INTERPLAY BETWEEN MYCOBACTERIA AND HOST SIGNALLING PATHWAYS

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Pathogenesis by mycobacteria requires the exploitation of host-cell signalling pathways to enhance the intracellular survival and persistence of the pathogen. The disruption of these pathways by mycobacteria causes impaired maturation of phagosomes into phagolysosomes, modulates host-cell apoptotic pathways and suppresses the host immune response. This review highlights the strategies employed by mycobacteria to subvert host-cell signalling and identifies key molecules involved in these processes that might serve as potential targets for new antimycobacterial therapies.

MACROPHAGES
Cells that belong to the mononuclear phagocyte system and are responsible for phagocytosis of foreign material.

PHAGOSOME
A vesicle that is formed by invagination of the plasma membrane during endocytosis and fuses with primary lysosomes to degrade engulfed material.

LYSOSOME
Membrane-limited cellular organelles with a low internal pH that contain acid hydrolases for the degradation of polymers such as proteins, RNA, DNA, polysaccharides and lipids.

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Tuberculosis (TB) is a major cause of mortality around the world, despite five decades of control programmes and the availability of efficacious drugs. TB still kills about two million people annually, and approximately one-third of the world's population is asymptomatically infected with *Mycobacterium tuberculosis*[1], the main causative agent of this disease. Although effective treatments are available, the spread of drug-resistant mycobacteria and the need for the extended use of current drugs means that there is an increasing need for the development of new therapeutic agents to combat TB.

M. tuberculosis belongs to the genus *Mycobacterium*, which comprises filamentous Gram-positive bacteria that are distinguished by complex surface lipids. The mycobacteria can be classified into species that cause TB in humans or in animals, including *M. tuberculosis* and *Mycobacterium bovis*, and species that are generally non-pathogenic, such as *Mycobacterium smegmatis* and *Mycobacterium vaccae*. Most mycobacteria, like *M. smegmatis*, can be readily isolated from environmental sources, such as soil and water. *M. tuberculosis*, however, is an obligate pathogen and has no natural reservoir outside humans, where its primary target cells are MACROPHAGES.

The successful parasitization of macrophages by pathogenic mycobacteria involves the inhibition of several host-cell processes, which allows them — unlike non-pathogenic species — to survive inside host cells (FIG. 1). The host processes that are inhibited by pathogenic bacteria include the fusion of PHAGOSOMES with LYSOSOMES, antigen presentation, apoptosis and the stimulation of bactericidal responses due to the activation of pathways involving mitogen-activated protein kinases (MAPKs), INTERFERON-γ (IFN-γ) and calcium (Ca^{2+}) signalling.

The modulation of host signalling mechanisms is a dynamic process requiring bacterial molecules that interfere with these pathways. As has been shown for several bacterial pathogens, the secretion of virulence mediator molecules is required for the modulation of host bactericidal responses (reviewed in REF. 2). A heterogeneous mixture of lipids and glycolipids are released from mycobacterial cells, in a vesicle-bound form, into the host cytoplasm, where they accumulate in late endosomal/lysosomal organelles[3]. These molecules might interfere with host signalling pathways, leading to an arrest of phagosomal maturation, modulation of host-cell apoptotic processes and suppression of the bactericidal response. The best-studied mycobacterial virulence factor is a cell-wall glycolypid, lipoarabinomannan (LAM), which has a phosphatidylinositol moiety that anchors it to the cell wall[4]. LAM contains multiple, branched arabinofuranosyl side chains that are either modified with mannose residues, to form

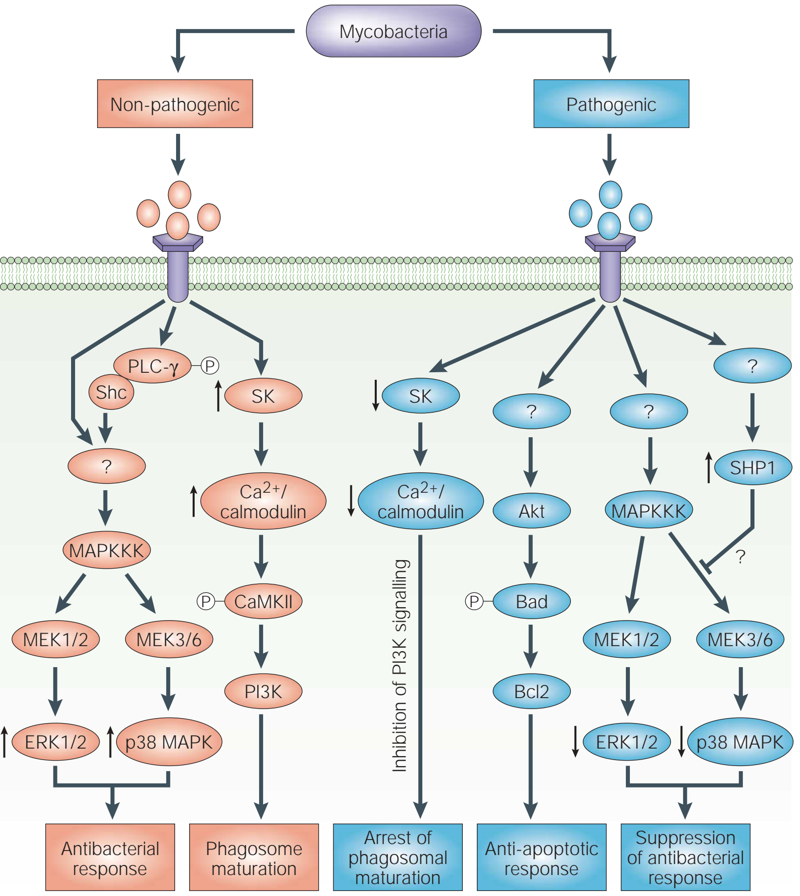

Figure 1 | **Overview of the differential regulation of host-cell signalling by pathogenic and non-pathogenic mycobacteria.** Pathogenic mycobacteria modify several host signalling pathways to enable them to survive inside host cells, including blocking phagosomal maturation, preventing apoptosis and suppressing the antibacterial immune response. By contrast, non-pathogenic or dead mycobacteria activate host signalling pathways that induce antibacterial responses and promote phagosome maturation. Pathogenic mycobacteria, in constrast to non-pathogenic mycobacteria, limit the activation of mitogen-activated protein kinase (MAPK) pathways in macrophages, thereby impairing the bactericidal immune response. Furthermore, it has been shown, that attenuated mycobacteria activate MAPK pathways in neutrophils by phosphorylation of phospholipase C-γ (PLC-γ)[58]. Heat-killed mycobacteria activate sphingosine kinase (SK), resulting in increased Ca^{2+}/calmodulin levels, which in turn leads to the maturation of phagosomes through the stimulation of calmodulin-dependent kinase II (CaMKII) activity and subsequent activation of phospatidylinositol-3-kinase (PI3K) signalling. By contrast, the Man-LAM virulence factor of pathogenic mycobacteria inhibits the rise in Ca^{2+}/calmodulin concentration, thereby preventing phagosomal maturation. Pathogenic bacteria also suppress host apoptotic pathways. Man-LAM promotes the phosphorylation of Bad through Akt, leading to its dissociation from Bcl-2, which is then able to exert its anti-apoptotic effects in the infected cells[40]. Man-LAM also activates Src-homology 2 (SH2) domain-containing tyrosine phosphatase 1 (SHP1), which — probably by dephosphorylation of certain host proteins such as MAPKs — inhibits the production of antibacterial agents in infected cells[60]. ERK, extracellular signal-related kinase; Man-LAM, mannose-capped lipoarabinomannan; MAPKKK, MAPK kinase kinase; MEK, MAPK/ERK kinase.

INTERFERON
A cytokine that activates the innate immune response, thereby preventing replication of pathogens.

Man-LAM, or with inositolphosphates, to form Ara-LAM. Man-LAM is abundant in slow-growing pathogenic mycobacteria, such as *M. tuberculosis*, whereas Ara-LAM is abundant in non-pathogenic mycobacteria. Mycobacteria also produce several proteins that undermine the host immune response, including eukaryotic-like kinases and protein tyrosine phosphatases.

This review highlights interference strategies that are used by mycobacteria to achieve intracellular survival, and explores how our knowledge of the molecules that are involved in the host–pathogen interaction can lead to the identification of new drug targets and the development of more efficient therapies for mycobacterial disease.

Mycobacteria affect phagosome maturation

Mycobacteria gain entry into macrophages through several cell-surface molecules, including members of the integrin family (such as the complement receptors (CRs) 1, 3 and 4), mannose receptors and Fcγ receptors[5]. Complement receptors are important phagocytic receptors of *M. tuberculosis* in macrophages, as shown by the fact that antibodies against CR3 inhibit mycobacterial internalization by 80% (REF. 6). Engagement of CRs by several bacterial pathogens blocks the production of harmful reactive oxygen intermediates by inhibiting the recruitment of NADPH oxidase to phagosomes[7,8]. However, current evidence indicates that although CR3 is important for internalization of *M. tuberculosis*, CR3-mediated phagocytosis has only a minor role in the intracellular survival and growth of mycobacteria. Infection of macrophages that were derived from *Cr3*-knockout mice showed no apparent alteration in mycobacterial survival[9]. So mycobacteria can survive and replicate intracellularly through other strategies that protect them from subsequent attack by antimicrobial components in the phagosomal maturation pathway.

Phagosomal maturation involves a series of sequential fusion events with various vesicles from the endocytic pathway, by which nascent phagosomes attain microbicidal properties and become phagolysosomes (FIG. 2). Phagolysosomes are acidic organelles that are rich in hydrolytic enzymes and which digest engulfed bacteria and other ingested particles. Immediately after phagocytosis, the phagosome acquires markers, such as Rab5 (a small GTPase) and EEA1 (early endosomal antigen 1), which direct the fusion of phagosomes with early endosomal vesicles (reviewed in REF. 10). During the course of maturation, the phagosomes lose Rab5 and acquire Rab7, another GTPase, which also functions in vesicular fusion. Late phagosomes acquire lysosomal markers, such as lysosome-associated membrane protein 1 (LAMP1), and acid hydrolysases, such as cathepsin D, through fusion with lysosomal vesicles[11]. Phagosomal maturation also involves the acquisition of vacuolar proton-ATPase molecules, which results in the acidification of phagolysosomes[12].

Pathogenic mycobacteria are directed to phagosomes that subsequently fail to fuse to lysosomes[13]. These phagosomes do not undergo further acidification, due to the absence of proton-ATPase molecules from the vacuolar membrane, and this reduced level of acidification allows the intracellular survival and growth of mycobacteria[12] (reviewed in REF. 14). Mycobacterial phagosomes are characterized by the presence of certain cellular proteins on their membranes, including tryptophane aspartate-containing coat protein (TACO; also known as mouse coronin 1)[15]

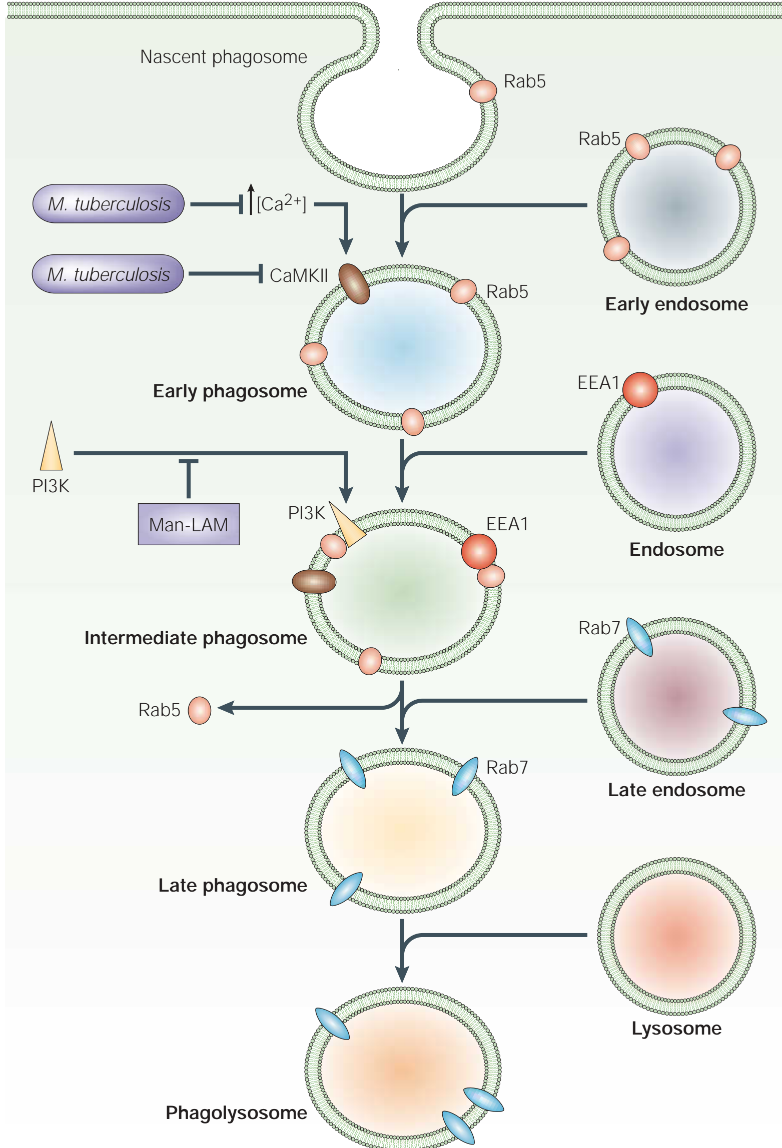

Figure 2 | **Pathogenic mycobacteria block the phagosomal maturation pathway.** On phagocytosis, nascent phagosomes acquire the GTPase Rab5 either from the plasma membrane or by fusion with early endosomes. Rab5 recruits phosphatidylinositol-3-kinase (PI3K), which generates phosphatidylinositol-3-phosphate (PI3P); PI3P mediates the recruitment of early endosomal antigen (EEA1) from endosomes. EEA1 is a Rab5 effector that triggers fusion of phagosomes with late endosomes. During the course of phagosomal maturation, early endosomal markers, such as Rab5 and EEA1, are lost from the intermediate phagosome, which then fuses with late endosomes and thereby acquires a second GTPase, Rab7. Late phagosomes fuse with lysosomes to form phagolysosomes, which are characterized by the presence of hydrolytic proteases, such as cathepsin D, and an acidic pH. Phagosomal maturation takes less than one hour. Ca^{2+} is a key regulator of phagosome maturation because it activates calmodulin and the calmodulin-dependent protein kinase CaMKII — which are necessary for recruitment of PI3K. To prevent phagosomal maturation, pathogenic mycobacteria block the rise in cellular Ca^{2+} concentration, $[Ca^{2+}]$, and thereby affect the association of phosphorylated CaMKII with the phagosomal membrane. In addition, Man-LAM (mannose-capped lipoarabinomannan) from *Mycobacterium tuberculosis* blocks the Ca^{2+}/calmodulin recruitment of PI3K to the phagosomes and thereby further obstructs phagosomal maturation.

and certain small GTP-binding proteins[16] (FIG. 3). TACO is recruited to and retained on the membranes of phagosomes that contain *M. bovis* bacille Calmette–Guérin (BCG); it is not present on the membranes of phagosomes that harbour killed mycobacteria, and is also absent from endosomal vesicles in uninfected cells (FIG. 3). The stable association of TACO with mycobacterial phagosomes is thought to inhibit the fusion of these phagosomes with lysosomal vesicles. However, one study indicated that although TACO is involved in the uptake of *M. bovis* BCG in human macrophages, it does not remain associated with phagosomes containing viable bacteria, and therefore does not render mycobacterial phagosomes unable to fuse with lysosomal vesicles[17]. In phagosomes harbouring *M. bovis* BCG, the loss of Rab5 from the membrane — which is seen in normal phagosomal maturation — does not take place, and Rab7 is selectively excluded from these phagosomes[18]. Furthermore, mycobacterial phagosomes do not recruit EEA1, which is essential for the fusion of lysosomal vesicles with phagosomes[19] (FIG. 2).

Ca^{2+} and PI3K signalling

Changes in the concentration of intracellular Ca^{2+} and PHOSPHATIDYLINOSITOL-3-KINASE (PI3K) activity are essential for proper phagosomal maturation[20–22], and pathogenic mycobacteria have been shown to interfere with Ca^{2+} and PI3K signalling pathways to impair this process. Alteration of the intracellular Ca^{2+} concentration is an important signalling mechanism in many cellular systems[23]. Ca^{2+} is a key second messenger that is released from intracellular stores and is involved in processes such as synaptic transmission, macrophage activation and apoptosis. The cytosolic Ca^{2+} concentration affects the phagosomal maturation process by modulating membrane fusion between phagosomes and lysosomal vesicles — which it does by regulating the activities of two Ca^{2+}-dependent effector proteins, calmodulin and the multifunctional serine/threonine protein kinase CaMKII[24] (FIGS 1 and 2). An increase in the intracellular concentration of Ca^{2+} leads to a change in the conformation of calmodulin, which in turn induces autophosphorylation and the subsequent activation of CaMKII[25]. CaMKII activation is required for the recruitment of EEA1 to the phagosomal membrane and for the regulation of bilayer fusion between endosomal vesicles[26].

Interference with Ca^{2+} signalling by mycobacteria. Macrophages that are infected with killed or antibody-opsonized *M. tuberculosis* show a sustained increase in cytosolic Ca^{2+} concentration compared with macrophages that are infected with live *M. tuberculosis*[27] (FIGS 1 and 2). Furthermore, a reduced viability of *M. tuberculosis* was seen in macrophages that were treated with a Ca^{2+} ionophore, which artificially increases cytosolic Ca^{2+} concentration. In addition, macrophages that were infected with live *M. tuberculosis* showed a significant reduction in the amounts of Ca^{2+}-bound calmodulin and phosphorylated CaMKII that were associated with the cytosolic face of the phagosomal membranes compared with phagosomes containing dead bacteria[24]. The delivery of lysosomal components to mycobacterial phagosomes can be blocked by using inhibitors of CaMKII or by chelating

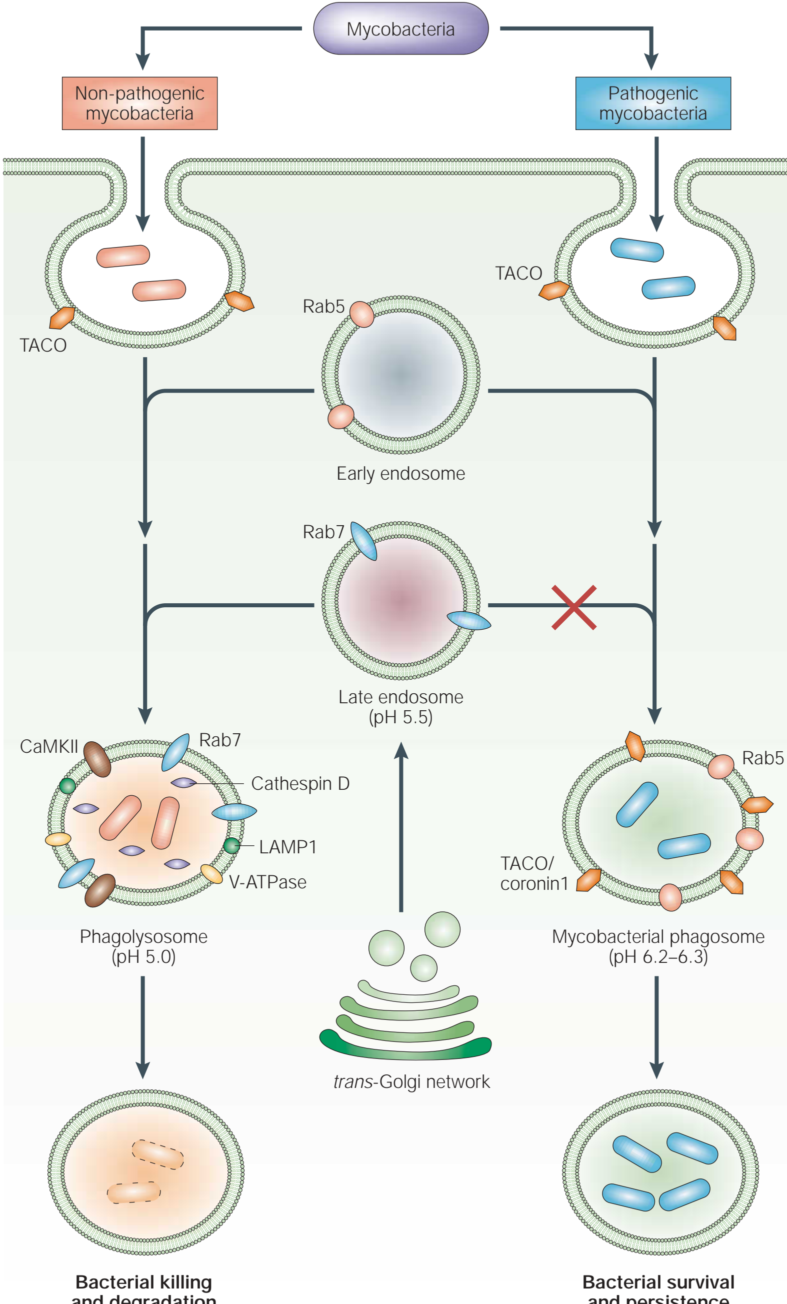

Figure 3 | **Comparison of phagosomes harbouring pathogenic or non-pathogenic mycobacteria.** Vacuoles containing pathogenic mycobacteria permanently display an actin-binding protein — tryptophane aspartate-containing coat protein (TACO) — and a small GTPase — Rab5 — on their outer membranes. These vacuoles contain little of the phosphorylated and activated form of calmodulin-dependent protein kinase II (CaMKII). Vacuoles containing inert particles or non-pathogenic mycobacteria show early endosomal markers, such as Rab5 and early endosomal antigen (EEA1). These vacuoles fuse with late endosomal vesicles and acquire proteins such as the proton-ATPase pump (V-ATPase), lysosome-associated membrane glycoprotein 1 (LAMP1) and lysosomal hydrolases such as cathepsin D — an aspartyl protease. Although vacuoles containing pathogenic mycobacteria do not fuse with lysosomes/late endosomes (represented by a red cross), they still acquire immature pro-cathepsin D from the *trans*-Golgi network, which provides an indication of their dynamic nature and accessibility to components of the endosomal pathway[12]. Finally, pathogenic mycobacterial are able to survive and replicate in the mycobacterial phagosome, whereas non-pathogenic mycobacteria are readily killed in phagolysosomes, which are rich in hydrolytic enzymes, have extremely low pH and possess several bactericidal peptides.

cytosolic Ca^{2+}. These data indicate that pathogenic mycobacteria are able to suppress the increase in cytosolic Ca^{2+} that results from host cell infection, and thereby inhibit Ca^{2+} signalling pathways, which would otherwise lead to phagosomal maturation. Inhibition of the increase in cytosolic Ca^{2+} concentration by *M. tuberculosis* is mediated by the lipid effector molecule Man-LAM, which is able to inhibit ionophore-induced increases in Ca^{2+} concentration in macrophages[28]. This effect is specific for LAM from pathogenic mycobacteria, as LAM from *M. smegmatis* does not block this increase.

Recent evidence indicates that *M. tuberculosis* prevents an increase in cytoplasmic Ca^{2+} concentration by the inhibition of a lipid kinase — sphingosine kinase (SK)[29] (FIG. 1). SK phosphorylates a host lipid, sphingosine, to form sphingosine-1-phosphate (S1P)[30], which is a ligand for specific G-PROTEIN COUPLED RECEPTORS and also regulates intracellular Ca^{2+} homeostasis by releasing Ca^{2+} from cytoplasmic organelles. Live *M. tuberculosis*, but not heat-killed bacteria, inhibits SK activity, which results in decreased production of S1P, and therefore, a reduced cytosolic Ca^{2+} concentration. Dihydroxysphingosine, a specific SK inhibitor, impairs phagosomal maturation. It remains to be determined whether Man-LAM inhibits SK activity to prevent the increase in cytosolic Ca^{2+} concentration.

Interference with PI3K signalling by mycobacteria. PI3K has been implicated in the recruitment of EEA1 to endosomes or phagosomes, as cells treated with wortmannin — a PI3K inhibitor — show reduced levels of EEA1 associated with early endosomes[31]. Recent evidence indicates that the recruitment of PI3K to the phagosomal membrane is dependent on its interaction with Ca^{2+}-bound calmodulin[32]. As Man-LAM inhibits the increase in cytosolic Ca^{2+}/calmodulin concentration, it blocks the association of Ca^{2+}/calmodulin with PI3K and thereby prevents the recruitment of EEA1 to phagosomes. In addition, inhibition of the PI3K pathway by Man-LAM also blocks the delivery of lysosomal proteins, such as hydrolases (for example, cathepsin D) and the membrane-docking fusion protein syntaxin 6, from the *trans*-Golgi network to phagosomes[33] (FIG. 3).

These findings indicate that Man-LAM blocks phagosome maturation by inhibiting a signalling cascade that consists of Ca^{2+}, calmodulin and PI3K. The arrest of phagosomal maturation by Man-LAM represents an effective mechanism that is used by mycobacteria for long-term survival in host cells. In view of the central role of Man-LAM in mediating the intracellular survival of mycobacteria, the genes that are involved in the biosynthetic pathway of Man-LAM represent potential targets for novel anti-TB drugs.

Interefence with host lipid signalling by mycobacteria. Recent studies indicate that mycobacteria might also inhibit phagosomal maturation by inhibiting host lipid-signalling pathways. This was shown in a recent study in which lipid molecules, such as ceramide or S1P, were added to cells that were infected with mycobacteria[34]. These lipids induce the assembly of actin molecules

Box 1 | Apoptosis and bacterial infection

Apoptosis, or programmed cell death, is a conserved physiological response to a wide variety of stimuli, which ultimately leads to the fragmentation and packaging of the cellular contents into vesicles — known as apoptotic bodies. These are taken up and recycled by neighbouring cells or macrophages of the immune system. The apoptotic programme is mainly activated by: the extrinsic pathway, which is initiated by the binding of ligands to death receptors; and the intrinsic pathway, which involves translocation of cytochrome *c* from mitochondria to the cytosol (FIG. 4). In both cases, the activation of the caspase (aspartate-specific cysteine protease) cascade and degradation of genomic DNA are characteristics of apoptotic cell death. Apoptosis is precisely controlled on many levels, and this involves the *Bcl-2* gene family, which consists of pro-apoptotic and anti-apoptotic members that are involved in regulating the release of cytochrome *c* (FIG. 4).

At present, the exact role of apoptosis in mycobacterial pathogenesis is unclear. Activation of anti-apoptotic proteins — for example, activation of Bcl-2 through the NF-κB pathway — might prolong host-cell survival, thereby offering a potential advantage to bacteria that persist and replicate. Conversely, inducing apoptosis might provide an advantage to bacteria by recruiting more immune cells to the site of infection, where they could become infiltrated by taking up apoptotic bodies containing pathogens. Whether macrophage death offers substantial advantages either to the bacteria or to the host is still uncertain[117]. However, it is likely that bacteria prevent apoptosis in the early phase of infection to allow them to replicate efficiently, but that they induce or are unable to prevent cell death in the later phase, which might facilitate their systemic dissemination through uptake into immune cells.

Apoptosis does not only involve the killing of pathogen-infected cells, but also contributes to the presentation of bacterial antigens to neighbouring antigen-presenting cells (APCs), which leads to T-cell stimulation. In the case of macrophages infected by *Salmonella* spp., apoptosis represents a mechanism by which antigens are presented to dendritic cells and T cells[118]. Recent evidence indicates that mycobacterial antigens are presented to APCs through small, extracellular apoptotic vesicles, which are secreted by infected macrophages[119]. The apoptotic presentation of antigens stimulates a broad spectrum of T-cell activity, including secretion of interferon-γ (IFN-γ). However, IFN-γ is unable to activate the bactericidal response in cells that are already infected with *M. tuberculosis*, but it might activate the antibacterial response in neighbouring uninfected cells.

around phagosomes, which is crucial for their fusion with endocytic organelles[35]. Actin molecules associate with endosomes and lysosomal organelles and guide their movement during vesicular fusion. Disruption of actin filaments abrogates the fusion of endosomes with lysosomes. Interestingly, phagosomes containing living, pathogenic mycobacteria failed to induce the formation of actin structures, whereas those containing non-pathogenic or dead mycobacteria readily induced actin assembly. Furthermore, the treatment of mycobacteria-infected cells with lipid molecules allowed actin assembly around the phagosomes and thereby induced fusion of the mycobacterial phagosomes with lysosomes. Consequently, by inhibiting the association of specific host lipid molecules with phagosomal membranes, mycobacteria block the fusion of phagosomes with lysosomes.

PHOSPHATIDYLINOSITOL-3-KINASE
(PI3K). PI3Ks are a conserved family of lipid kinases that phosphorylate the 3′-OH group of the inositol ring of membrane-bound phosphatidylinositides.

G-PROTEIN COUPLED RECEPTORS
(GPCRs). These cell surface receptors, which are characterized by seven transmembrane domains, are coupled to small G-proteins. Activation of GPCRs induces binding of GTP to the G-proteins, which leads to stimulation, or repression, of downstream signalling events.

Mycobacteria alter host apoptotic pathways

Macrophages that are infiltrated with potentially harmful bacteria activate their apoptotic programme to resolve the infection (BOX 1; FIG. 4). However, many bacterial pathogens alter host apoptotic pathways[36]. For example, infection of macrophages with virulent strains of *M. tuberculosis* induces much lower levels of apoptosis than does infection with attenuated strains[37].

Mycobacteria-induced macrophage apoptosis is a complex phenomenon that is modulated by mycobacterial virulence factors (reviewed in REF. 38), and mycobacteria are thought to influence the host apoptotic pathway through several mechanisms. First, Man-LAM has been shown to antagonize mycobacteria-induced apoptosis in murine macrophages by preventing the increase in cytosolic Ca^{2+} concentration that follows mycobacterial entry or treatment with a Ca^{2+} ionophore[28], as described above. Ca^{2+} is believed to facilitate apoptosis by increasing the permeability of mitochondrial membranes, thereby promoting the release of pro-apoptotic elements such as cytochrome *c*[39].

Man-LAM also stimulates the phosphorylation of the apoptotic protein Bad, which prevents it from binding to the anti-apoptotic proteins Bcl-2 and Bcl-X_L[40] (FIG. 4). Free, cellular Bcl-2 prevents the release of cytochrome *c* from the mitochondria, inhibits caspase activity and functions as an anti-apoptotic regulator in many systems[41], including mycobacteria-infected cells. The phosphorylation of Bad that is stimulated by Man-LAM involves activation of the Akt (protein kinase B) cascade, as shown by experiments in which phosphorylation of Bad was abrogated in macrophages transfected with a kinase-inactive mutant of Akt. Akt has an amino-terminal pleckstrin-homology (PH) domain, which is involved in the activation of Akt by binding several lipid molecules[42]. Binding of Man-LAM to the PH domain might stimulate the kinase activity of Akt and thereby block pro-apoptotic signals (FIG. 4).

M. tuberculosis also limits macrophage apoptosis by inducing the production of the immunosuppressive cytokine interleukin-10 (IL-10)[43]. IL-10 was shown to block the synthesis of tumour-necrosis factor-α (TNF-α) — a stimulator of apoptosis — in infected macrophages. TNF-α binds to death receptors to activate the apoptotic program (FIG. 4). IL-10 inhibits TNF-α activity by inducing the release of the soluble TNF receptor type 2 protein (TNFR2), which forms an inactive complex with TNF-α that prevents the induction of TNF-mediated apoptosis.

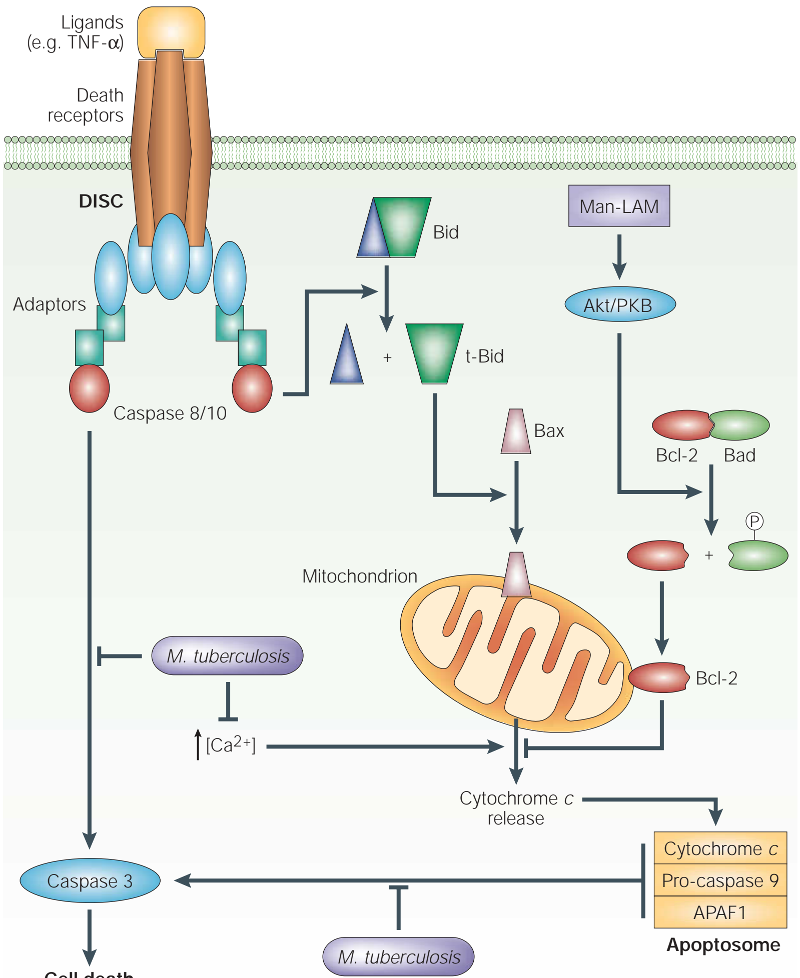

Figure 4 | **Inhibition of host apoptotic pathways by pathogenic mycobacteria.** The extrinsic pathway of apoptosis is initiated by the binding of ligands, such as tumour-necrosis factor-α (TNF-α), to death receptors. These receptors interact with adaptor proteins, which recruit and activate caspase 8 and/or caspase 10 to form the DISC (death-inducing signalling complex). This leads to the initiation of a caspase cascade, which ultimately leads to the activation of caspase 3 and other 'executioner' caspases, which digest important substrates in the cell to induce cell death. This extrinsic pathway of apoptosis can be amplified by activating an intrinsic apoptotic pathway, which is activated by cellular stress, for example. The link is mediated by the cleavage of Bid (a member of the Bcl-2 family) by caspases 8/10 to produce t-Bid. t-Bid mediates the assembly of pro-apoptotic members of the Bcl-2 family (for example, Bax and Bak) into hetero-oligomeric complexes that form pores in the outer membrane of the mitochondria, resulting in the release of apoptosis-regulating factors such as cytochrome *c*. Together with pro-caspase 9 and APAF1 (apoptosis-activating factor 1), cytochrome *c* forms the apoptosome, which induces activation of caspases 9 and 3 and triggers cell death. The release of cytochrome *c* can be inhibited by Bcl-2 and related anti-apoptotic proteins. Bcl-2 is regulated by binding to Bad, and for Bcl-2 to exert its anti-apoptotic activity, the Bcl-2/Bad complex must be broken down by phosphorylation of Bad by Akt. *Mycobacterium tuberculosis* induces Akt activity through the Man-LAM virulence factor to block activation of the intrinsic apoptotic pathway. *M. tuberculosis* can also prevent the activation of caspases[38], and inhibit the extrinsic apoptotic pathway by stimulating the release of IL-10 from infected macrophages, which leads to inhibition of TNF-α production.

NEUTROPHILS
Polynuclear leucocytes belonging to the myeloid lineage that migrate to sites of infection or wounds and mediate the inflammatory response.

In contrast to their inhibition of apoptosis during the early stages of infection, mycobacteria might induce apoptosis in the acute phase so as to infect neighbouring cells. Recent evidence indicates that mechanisms that are involved in generating an apoptotic response are mediated by activation of apoptosis signal-regulating kinase 1 (ASK1), which is a member of the MAPK family (discussed in detail in the next section)[44]. ASK1 activates the downstream p38 MAPK, which can induce the expression of pro-apoptotic proteins, such as caspases. Apoptosis of macrophages by *Mycobacterium avium* can be blocked by the transfection of cells with catalytically inactive mutants of ASK1 and p38 MAPK.

Interestingly, microarray data from NEUTROPHILS infected with a diverse group of bacterial pathogens — such as *Listeria monocytogenes* and *Streptococcus pyogenes* — revealed that ASK1 was upregulated in all infected cells[45]. This might represent a common pattern in the pathogen–host apoptosis differentiation programme and resolution of the host inflammatory response. However, we are still far from having a clear picture of how pathogenic, in comparison with non-pathogenic, mycobacteria are able to subvert the apoptosis signalling machinery of host cells. Future studies of the mechanisms that are involved in mycobacterial modulation of macrophage survival and death will provide a useful insight in understanding host–mycobacteria interactions and could lead to the identification of potential targets for the control of mycobacterial infections.

Interference with MAPK and JAK/STAT pathways

Pro-inflammatory CYTOKINES, such as IL-1, IL-6, TNF-α and interferons, induce a cellular INNATE IMMUNE RESPONSE when invading bacteria are detected. The release of pro-inflammatory cytokines results in local tissue damage and enhanced recruitment of potential defence cells to the site of infection. The activation of host-cell signalling cascades, such as the MAPK or JAK/STAT (Janus kinase/signal transducer and activator of transcription) pathways, results in the production of pro-inflammatory cytokines and chemokines. Pathogenic — but not non-pathogenic — mycobacteria have evolved mechanisms to suppress these signal transduction cascades and thereby attenuate the cytokine-induced immune response.

Modulation of MAPK signalling. MAPKs are evolutionarily conserved enzymes that are important in cellular signal transduction. Three main families of MAPKs are found in mammalian cells: the c-Jun N-terminal kinases (JNKs 1, 2 and 3); the extracellular signal-related kinases (ERKs 1 and 2); and the p38 MAPK (p38 α, β γ and δ)[46]. ERK1/2 and p38 become activated through the phosphorylation of crucial tyrosine and threonine residues by upstream kinases (FIG. 5). MAPKs themselves phosphorylate a range of substrates, including transcription factors such as activator protein 1 (AP1), thereby controlling a wide spectrum of cellular responses, such as the synthesis of pro-inflammatory cytokines like IL-1, TNF-α and IL-12.

The activation of MAPK signalling in macrophages that are infected with non-pathogenic mycobacteria leads to the synthesis of various microbicidal molecules, including TNF-α, which mediate antibacterial and inflammatory immune responses[47]. Inhibitors of ERK1/2, such as PD98059, and of MAPK/ERK kinase 1 (MEK1), such as U0126, lead to decreased secretion of TNF-α and

further enhance the growth of pathogenic mycobacteria in human macrophages[48]. These observations are supported by a study that demonstrated that the secretion of TNF-α by macrophages infected with *M. avium* is dependent on MEK1 and ERK1/2 activation[49]. A high level of TNF-α is a crucial factor for controlling primary infection, as it induces the expression of other pro-inflammatory cytokines — such as IL-1 — and of several chemotactic cytokines, which attract immune cells to the site of infection.

In a number of bacterial species, the modulation of MAPK activity is thought to be an effective virulence strategy[50]. For example, in *Yersinia pseudotuberculosis*, a secreted cysteine protease, YopJ, inhibits the innate immune response by blocking activation of the MAPK and nuclear factor-κB (NF-κB) pathways, thereby inhibiting the synthesis of pro-inflammatory cytokines such as TNF-α[51]. YopJ disrupts post-translational modifications — such as ubiquitylation — of several proteins that are involved in the MAPK pathway, and also blocks the phosphorylation of MAPK kinases (MAPKKs), thereby impairing cellular signalling[52]. Similarly, in the case of *Salmonella enterica* serovar Typhi, a protein known as SptP, which has both tyrosine phosphatase activity and GTPase-activating protein (GAP) activity, inhibits the activation of Raf — a MAPKK kinase (MAPKKK) — and thereby blocks MAPK pathways[53].

Mycobacteria also modify MAPK signalling to promote their survival in host cells. The regulation of MAPK pathways by mycobacteria has been analysed by comparing changes in host gene expression that are induced by virulent and attenuated strains. One example of this is a study using an ISOGENIC pair of *M. avium* MORPHOTYPES, SmT and SmO, which represent a more virulent and a less virulent phenotype, respectively[54]. It was shown that during the first 15 minutes following infection, the induction of p38 phosphorylation in murine macrophages was similar for both strains. However, only less virulent strains elicited a sustained activation of p38 (FIG. 1). Another study has shown that entry of the virulent *M. avium* strain causes early activation of the p38 and ERK1/2 pathways, which, in contrast to infections with the non-pathogenic *M. smegmatis* or *Mycobacterium phlei*, is quickly lost[47].

In vivo data about the role of the p38 cascade in mycobacterial infections originate from a study involving the treatment of mice that had been infected with pathogenic mycobacteria with the p38 inhibitor SB203580 (REF. 55). The treated mice showed enhanced survival of pathogenic mycobacteria in various organs, but also increased cytokine levels. These results imply that the inhibition of p38 has a role in enhanced bacterial survival. The observed increase in cytokine levels might be due to the inhibition of kinases other than p38 by SB203580 (REF. 56). In summary, pathogenic mycobacteria have evolved mechanisms to prevent a sustained activation of the ERK1/2 and p38 cascades, and this accounts, at least in part, for their survival.

Although mycobacteria reside mainly in macrophages, and activated macrophages are central to protection against *M. tuberculosis*, polymorphonuclear granulocytes have a significant protective role in the early phase of TB infection[57]. Infection of neutrophils with the attenuated *M. tuberculosis* H_{37}Ra strain leads to the tyrosine phosphorylation of several host proteins, including phospholipase C-γ2 (PLC-γ2)[58] (FIG. 1). PLC-γ2 is a lipid-metabolizing enzyme that regulates several functions of neutrophils, such as the generation of bactericidal compounds, including reactive oxygen intermediates[59]. Activation of PLC-γ2 leads to its

Figure 5 | **Disruption of macrophage signalling pathways by mycobacteria.** Mitogen-activated protein kinase (MAPK) signalling pathways are activated by stimuli such as pathogen entry, cytokines and growth factors, which lead to a cascade of kinase activity that ultimately results in the activation of MAPKs — for example, p38, extracellular signal-related kinases (ERKs) and Jun N-terminal kinases (JNKs). Activated MAPKs phosphorylate substrates such as transcription factors — for example, activator protein 1 (AP1) and nuclear factor (NF)-κB — which leads to the production of inflammatory mediators like tumour-necrosis factor-α (TNF-α) and interleukin (IL)-1. Pathogenic mycobacteria suppress this host response by inhibiting the activation of p38 and ERK1/2. The binding of INF-γ to its receptor leads to the recruitment of Janus kinases (JAKs), which bind to the intracellular domain of the receptor, leading to its tyrosine phosphorylation and subsequent association with the signal transducer and activator of transcription (STAT) protein. Phosphorylated STAT is then translocated to the nucleus, where it activates the transcription of interferon (IFN)-γ target genes, leading to a potent anti-bacterial response. Pathogenic *Mycobacterium avium* interferes with the JAK/STAT signalling pathways by downregulating the expression of the IFN-γ receptor, whereas *M. tuberculosis* affects the DNA-binding activity of STAT1, which leads to reduced transcription of IFN-γ-responsive genes. MAPKK, MAPK kinase; MAPKKK, MAPK kinase kinase; MEK, MAPK/ERK kinase.

CYTOKINES
Low-molecular-weight proteins that are important for immunity, inflammation and development, and which contribute to the pathophysiology of acute and chronic infections.

INNATE IMMUNE RESPONSE
A cellular defence reaction to counteract invading pathogens such as bacteria and viruses. It uses interferon-dependent signalling and leads to the activation of genes that are responsible for bactericidal or antiviral responses.

ISOGENIC
Having identical genotypes.

MORPHOTYPE
A member of one form of a polymorphic species.

association with an adaptor protein, Shc, and signal transduction through the small GTP-binding protein Ras, which in turn activates a cascade composed of MAPKKs — for example, Raf — and downstream MAPK elements[58]. It will be interesting to determine whether virulent mycobacteria inhibit the tyrosine phosphorylation of PLC-γ2 to downregulate MAPK signalling pathways.

In addition, Man-LAM is able to block phorbol acetate-induced phosphorylation of MAPK in a human monocytic cell line and also induces the tyrosine phosphorylation and increased phosphatase activity of Src-homology 2 (SH2)-domain-containing tyrosine phosphatase 1 (SHP1)[60]. Activated SHP1 was able to dephosphorylate MAPK *in vitro*, which indicates that pathogenic mycobacteria might limit activation of MAPK in infected cells by the upregulation of SHP1 activity. However, it is unclear whether activation of SHP1 is essential for mycobacterial survival in host cells.

Modulation of JAK/STAT signalling. Tyrosine phosphorylation of JAK and STAT has been shown to be essential for the antibacterial response[61]. Phosphorylation of JAK1/2 and STAT is mediated by the binding of IFN-γ to its cell surface receptor (FIG. 5), which leads to the activation of a strong bactericidal response, including the production of reactive oxygen and nitrogen intermediates, and the synthesis of cytokines, such as IL-12 and TNF-α (REF. 62). A recent study indicates that IFN-γ also hinders the replication of mycobacteria by inducing the expression of a GTPase, LRG-47, which promotes phagosomal maturation[63].

Pathogenic mycobacteria have evolved mechanisms to suppress the IFN-γ and JAK/STAT signalling pathways[64]. Cells that are infected with virulent *M. avium* show decreased levels of the IFN-γ receptor, which impairs the tyrosine phosphorylation of JAK1/2 and reduces the DNA-binding activity of STAT. However, another study indicates that infection of macrophages with *M. tuberculosis* does not affect either the tyrosine phosphorylation of STAT or its nuclear translocation, although it does diminish its association with transcriptional co-activators, such as CREB, which leads to decreased expression of IFN-γ-regulated genes[65]. Moreover, the immune response to mycobacterial infection is impaired in TB patients with heterozygous germline mutations of *STAT*, which predispose individuals to TB and other intracellular infections[66].

The details of the mechanisms by which pathogenic mycobacteria suppress the activation of the IFN-γ signalling pathway remain to be determined. Recent evidence indicates that certain mycobacterial lipids, such as trehalose 6,6′-dimycolate — which is also known as cord factor and is an important component of the *M. tuberculosis* cell wall — induces expression of SOCS (suppressor of cytokine signalling) proteins, which directly bind and inactivate JAKs and thereby block JAK/STAT signalling pathways[67]. In summary, the inhibition of induction of IFN-γ-inducible genes might be one mechanism by which mycobacteria circumvent or modulate the IFN-γ-mediated host defence response.

Modulation of signalling in dendritic cells

Dendritic cells (DCs) have an important role in the ADAPTIVE IMMUNE RESPONSE to bacterial infections. Like macrophages, immature DCs phagocytose bacteria and consequently undergo considerable changes, resulting in DC maturation and activation and differentiation of T cells. Depending on the type of pathogen that is recognized by the DCs, T cells differentiate into T helper 1 (T_H1) cells, which secrete IFN-γ, or T_H2 cells, which secrete IL-4. IFN-γ induces the killing of intracellular pathogens, whereas IL-4 is effective against extracellular pathogens.

Toll-like receptors (TLRs) and C-type lectins are expressed on the surface of DCs (FIG. 6), and these interact with several pathogens (reviewed in REF. 68). TLRs are phylogenetically conserved receptors that recognize pathogen-associated molecular patterns to establish innate immunity and activate immune cells against these micoorganisms. They are linked to the NF-κB and MAPK pathways and are involved in DC maturation and the production of inflammatory cytokines. By contrast, C-type lectins recognize a wide variety of pathogens, such as yeast, viruses and bacteria, through their diverse carbohydrate structures. This leads to the internalization of the pathogen and processing of antigens for presentation by major histocompatibility complex (MHC) molecules to T cells. Several pathogens have evolved strategies to subvert the function of DCs and, therefore, suppress the immune response.

Viable mycobacteria or lipopolysaccharide (LPS) induce the maturation of human DCs — probably through TLR2- and TLR4-dependent signalling pathways[69]. Furthermore, mycobacterial lipoproteins, such as PIM (phosphatidylinositol monomannoside), Man-LAM and the 19-kDa antigen, stimulate TLR2 to produce a pro-inflammatory response, which can promote mycobacterial killing[70] or induce apoptosis in the infected cells[71,72].

DC maturation leads to the production of inflammatory cytokines and the activation of a T-cell response, which in turn leads to the killing of the pathogen. In cases in which the immune response is insufficient to kill the pathogen, increased secretion of Man-LAM by infected macrophages or DCs leads to the binding of Man-LAM to the C-type lectin DC-SIGN (DC-specific intracellular-adhesion molecule-grabbing non-intergrin) (FIG. 6). This blocks the maturation of DCs that are attracted to the site of infection and thereby suppresses T-cell activation[73,74]. Binding of Man-LAM to DC-SIGN blocks the *M. bovis* BCG-induced or LPS-induced maturation of DCs. Preventing this binding using DC-SIGN-specific antibodies allows *M. bovis* BCG-induced or LPS-induced DC maturation to occur. Man-LAM inhibits the LPS-induced production of IL-12 by DCs, which indicates that it interferes with LPS signalling pathways that are mediated by TLRs[75]. So, Man-LAM interferes with DC maturation signalling to prevent an appropriate immune response.

Binding of Man-LAM to DC-SIGN also induces a signalling cascade that results in the secretion of the

CREB
cAMP response element (CRE)-binding protein. It stimulates the basal transcription of CRE-containing genes and mediates induction of transcription following phosphorylation by protein kinases.

ADAPTIVE IMMUNE RESPONSE
This involves specificity and immunological memory. It is mediated by T and B cells through activation of cytotoxic $CD8^+$ T cells for pathogen killing, or by interaction with $CD4^+$ T cells for antibody production.

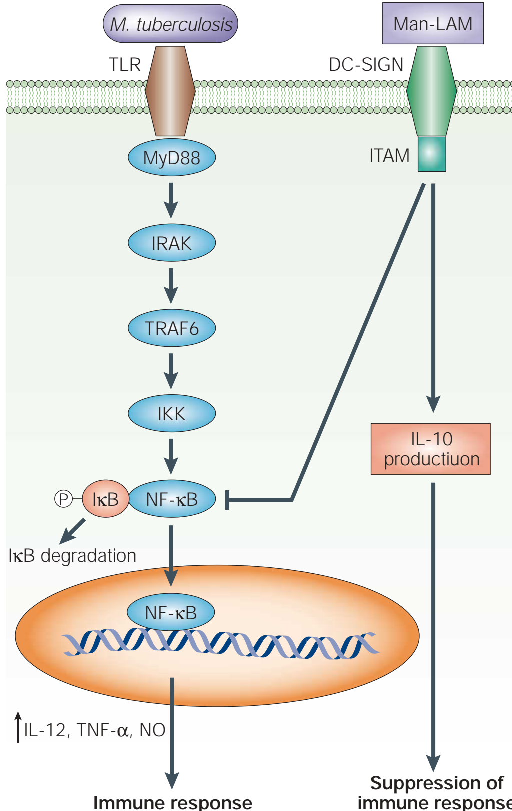

Figure 6 | **Disruption of dendritic-cell signalling pathways by mycobacteria.** Dendritic cells (DCs) express DC-SIGN (DC-specific intracellular adhesion molecule-grabbing non-integrin) and Toll-like receptors (TLRs) on their surfaces. TLRs initiate signalling by binding an adaptor protein, MyD88, which in turn recruits a serine/threonine kinase, IRAK (interleukin-1 receptor-associated kinase). IRAK then associates with the adaptor protein TRAF6 (TNF-receptor-associated factor 6). This leads to the activation of the IKK (inhibitor of κB kinase) complex, which phosphorylates IκB, an inhibitor of NF-κB. Phosphorylation of IκB causes its degradation and thereby allows translocation of NF-κB to the nucleus. Binding of *M. tuberculosis* to TLRs results in activation of NF-κB, which leads to DC maturation and production of immunostimulatory cytokines that activate T cells and mediate killing of pathogenic mycobacteria. However, Man-LAM from pathogenic mycobacteria binds to DC-SIGN and inhibits TLR signalling. This blocks DC maturation, enhances the production of the immunosuppressive cytokine IL-10 and, as a consequence, the activation of T cells is impaired. ITAM, tyrosine-containing activation motif.

anti-inflammatory and immunosuppressive cytokine IL-10 (REF. 73). IL-10 is an inhibitor of activated DCs and macrophages and, as such, controls innate as well as cell-mediated immunity. IL-10 blocks the production of pro-inflammatory cytokines, such as IL-12 and TNF-α, and reduces the expression of MHC class II molecules, which are required for antigen presentation[76]. The production of IL-10 that is induced by Man-LAM in DCs impairs the maturation of DCs. So, mycobacterial subversion of TLR signalling pathways through the activation of DC-SIGN imparts a selective advantage to the bacteria in avoiding clearance through recognition by T cells. The balance between the stimulation of TLRs and C-type lectins by pathogens might be implicated in the overall immune response — immune activation or immune suppression.

The DC-SIGN signalling pathways that lead to production of anti-inflammatory molecules are not well understood. However, tyrosine-containing activation motifs (ITAMs) at the carboxy terminus of DC-SIGN might be involved in its signalling pathways[77]. ITAMs are involved in the phosphorylation of specific receptor proteins that interact with the SH2 domain of some tyrosine kinases and phosphatases[78]. The interaction of Man-LAM with the ITAM motifs of DC-SIGN receptors might be important for mediating signals that lead to the production of anti-inflammatory responses and inhibition of TLR signalling pathways. Further studies designed to elucidate mycobacterium-induced signalling pathways through DC-SIGN are expected to contribute to an understanding of the mechanism by which the adaptive immune response is suppressed in infected patients.

It is already known that pathogenic mycobacteria prevent the adaptive immune response by interfering with antigen processing and presentation in antigen-presenting cells, such as macrophages or DCs (reviewed in REF. 14). Mycobacteria use several strategies for suppressing antigen presentation, including sequestering mycobacterial antigens from molecules that are required for T-cell activation[79] and downregulation of the expression of MHC class II molecules[80,81], and co-stimulatory molecules like CD1 (REFS 81,82). Mycobacterial lipids, such as the 19-kDa lipoprotein, downregulate expression of MHC class II molecules and interfere with the presentation of antigens in infected macrophages[83,84]. So, the capacity of pathogenic mycobacteria to alter the process of antigen presentation represents an effective strategy for inhibiting the immune response.

Mycobacterial kinases and phosphatases

Protein kinases are essential for virulence in a number of bacterial species. For example, *Yersinia* spp. secrete a kinase (YpkA) into the host cytoplasm, where it phosphorylates specific proteins to prevent bacterial uptake, and thereby allows the bacteria to avoid killing by macrophages[85]. *Yersinia* mutants that lack YpkA are avirulent in mice[86]. Examination of the *M. tuberculosis* genome sequence shows the presence of several eukaryotic-like protein kinases and phosphatases[87], which might mediate signalling between mycobacteria and host cells to establish an environment that is favourable for replication and survival of mycobacteria.

Mycobacterial serine/threonine protein kinases. The *M. tuberculosis* genome contains eleven eukaryotic-like serine/threonine protein kinases (STPKs), of which five have been characterized in detail[88–91]. Comparative genomic analysis of *Mycobacterium leprae* and *M. tuberculosis* showed extensive gene decay in *M. leprae* that has removed or inactivated about 2,400 genes. These include the genes that encode the STPKs, with the

exception of those that encode PknA, PknB, PknG and PknL[92], which implies that these kinases are essential for mycobacterial growth or pathogenesis. To identify the genes that are required for optimal mycobacterial growth, a library of transposon-insertion mutants of *M. tuberculosis* was constructed[93]. Using this technique, only PknA, PknB and PknG out of the 11 mycobacterial kinases seem to affect growth *in vitro*. However, it is possible that the other STPKs, which are not essential for *in vitro* growth, might help the bacteria to adapt to the hostile intracellular environment.

Mutant *M. tuberculosis* with an inactivated *pknG* gene are highly attenuated in immunocompetent mice, and infection with these bacteria results in delayed mortality in immunodeficient mice[94]. So, PknG mediates mycobacterial survival in host cells. PknG might block the maturation of phagosomes by phosphorylating cellular proteins, thereby mediating the survival of mycobacteria in host cells. To elucidate the underlying mechanism for disruption of phagosomal maturation, the cellular substrates of PknG need to be identified.

Mycobacterial protein tyrosine phosphatases. Phosphatases have central roles in signal pathways as they oppose the effects that are mediated by protein kinases. Protein tyrosine phosphatases (PTPases) have been identified in a number of bacterial species and are essential for their development and pathogenesis[95]. *Y. pseudotuberculosis*, an extracellular pathogen, secretes the PTPase YopH, which dephosphorylates host focal adhesion proteins, such as p130^{cas}, paxillin, and focal adhesion kinase. This leads to destabilization of focal adhesions that are involved in the internalization of bacteria by eukaryotic cells[96,97]. So, YopH prevents uptake of bacteria by the host immune cells, thereby allowing the pathogen to replicate extracellularly. Similarly, during the internalization of *Salmonella enterica* serovar Typhimurium into intestinal cells, a bacterially encoded PTPase — SptP — mediates the reversal of the actin cytoskeleton reorganization that is induced by bacterial entry. SptP interacts with small GTPase-binding proteins — namely, Cdc42 and Rac1 — and thereby restores the normal actin cytoskeletal architecture of the host cells despite the uptake of a large number of internalized bacteria[98].

M. tuberculosis has two functional PTPases — MptpA and MptpB — which are secreted into the culture supernatant by growing mycobacterial cells[99]. As the mycobacterial genome lacks tyrosine kinases, the presence of the two secretory tyrosine phosphatases indicates that they might be involved in the dephosphorylation of host proteins. Indeed, when the *mptpB* gene was deleted from *M. tuberculosis*, the mutant strain was attenuated in the lung and spleen of infected animals[100]. Furthermore, wild-type and mutant *mptpB* strains were equally able to survive in resting macrophages, but the ability of *mptpB* mutants to survive in macrophages activated with IFN-γ was highly impaired. This indicates that MptpB might mediate mycobacterial survival in host cells by dephosphorylating proteins that are involved in IFN-γ signalling pathways.

Novel targets for TB therapeutics

No new compound has been developed for the treatment of TB since the introduction of the antibiotic rifampicin in 1962. At present, treatments for TB are far from adequate, requiring the administration of up to four drugs for 6–9 months. Furthermore, the spread of multidrug-resistant mycobacteria adds to the urgent need for the discovery of new drug targets. Any new therapy should also address the problem of efficacy against persistent TB bacteria, which persist within infected patients for undefined periods of time without displaying any symptoms of clinical disease[101]. The prolonged therapy that is required for the treatment of TB is a consequence of the presence of persistent bacteria, as TB drugs that are available at present are effective primarily against actively replicating bacteria.

The sequencing of the complete genome of *M. tuberculosis* has greatly increased the number of possible targets against which new antimycobacterial agents can be developed. However, after target identification and validation, potent modulators of the target need to be identified, optimized and finally tested in an animal model for the development of a clinical drug candidate (BOX 2).

Mycobacterial kinases and phosphatases as drug targets. Kinases and phosphatases are attractive therapeutic targets owing to the ease with which specific inhibitors against these molecules can be developed, and their central role in cellular signalling. Several kinase and phosphatase inhibitors have been identified in the development of new drugs for the treatment of several diseases, such as cancer[102].

Inhibitors of protein kinases can prevent the uptake of *M. leprae* by peritoneal macrophages in mice[103]. Although the inhibitors that were used in this study — for example, staurosporine — were relatively non-selective, this study provided the first indication that protein kinases might be important in regulating the entry and phagocytosis of mycobacteria in macrophages. Subsequently, a small-molecule kinase inhibitor — 1-(5-isoquinolinesulphonyl)-2-methylpiperazine, a sulphonyl compound belonging to the H-series — was found to inhibit *in vitro* growth of *M. bovis* BCG, and also inhibited the kinase activity of the *M. tuberculosis* kinase PknB[104]. As PknA, PknB and PknG are required for the growth of mycobacteria *in vitro*[93], any compound that specifically blocks these kinases might be a potential candidate for a new antimycobacterial agent. In addition, rational design of PknB-specific inhibitors can be undertaken with the information that is available from the recently described PknB X-ray crystal structure, which will greatly accelerate the development of PknB inhibitors[105]. Furthermore, the use of the available knowledge about the characteristic structures of certain protein kinases, such as the ATP-binding pocket of PknB, enables the development of inhibitors for other members of this target family, which might be useful across different therapeutic indications[106].

Box 2 | **Development of drugs against bacterial kinases and phosphatases**

The characterization of protein kinases and phosphatases has revealed new targets for the development of drugs for several indications, including diabetes, inflammatory disorders and cancer. The identification of several kinases and phosphatases as being essential for mycobacterial pathogenesis makes them attractive targets for antimycobacterial therapies.

The development of a novel antimicrobial drug begins with the identification of a target protein, the modulation of which might inhibit or reverse disease progression. Several techniques are used for the identification of novel targets — such as allelic-exchange mutagenesis or high-density mutagenesis — that can be used to knock out a particular gene from the bacterial genome. Furthermore, the use of antisense RNA to downregulate mRNA expression might help to validate essential genes involved in bacterial growth or pathogenesis.

After the validation of a kinase or a phosphatase as a drug target, small compounds that modify their activity can be identified by screening compound libraries using purified enzyme. Hits from these biochemical screens are further selected by criteria such as physical properties — for example, cellular permeability, microsomal stability and solubility. The most promising compounds are then tested for their ability to inhibit mycobacterial growth *in vitro* and to determine the minimal inhibitory concentration (MIC). In addition, these compounds are further evaluated for cytotoxicity in cultured cell lines (toxicity profiling) and are also used for identifying inhibition of related host kinases or phosphatases (selectivity profiling).

Potent hits are then tested in macrophages that are infected with pathogenic mycobacteria. Compounds that perform well in these infection assays are further selected on the basis of a favourable eADME (early drug absorption, distribution, metabolism and excretion) profile. This helps to optimize compounds with promising pharmocokinetic and pharmacodynamic properties. Positive candidates, known as 'lead compounds', are tested in a low-dose aerosol infection mouse model of TB, which measures the bacillary load in lungs of infected mice. Successful compounds — so-called 'pre-clinical candidates' — are then further evaluated in clinical settings.

In addition to STPKs, the mycobacterial genome encodes several two-component systems, which consist of histidine kinases and their associated response regulators. These control the expression of target genes in response to stimuli that are involved in chemotaxis, phototaxis, osmosis, nitrogen fixation and intracellular survival[107]. The histidine kinases from various bacteria also present novel targets for the development of new kinase inhibitors. MtrA[108] and SenX3[109], histidine kinases that are essential for mycobacterial virulence and persistence in mice, could also be good targets for the development of new drugs for persistent TB bacteria.

Recent advances in the development of inhibitors specific for PTPases for the treatment of diseases such as type 2 diabetes have greatly enhanced our knowledge of phosphatase inhibitor design and function, and have shown that phosphatases are indeed good drug targets[110]. As a result, there is growing interest in the development of potent and specific inhibitors of these enzymes to treat several bacterial diseases. For example, several PTPase inhibitors have already been reported that could potentially be developed as novel drugs against *Salmonella* and *Yersinia* infections[111]. In view of the important role of PTPases in the survival of mycobacteria in mice, MptpB might be a valuable TB target.

Other potential targets for antimycobacterial drugs. In recent years, scientists have identified and characterized several new *M. tuberculosis* enzymes — such as isocitrate lyase (ICL), malate synthase(MS) and cyclopropane synthases (CS) — which could be potential drug targets (reviewed in REFS 111,113). ICL and MS are enzymes of the GLYOXYLATE SHUNT and are required for establishment of a persistent infection by mycobacteria. CS belongs to a family of enzymes that modify cell envelope lipids with different cyclopropane rings, which are important for mycobacterial pathogenesis and persistence. Other potential targets include proteins that are involved in mycobacterial virulence or the biosynthesis of cell wall components (reviewed in REF. 114).

Recent advances in our understanding of the fundamental aspects of the interaction of mycobacteria with host cells, as described in this review, provide a platform for a rational approach to the development anti-TB drugs. For example, the genes that are required by *M. tuberculosis* to resist the harmful effects of reactive nitrogen intermediates generated in phagolysosomes have been identified and could be useful targets[115]. The products of these genes form a proteasome-like organelle, which degrades or repairs mycobacterial proteins that are damaged by reactive nitrogen intermediates. A comparison of the intraphagosomal gene-expression profile of *M. tuberculosis* in both resting and IFN-γ-activated macrophages using mycobacteria grown in broth culture identified several genes that are involved in induction of persistence, fatty-acid metabolism, and resistance to nitric oxide (NO)[116]. Further characterization of these gene products will provide information about the survival strategies of this pathogen and also help to identify new targets.

Host cell proteins as drug targets. The targeting of host signalling molecules that are involved in the host–pathogen interaction might provide an alternate strategy for treating several bacterial and viral diseases. Inhibition of the p38 and ERK1/2 signalling pathways in macrophages that are infected with pathogenic mycobacteria has a significant role in suppression of the host defence in response to mycobacterial infections (FIG. 1). The activation of the MAPK pathways might therefore prove useful in promoting a bactericidal response. However, it is more difficult to activate than to inhibit a protein kinase signalling pathway. Moreover, such a strategy might be non-specific owing to the wide

GLYOXYLATE SHUNT
A biochemical pathway that is used by plants and microorganisms to metabolize acetate or long-chain fatty acids as a source of energy.

range of processes in which MAPK signalling is involved, and harmful side effects might arise from using compounds that activate MAPK activity. Nevertheless, selective inhibitors for kinases such as p38, JAK, PI3K and JNK are in pre-clinical and clinical development. As well as being used for therapeutic purposes, they might also serve as useful tools for elucidating the physiological roles of specific signalling pathways during mycobacterial infection.

Conclusion

Mycobacterial species are well adapted to the hostile environment of phagocytic cells, and they use several strategies for survival within host cells that are not seen in other bacteria. Our understanding of the mechanisms of interaction between mycobacteria and host cells, and of the consequent changes that are induced by mycobacteria in the host signalling machinery, is still incomplete. However, it is clear that some of the strategies that are used by mycobacteria for intracellular survival involve disruption of the host signalling machinery. To gain a better understanding of the proteins involved in the survival of mycobacteria within host cells, methods such as RNA interference for the suppression of host protein expression or genetic disruption of bacterial genes might be useful. Further studies, with the help of new techniques in genomics and proteomics, will elucidate the precise mechanisms by which pathogenic mycobacteria are able to down-regulate host-signalling pathways involving TLRs, MAPKs and JAK/STATs.

Mycobacterial gene products that disrupt host defences during infection represent potential drug targets. In this regard, studies of the inhibition of host cell functions — such as phagosome and DC maturation — and of apoptosis by mycobacteria, offer new strategies for therapeutic interventions, and new drugs could be designed to reverse the inhibition of the MAPK and JAK/STAT signalling pathways in infected cells. In addition, genes that are involved in the biosynthesis of Man-LAM or mycobacterial kinases and phosphatases might be useful targets.

Kinases and phosphatases are important targets for the development of new drugs for several diseases — such as cancer and inflammatory diseases — and mycobacterial kinases and phosphatases could be potential targets for new TB drugs. With the use of the latest integrative tools in structural biology, pharmaceutical chemistry and assay systems, it will be possible to obtain new potent and selective inhibitors of protein kinases and phosphatases. Specific inhibitors are also valuable tools for understanding the physiological roles of protein kinases and phosphatases in mycobacterial pathogenesis and will help us to elucidate novel features of the pathogenic strategies that are used by these lethal bacteria.

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Competing interests statement

The authors declare that they have no competing financial interests

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