from mouse macrophage cell line RAW 264.7.

Box 1 | **Interaction between phagocyte-derived ROS and RNI**

Nitric oxide (NO·) is generated by nitric oxide synthase 2 (also known as iNOS). Superoxide ($O_2^{\cdot-}$) is generated by the phagocyte NADPH oxidase. Although reactive nitrogen intermediate (RNI) production is not an immediate response to infection, unlike reactive oxygen species (ROS), NO· can diffuse across membranes and can act on bacteria in both vacuoles and the cytosol over a longer period of time than the short-lived oxidative burst. Although NOS2 and the phagocyte NADPH oxidase are differentially regulated, these systems could be co-stimulated by inflammatory stimuli (for example, interferon-γ). Simultaneous production of RNI and ROS could lead to the formation of a range of antimicrobial molecular species (TABLE 1), each with distinct stability, compartmentalization and reactivity. Macrophages can produce $O_2^{\cdot-}$ and NO· in equimolar amounts, leading to the generation of the particularly potent effector, peroxynitrite ($ONOO^-$)[75].

Mice with phagocytes that are deficient in the production of either ROS or RNI are markedly more susceptible to a range of infections[74]. However, mice deficient in both NADPH oxidase and NOS2 are much more susceptible to severe spontaneous infections with endogenous bacteria and fungi than mice deficient in either pathway alone[95].

The major source of ROS is the multicomponent enzyme NADPH oxidase. In resting cells, the components of this complex are present but are dissociated from each other and inactive. On phagocytosis, the NADPH oxidase components are translocated to the phagosome in a Rac-dependent manner to facilitate the formation of superoxide ($O_2^{\cdot-}$) in the phagosomal lumen[70].

In mammals, nitric oxide (NO·) and RNI are generated by nitric oxide synthases (NOS)[71]. In macrophages NOS2 (also known as iNOS) is important for the generation of RNI during inflammation, immune regulation[71–73] and infection with intracellular pathogens[74,75]. The gene encoding NOS2 is not transcribed in uninfected cells, but its expression can be induced by type I and type II interferons (IFNs), pro-inflammatory cytokines and lipoteichoic acid[74,75]. Stimulation results in the activation of signalling pathways, including the p38 MAPK pathway, the NF-κB pathway and the Janus kinase–signal transducer and activator of transcription–IFN regulatory factor 1 (JAK–STAT–IRF1) pathway, leading to NOS2 transcription[75].

Although *S.* Typhimurium and *M. tuberculosis* induce the production of NOS2 at the site of the infection, they have developed mechanisms to detoxify ROS and RNI. In *Salmonella* spp. periplasmic superoxide dismutases (SodCI and SodCII) detoxify ROS[76–79]. The virulence of *Salmonella* spp. lacking superoxide dismutase can be rescued by either NOS2 inhibition or the absence of $O_2^{\cdot-}$-generating phagocyte NADPH oxidase[76], indicating that peroxynitrite production from RNI and ROS (BOX 1) is important for bacterial killing.

Glucose 6-phosphate dehydrogenase is required for NADPH synthesis and therefore for the maintainence of the redox state in *Salmonella* spp.. NADPH is the electron source for several reductases that repair oxidative damage and regenerate antioxidant species, including glutathione reductase, thioredoxin reductase and methionine sulphoxide reductase[79,80]. *S.* Typhimurium containing a mutation in *sspJ* are hypersensitive to $O_2^{\cdot-}$ *in vitro*, show attenuated virulence in mice and show deficient growth in macrophages[81]. The virulence of the *sspJ*$^-$ mutant and its ability to grow in macrophages is restored when infecting mice deficient in NADPH oxidase (*p47phox*$^{-/-}$ mice)[82].

Bacterial peroxiredoxins are found in a range of species and have been directly linked to the decomposition of peroxynitrite[83]. The peroxiredoxin alkyl-hydroperoxide reductase subunit C (AhpC) from *S.* Typhimurium catalytically detoxifies peroxynitrite to nitrite fast enough to forestall the oxidation of bystander molecules such as DNA[84]. Results are similar with peroxiredoxins from *M. tuberculosis*, *H. pylori* and *L. pneumophila*. Recently, it was found that the accumulation of NADH following the NO-mediated inhibition of *S.* Typhimurium electron transport chain (or respiratory arrest) is a newly described antioxidant strategy[85].

M. tuberculosis lacks a glutathione-dependent detoxification system. Instead, catalase and peroxidases probably contribute to resistance to oxidative and nitrosative stress[86,87]. Clinical isolates lacking catalase are virulent and resistant to isoniazid because the enzyme is required to potentiate this drug. The survival and virulence of such strains are attributed to the peroxiredoxin-type peroxidases alkyl hydroperoxide reductase[88] and thioredoxin peroxidase[86]. Alkyl hydroperoxide reductase has a specific role in antioxidant defences involving peroxynitrite in macrophages, because they contribute to survival on exit from the stationary phase during dormancy (latent infection) or on transmission to a new host. Mycobacteria also increase the production of the lipid trehalosedimycolate (TDM) in response to their intracellular environment[89]. TDM could slow down phagosome maturation and may scavenge ROS and RNI, thereby protecting mycobacteria from macrophages. However, the virulence function of TDM is fully abolished by NOS2 following the activation of macrophages by IFNγ and LPS[47]. RNI directly inactivates TDM's virulence function by altering its hydroxyl groups.

The *S.* Typhimurium flavohaemoglobin Hmp mediates the aerobic detoxification of NO· to $NO3^-$. Studies in mice and *in vitro* suggest that Hmp is essential for persistence and virulence[90]. However, under aerobic conditions and in the absence of nitrosactive stress, enhanced Hmp expression increases the susceptibility of *S.* Typhimurium to hydrogen peroxide, resulting in flavin adenine dinucleotide-dependent and iron-dependent damage. Therefore, Hmp requires precise regulation. *hmp* is in fact induced by NO· and repressed when intracellular free iron concentrations are increased. This regulation allows Hmp to play a central part in NO· detoxification without contributing to oxidative stress[90].

Mycobacterium spp. mutants defective in DNA repair (for example, those lacking *uvrB*) or synthesis of a flavin cofactor (for example, those lacking *fbiC*) were highly susceptible to acidified nitrite. Mice infected with the *uvrB* mutant have attenuated infections compared with those infected with wild-type mycobacteria, but this attenuated phenotype can be rescued by genetic inactivation of NOS2 or loss of both NOS2 and NADPH oxidase[91]. Mutant strains of *S. enterica* deficient in RecBC are also more susceptible to killing by RNI.

Various pathogens can also avoid damage mediated by NOS2. Intracellular proliferation of *Francisella tularensis* is inhibited by NO·. However, this pathogen is capable

Table 1 | **Innate immune effectors used by host cells to control intracellular pathogens**

Effector	Action	Protective bacterial proteins	Protective bacterial mechanisms
ROS (superoxide, hydrogen peroxide and hydroxyl radical)	Generation of oxidative stress into the bacterial phagosome	Catalase, superoxide dismutase, heat shock proteases and mycobacterial glycolipids	NADH and NADPH redox pathways, DNA repair systems and inhibition of the recruitment of oxidase components to the phagosome
Peroxynitrite	Potent oxidant	Superoxide dismutase and peroxiredoxins	NA
RNI (nitric oxide, NONOates, S-nitrosothiols, nitrite and nitrous acid)	Diffusion into bacterial phagosome and generation of oxidative stress	Flavohaemoglobin, heat shock proteases and mycobacterial glycolipids	DNA repair systems, avoidance of NOS2 induction and blockage of active NOS2 accumulation at the phagosome
Antimicrobial peptides (defensins, cathelicidin, ubiquitin, ubiquicidin, histones and HMGN2)	Disruption of bacterial membranes; can be found in phagosomes and the cytosol or can be secreted	NA	Escape from the phagosome, intracellular motility, interaction with endocytic pathway and control of vesicular trafficking
Lysosome (vacuolar ATPase, cathepsin G and lysozyme)	Acidification of lysosomal lumen and disruption of bacterial membranes	NA	Inhibition of fusion with lysosome and escape from the phagosome
NRAMP and ferroportin	Depletion of key nutrients and divalent metals from phagosomes	Iron chelators and siderophores	Interaction with endocytic pathway and control of vesicular trafficking

HMGN2; high-mobility group nucleosome-binding domain-containing protein 2; NOS2; nitric oxide synthase 2; NA, not applicable; NRAMP, natural resistance-associated macrophage protein; RNI, reactive nitrogen intermediate; ROS, reactive oxygen species.

of phase variation, resulting in the production of altered LPS[92]. Altered LPS of *F. tularensis* is no longer a potent stimulus of NOS2 induction, and this allows the bacterium to replicate intracellularly in macrophages[92]. A reduction in NOS2 expression was also observed in cells infected with *Salmonella* spp., which exhibit an altered LPS, indicating that the modification of LPS might be a common strategy to minimize NO· synthesis and killing by RNI[93].

In the case of *S. enterica*, although rapid clearing has been attributed to ROS rather than RNI, animal studies indicate that NO· plays an important part in clearing the bacterial burden at later time points of infection[94,95]. By contrast, *S.* Typhimurium can suppress the synthesis of RNI and ROS. *S.* Typhimurium seems to interfere with the recruitment of oxidase components to the phagosome through its SPI2 T3SS[94,96,97]. Indeed, in the absence of a functional SPI2 T3SS, wild-type but not NADPH oxidase-deficient macrophages can restrict *S.* Typhimurium infection, demonstrating a crucial role for NAPDH oxidase in controlling intracellular bacterial growth. The mechanism used by the bacterial effectors to manipulate the localization of the oxidase components is still unknown. *S.* Typhimurium induces NOS2 production in macrophages by the effector SopE2, which is secreted by T3SS1 (REF. 98). The O antigen of *S.* Typhimurium LPS also promotes host cell NO synthesis in J774-A.1 cells[99]. By contrast, SPI2 T3SS of *S.* Typhimurium plays a part in limiting the bacteriostatic effects mediated by NOS2 (REF. 100); in infected macrophages, NOS2 localization and peroxynitrite formation are associated with an intravacuolar SPI2 mutant but not wild-type *S.* Typhimurium[100]. These results indicate that *S.* Typhimurium block the accumulation of active NOS2 in *Salmonella*-containing vacuoles through the SPI2 T3SS.

Recently, it was reported that *M. tuberculosis* inhibits trafficking of NOS2 to the mycobacterial phagosome[101,102]. Following macrophage activation by IFNγ, NOS2 is delivered and kept in the vicinity of phagosomes. Its positioning in the cell depends on a functional actin cytoskeleton[101] and is dictated by interactions with the scaffolding protein ezrin–radixin–moesin-binding phosphoprotein 50 (EBP50; also known as NHRF1). *M. tuberculosis* interferes with the process of EBP50-guided positioning of NOS2, thereby avoiding delivery and accumulation of this enzyme and its noxious products near the phagosome, where NO· would have the best chance of inhibiting intracellular mycobacteria[102].

Another important strategy of the innate immune system that promotes resistance to intracellular infection is to sequester key nutrients such as iron from the invading bacteria. Ferroportin, natural resistance-associated macrophage protein 1 (NRAMP1) and NRAMP2 (also known as DMT1) act as iron transporters to move ferrous iron from the lumen of the phagosome into the cytosol[103,104]. To allow their replication, many intracellular bacteria produce high affinity iron chelators, known as siderophores, which can 'steal' iron from host proteins[105–107] (TABLE 1). In addition, intracellular bacteria upregulate genes that are characteristic of nutritional stress, such as limitation of iron, magnesium, glucose, amino acid and phosphate[108,109].

Autophagy

Under conditions ranging from hormone and IFNγ treatment to starvation or infection, the host cell can use an intracellular self-eating mechanism to eliminate microorganisms; this process is known as autophagy[110–112]. However, numerous pathogenic bacteria have evolved to use autophagosomes as a niche for survival (FIG. 4). *Porphyromonas gingivalis*, *Coxiella burnetii* and

Ezrin–radixin–moesin-binding phosphoprotein 50
A scaffolding protein that is responsible for the anchoring of various cellular proteins to the actin cytoskeleton through its linkage to ezrin, radixin and moesin.

Autophagosome
An intracytoplasmic vacuole that contains components of the cytoplasm. It fuses with a lysosome to form an autophagolysosome, thereby subjecting its contents to enzymatic digestion.

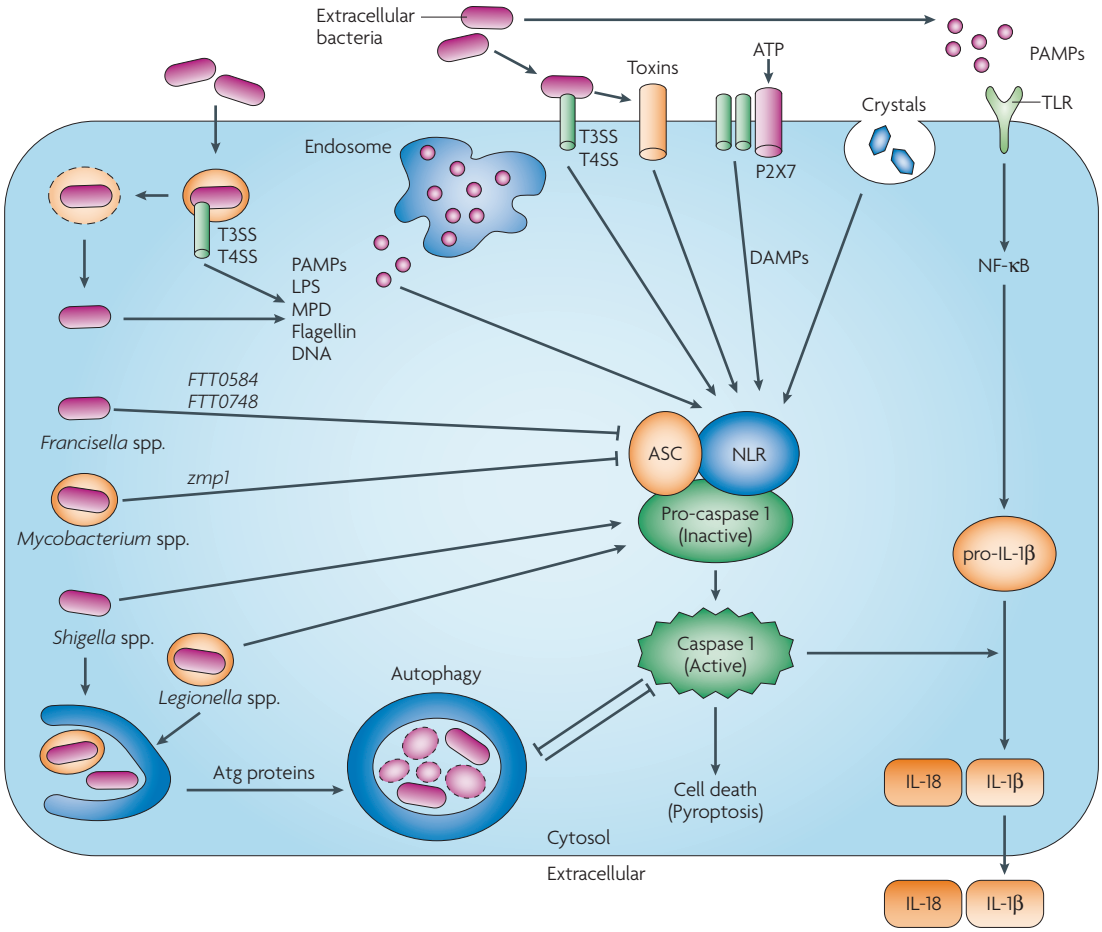

Figure 4 | **Autophagy and inflammasome activation can be manipulated by intracellular pathogens.** The inflammasome consists of a NOD-like receptor (NLR), apoptosis-associated speck-like protein containing a CARD (ASC) and pro-caspase 1; ASC bridges the NLR and pro-caspase 1. Inflammasome activation begins with the processing of pro-caspase 1 into its mature, active form, caspase 1, which promotes the maturation of the pro-inflammatory cytokines interleukin-1β (IL-1β) and IL-18. Active caspase 1 induces a robust innate immune response against pathogens and cell death. Inflammasome activation is mediated by the presence of ATP, toxins, crystals and intracellular bacteria or their products (for example, injectisomes (T3SS and T4SS), pathogen-associated molecular patterns (PAMPs), lipopolysaccharide (LPS), muramyl dipeptide (MPD), flagellin and DNA). However, some bacteria such as *Francisella* spp., *Mycobacterium* spp. and *Shigella* spp. can can prevent this process. During autophagy a portion of the cytosol, a damaged organelle or a pathogen is sequestered in a structure that is termed the phagophore, which elongates and seals to form an autophagosome (with a double membrane) that maturates and fuses with the lysosome to degrade the confined material. The autophagic pathway is regulated by a set of autophagy-related proteins (Atg). Intracellular pathogens (bacteria, parasites and viruses) that are either free inside the cytosol, inside phagosomes or inside pathogen-containing vacuoles can be degraded by the autophagic machinery. However, numerous pathogenic bacteria use autophagosomes as niche of survival and replication. NF-κB, nuclear factor-κB; P2X7, P2X purinoceptor 7; T3SS, type III secretion system; TLR, Toll-like receptor.

C. trachomatis can survive in a multimembranous compartment enriched in ER markers and in the autophagosome marker ATG7 (REFS 113,114). Autophagosomes are also permissive to *L. monocytogenes* replication[115]. In macrophages, listeriolysin O is responsible for lysing the pathogen-containing vacuole in cells infected with *L. monocytogenes*. However, some bacteria express low levels of listeriolysin O and are targeted to the autophagosome, where they can replicate efficiently[115].

When *L. pneumophila* is ingested by mouse macrophages, the bacterium secretes microbial T4SS effectors into the host cytoplasm, which are detected by host NAIP5, a NOD–LRR protein system, leading to inflammasome assembly and caspase 1 activation[116]. When the infection is limited, macrophages increase autophagy to capture the pathogen for further delivery to lysosomes. If autophagy fails to contain the pathogen, caspase 1 activity exceeds the threshold that is required to induce pyroptosis[116] (FIG. 4). Thus, NAIP5 bridges autophagy and pyroptosis, two important immune responses to intracellular pathogens.

In macrophages, *Shigella* spp. infection triggers a complex crosstalk between the inflammasome (see below) and autophagy. *Shigella* spp. promotes caspase 1 activation and IL-1β processing mediated by NLRC4 and the adaptor protein apoptosis-associated speck-like protein containing a CARD (ASC)[117]. Cholesterol[118], NLRC4 and caspase 1 were shown to be crucial for pyroptosis, whereas

Apoptosis-associated speck-like protein containing a CARD (ASC). A protein that contains a CARD domain in its carboxy-terminal region and a pyrin domain in the amino-terminal region. Both domains allow ASC to recruit other pyrin domain- and CARD-containing proteins through homotypic protein–protein interactions.

ASC is dispensable. Notably, infection of macrophages with *Shigella* spp. also induces autophagy, which is markedly increased in macrophages deficient for caspase 1 or NLRC4, but not ASC[117]. Furthermore, autophagy induction is associated with a transient resistance to pyroptosis.

Although autophagy is a defence mechanism, some pathogens use autophagy for their own benefit. In macrophages, *Shigella* spp. infection induces pyroptosis through caspase 1 activation and autophagy induction, and these events may be reciprocally regulated[119] (FIG. 4). It has been suggested that autophagy mediates viability of macrophages infected with *Shigella* spp.[119]. However, activation of caspase 1 suppresses autophagy and promotes rapid pyroptosis of infected macrophages. In addition, IcsA, which is essential for mediating actin-based motility of *Shigella* spp., is a target for autophagy (ATG5 binds IcsA and induces autophagy). However, through its T3SS, *Shigella* spp. secretes IcsB, an effector that plays a crucial part in the camouflage against autophagic recognition[120]. IcsB also binds IcsA and competes with ATG5 for the same region of IcsA, suggesting that IcsB has a role in preventing ATG5 binding.

Control of caspase 1 activation

TLRs recognize PAMPs on the cell surface or in endosomes, whereas NLRs recognize PAMPs in intracellular compartments[121–123]. Both TLR- and NLR-mediated recognition of PAMPs leads to the activation of host cell signalling pathways and subsequent innate and adaptive immune responses. On recognition of microbial molecules some NLRs can trigger the activation of caspase 1 in a multiprotein complex formed in the cytosol of certain cells, which is termed the inflammasome[124].

The inflammasome consists of caspase 1, ASC and different NLRs; ASC bridges NLR proteins and the inactive procaspase 1 (REFS 125,126). Inflammasomes are designated by their NLR specificity: NLRP1 inflammasome, NLRP3 inflammasome and NLRC4 inflammasome. NLRC4 and ASC are part of different inflammasomes[127]: NLRC4 interacts with caspase 1 through its caspase-recruitment domain (CARD), and ASC is an adaptor protein or activity enhancer of the inflammasome. ASC can also self-associate through its CARD and pyrin domain to form the ASC pyroptosome (a large supramolecular assembly of ASC) and activate caspase 1 (REF. 128).

The assembly and activation of the inflammasome results in the catalytic processing of the 45 kDa pro-caspase 1 into its mature and enzymatically active form, a heterodimer composed of subunits p20 and p10 (REF. 124). This activation in conjunction with TLR activation generates an increase in pro-IL-1β production. The action of caspase 1 promotes the maturation of several ILs such as IL-1β, IL-18 and IL-33 (REF. 129). IL-1β produced by activated macrophages and monocytes induces a vigorous host response against pathogens, with a concomitant production of ROS and RNI, activation of other pro-inflammatory cytokines, recruitment of inflammatory cells to sites of infection[130], protein secretion and pyroptosis[131,132] (FIG. 4).

Two distinct signals are required for the secretion of IL-1β. The first signal comes from various extracellular or endosomal PAMPs, which engage their specific TLRs and thereby activate the NF-κB signalling pathway, ultimately triggering gene expression and synthesis of pro-IL-1β[126]. These PAMPs include LPS (a TLR4 agonist), lipoteichoic acid (a TLR2 agonist), CpG dinucleotides (TLR9 agonists), the lipopeptide Pam3CysSerLys4 (a TLR2 agonist) and R848 (also known as resiquimod; a TLR7 agonist)[126]. The second signal is provided by different danger-associated molecular patterns (DAMPs), such as extracellular ATP, uric acid crystals, calcium pyrophosphate dihydrate and different pore-forming toxins, and activates the inflammasome[129] (FIG. 4).

The NLRP3 inflammasome is a highly conserved microorganism-recognizing cell component[133,134]. Although PAMPs are generally considered to be just the first signal that is required for the stimulation of pro-IL-1β production, certain PAMPs also provide the second signal that activates inflammasomes to secrete IL-1β. MDP (through NLRP1, NOD2 and NLRP3)[135–138], cytosolic flagellin (through the NLRC4)[139,140], RNA (through NLRP3)[141] and cytosolic LPS in the presence of a pore-forming molecule streptolysin O (through NLRP3)[142] can activate the inflammasome through different NLRs. Furthermore, extracellular PAMPs, such as LPS, MDP, zymosan and flagellin, induce not only the production of pro-IL-1β by engaging the TLR–NF-κB pathway but also the extracellular release of endogenous ATP from human monocytes, which could activate the P2X purinoceptor 7 (P2X7), leading to ATP–P2X7-mediated caspase 1 activation and IL-1β secretion[143].

Although NLRs have been proposed to be sensors of cytosolic ligands, direct interaction between NLRs and PAMPs has never been confirmed. The main mechanism of NLR-mediated inflammasome activation may include pore formation, K^+ efflux, ROS production and lysosome damage[129]. Microbial molecules or intermediary signals induced by these molecules are thought to stimulate response mechanisms that lead to inflammasome activation.

Controlling caspase activation and cell death is an important aspect of virulence for several intracellular pathogens (FIG. 4). Recently, two *Francisella* spp. virulence genes (*FTT0584* (which encodes a protein of unknown function) and *FTT0748* (which encodes a putative transcription factor)) were found to contribute to growth and/or survival of the pathogen in mice[144]. However *Francisella* spp. mutants lacking these two genes replicate intracellularly with the same kinetics as wild-type bacteria. Surprisingly, *FTT0584–FTT0748* mutants induce higher levels of cell death and IL-1β secretion from activated macrophages compared with wild-type bacteria. These effects are ASC and caspase 1 dependent, suggesting that these two genes directly or indirectly inhibit or delay inflammasome activation by an as yet undefined mechanism[144].

Mycobacteria also prevent caspase 1 activation and secretion of IL-1β. The gene *zmp1* (also known as *Rv0198c*), which encodes a putative Zn^{2+} metalloprotease in *M. tuberculosis* and *Mycobacterium bovis*, is required for this process[145]. Infection of macrophages with mycobacteria that lack *zmp1* triggers the activation of caspase 1, resulting in increased IL-1β secretion,

improved mycobacterial clearance by macrophages and lower bacterial burden in the lungs of aerosol-infected mice[145]. Moreover, the ability of mycobacteria to maintain phagosome maturation arrest is lost in these mutants. In addition to preventing IL-1β activation, mycobacteria downregulate the synthesis of pro-IL-1β. There is also a substantial increase in the levels of accumulated $O_2^{\cdot-}$ during infection of macrophages with mycobacteria that lack *zmp1* when compared with infection with wild-type bacilli. The activation of caspase 1 in the absence of *zmp1* depends on NLRC4 and ASC[145]. The inhibition of caspase 1 or IL-1β expression using RNA interference prevents increased mycobacterial phagosome maturation, and this is associated with the *zmp1* mutation.

Yersinia spp. inject T3SS effectors into the host cell. The *Yersinia* spp. effector YopP efficiently shuts down NF-κB-dependent signalling pathways, preventing the production of pro-inflammatory cytokines (such as pro-IL-1β)[146,147]. Aside from the action of YopP, YopE and YopT on the Rho GTPase RAC1, *Yersinia* spp. interferes with the caspase 1-mediated maturation of pro-IL-1β in macrophages[148]. RAC1 can modulate the ASC-induced proteolytic activation of caspase 1 and corresponding pro-IL-1β maturation[148]. Furthermore, infection of naive macrophages by *Yersina* spp. results in a YopJ-dependent apoptosis, in which YopJ and TLR4 signalling are required for maximal activation of caspase 3 (REFS 146,147,149). This process could be advantageous for *Yersinia* spp. growth during early stages of infection. However in response to *Yersinia* spp. infection, activated macrophages suppress YopJ-dependent apoptosis and caspase 3 activation and simultaneously enhance pyroptosis, resulting in increased membrane permeability and release of active IL-18. In this case pyroptosis requires the bacterial T3SS but none of its known translocated effectors. Thus, regulating the mechanism of cell death is important for effective responses to *Yersinia* spp. infection, and activated macrophages resisting apoptosis are redirected to use pyroptosis facilitating host resistance. The redirection of macrophage death is a generalized host response to TLR stimulation[150]. Macrophage activation may overcome the ability of translocated *Yersinia* spp. effector proteins to inhibit the activation of caspase 1 (REF. 148).

Concluding remarks

The importance of PRRs for our immunity has become clear over the past decade. PRRS are crucial to detect different PAMPs and DAMPs and to coordinate signals that allow host cells to induce a range of defence mechanisms, including oxidative stress, autophagy, inflammasome activation and cell death. However, these PRRs are also targets for microorganisms to subvert both immune recognition and intracellular signalling. In addition to detecting components in the environment using two-component systems, bacteria have learnt how to avoid detection by PRRs. In fact, intracellular pathogenic bacteria have evolved sophisticated mechanisms of host pathway modulation. The coordinate actions of bacterial effectors allow bacteria to maintain extensive contacts with the host membrane compartments and host cytosol to maintain membrane homeostasis of vacuoles, acquire nutrients and avoid damage. These events are essential for their survival and for the establishment of an intracellular replicative niche. Therefore, the identification and the characterization of cellular and intracellular barriers is a priority for investigating new avenues for therapeutics.

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Competing interests statement

The authors declare no competing financial interests.

DATABASES

UniProtKB: http://www.uniprot.org
ActA | ASC | ATG7 | caspase 1 | EEA1 | IL-1β | IL-8 | NOS2 | listeriolysin O | NLRC4 | RAB5 | RAB7 | RAC1 | TLR3 | TLR7 | TLR8 | TLR9

Entrez Genome Project: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?DB=genomeprj
Brucella abortus | *Brucella melitensis* | *Chlamydia trachomatis* | *Escherichia coli* | *Francisella tularensis* | *Legionella pneumophila* | *Listeria monocytogenes* | *Mycobacterium bovis* | *Mycobacterium tuberculosis* | *S. enterica* subsp. *enterica* serovar Typhimurium | *Shigella flexneri* | *Yersinia pestis*

FURTHER INFORMATION

Jean-Pierre Gorvel's homepage: http://www.ciml.univ-mrs.fr/Lab/Gorvel.htm

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