Mycobacteria within its intracellular niche: survival of the pathogen or its host?

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Understanding how the host immune system signals in response to infection by pathogenic mycobacteria and how the bacteria activate immune evasion mechanisms is central to the global efforts to thwart mycobacterial infections, primarily tuberculosis. Rapid advances in the field of cellular microbiology and comparative genomics together with powerful techniques for studying the transcriptome and the proteome of the infected macrophage should aid in the understanding of the interplay between the host and the bacterium in its intracellular niche. This review summarizes the present state of knowledge in this field.

PATHOGENIC mycobacteria survive within the macrophage. Infections are acquired through inhalation of infective bacilli. Once in the lung, the mycobacteria are internalized by alveolar macrophages in which they replicate and set-up infection foci in the tissue of the alveolar wall. The local inflammatory response caused by phagocytosis of mycobacteria by alveolar macrophages leads to recruitment of mononuclear cells from neighbouring blood cells and eventually formation of a granuloma which consists of a kernel of infected macrophages surrounded by foamy giant cells with lymphocytes delineating the periphery of the structure. The bacterium survives within the macrophage using a variety of mechanisms including inhibition of phagosome-lysosome fusion, inhibition of the acidification of phagosomes and resistance to killing by oxygenated metabolites. The macrophage mounts its offence by producing an array of proinflammatory cytokines which trigger macrophage microbicidal activity[1]. On the other hand, the bacterium triggers processes to offset macrophage microbicidal activity and to create an environment favouring its own survival. In order to eventually design therapies whether chemotherapeutic or immunomodulatory, it is necessary to identify the mechanisms by which the mycobacteria are phagocytosed, the mechanisms activated by the host to counter mycobacterial survival and the mechanisms by which the mycobacteria moderate the host immune response. The ability of *Mycobacterium* species to persist in their hosts depends on avoiding or minimizing the induction of a productive immune response and suppressing the effector cascade once such a response has been developed. This review will focus on some aspects of the present state of knowledge of the innate immune response to mycobacteria and on how mycobacteria escape immune surveillance and survive within the macrophage.

Dendritic cells (DCs) play a crucial role in the immune response against bacterial pathogens. Immature DCs capture invading pathogens in the peripheral tissues and process them into peptide fragments[2]. They then migrate to lymphoid tissues, undergo maturation and present the antigens on major histocompatibility complexes (MHCs), enabling activation of naïve T cells. Depending on the pathogen recognized, naïve T cells can differentiate into T-helper-1 (Th1) cells (as in the case of pathogenic mycobacteria) which secrete interferon-*g* (IFN-*g*) or into T-helper 2 (Th2) cells which produce IL-4. IFN-*g* plays a central role in macrophage microbicidal activity. *M. tuberculosis* has developed molecular mechanisms to limit activation of macrophages by IFN-*g*[3]. The development of Th1 T cells is driven by IL-12 which is produced by macrophages and dendritic cells following phagocytosis of bacteria[2,4]. IL-12 is a crucial cytokine in controlling mycobacterial infection. IL-12 knockout mice are susceptible to *M. tuberculosis* infection[5] and individuals with mutations in the genes for IL-12 or its receptor have increased susceptibility to mycobacterial infection[6,7]. Comparative analysis of gene expression responses in macrophages infected with *M. tuberculosis* also suggests that inhibition of IL-12 production is a key response by which this organism survives host defenses[8].

Early interactions between lung dendritic cells (LDCs) and *M. tuberculosis* are thought to be critical for mounting a protective, anti-mycobacterial immune response and for determining the outcome of the infection. *M. tuberculosis* enters human monocyte-derived dendritic cells after binding to the lectin <u>DC</u>-<u>s</u>pecific <u>i</u>ntercellular adhesion molecule-3 *g*rabbing <u>n</u>on-integrin (DC-SIGN)[9]. Whereas the complement receptor CR3 and mannose receptor (MR) are the main receptors for *M. tuberculosis* on macrophages[10], DC-SIGN is critically important in dendritic cells. Lipoarabinomannan (LAM) is the key ligand of DC-SIGN[11]. A brief overview of the role of LAM and other mycobacterial lipids in immunity is given below.

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Role of LAM and other mycobacterial lipids in infection

LAMs are one of the modulators of macrophage/dendritic cell functions. LAMs are lipoglycans restricted to the mycobacteria[12]. LAM has a tripartite structure composed of a carbohydrate backbone, a glycosylphosphatidyl inositol (GPI) anchor and capping motifs. The D-mannan core and the D-arabinan domain constitute the carbohydrate backbone. At its reducing end, the mannan core is terminated by the glycosyl-phosphatidylinositol anchor. The arabinan domain is capped either by mannosyl[13–15] or phosphoinositide residues[16,17]. The Man-LAMs are characterized by the presence of mannosyl caps and are found in the slow-growing mycobacteria, *M. tuberculosis*, *M. leprae* and *M. bovis* BCG. The PI-LAMs contain phosphoinositide caps and are present in the fast-growing mycobacteria, e.g. *M. smegmatis*.

Binding of mannosylated LAM to immature DCs blocks both LPS-mediated maturation and IL-12 secretion[18]. Man-LAM binding to DC-SIGN interferes with DC maturation signals and also induces IL-10 secretion[19] thereby contributing to immune suppression. Targeting DC-SIGN is one of the strategies used by *M. tuberculosis* to escape immune surveillance[20]. Man-LAMs exert an anti-inflammatory effect attenuating LPS-induced tumour necrosis factor-*a* (TNF-*a*) and IL-12 p40 mRNA expression[21]. *M. bovis* BCG Man-LAM when added at the onset of monocyte culture subsequently inhibits both IL-12 mRNA (p40 and p30) expression and IL-12 p70 production by mature DCs[22]. Both manno-oligosaccharide caps and the GPI anchor fatty acid play a critical role in the inhibitory role of Man-LAM on IL-12 production[23].

Man-LAMs activate the tyrosine phosphatase[21] SHP-1. It is conceivable that Man-LAMs exert their antinflammatory functions by dephosphorylating kinases such as the mitogen-activated protein (MAP) kinases which are involved in TNF-*a* and IL-12 production. Accumulating evidence suggests that mycobacteria-infected macrophages undergo apoptosis[24–26]. Man-LAM is largely responsible for the inhibition of apoptosis in *M. tuberculosis*-infected macrophages. Man-LAM promotes Bad phosphorylation[27]. Bad is a proapoptotic member of the Bcl-2 family that inhibits Bcl-X_L function by sequestering it as a heterodimer[28]. Its phosphorylation dissociates it from the heterodimer and leads to its sequestration in the cytosol in association with 14-3-3. Man-LAM is also able to prevent *M. tuberculosis*-induced calcium influx and to consequently inhibit calcium-dependent events associated with apoptosis[29]. Ca^{2+} participates in signal transduction and gene expression through the c-AMP-response element binding protein (CREB) that is phosphorylated after Ca^{2+} influx[30]. c-AMP response element (CRE) is present in the promoter region of the TNF-*a* gene and CREB participates in IFN-*g*-dependent JAK/STAT signalling[31] also involved in apoptosis[32]. Man-LAM inhibits CREB function by inhibiting nuclear translocation of CREB[29]. Among the late events regulated by Man-LAM is the tilting of the TNF-*a*/IL-10 balance in favour of IL-10 which is considered to be an antiapoptotic cytokine[25]. Man-LAM has recently been suggested to be crucial in prevention of phagosomal maturation[33]. Some of the anti-inflammatory and anti-apoptotic signalling effects of Man-LAM are summarized in Figure 1.

Unlike Man-LAM, phosphatidylinositol dimannosides (PIM_2) released by intracellular mycobacteria elicit the production of the proinflammatory cytokines TNF-*a* and monocyte chemoattractant protein 1 (MCP-1) which are both strong promoters of a granulomatous response[34].

Genetic manipulations have helped in the understanding of the requirement of unique mycobacterial lipids in infection. Phthiocerol mycocerosate (PDIM) is one such lipid. PDIM is a peripheral lipid which is essential for maintenance of infection in the lung tissue[35,36]. These lipids are shed into infected macrophages[37], intercalate into the host cell membranes, are transported inside the cell and accumulate in a multivesicular lysosomal structure reminiscent of the MIIC compartment that is involved in antigen loading of MHC class II molecules. These vesicular structures are shed into the external medium through a constitutive exocytic process[38]. These exosomes induce a proinflammatory response comparable to that of intact bacilli and might function *in vivo* by expanding the influence of the bacterium beyond its host cell and helping in granuloma maintenance.
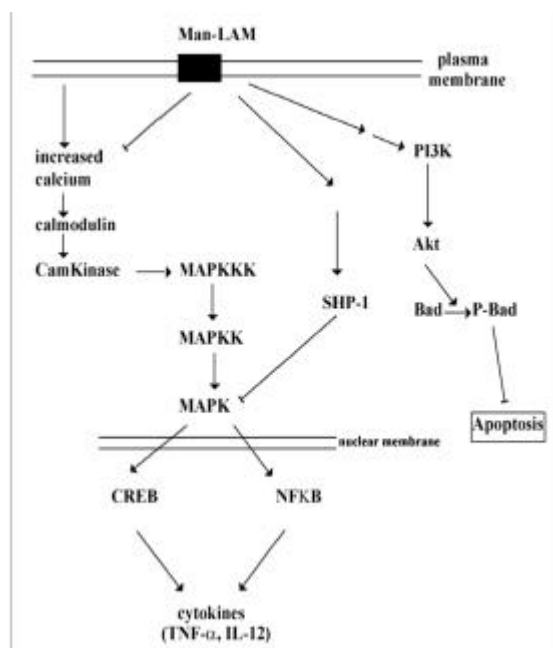
Figure 1. Schematic representation of the anti-inflammatory and anti-apoptotic effects of Man-LAM in macrophages.

Toll receptor signalling

The Toll-like receptor (TLR) proteins are key regulators of innate immune activation induced by *M. tuberculosis*[39]. The engagement of the Toll-like receptor 4 (TLR4) and the IL-1 receptor initiates a common signalling pathway, leading to the sequential activation of the adapter protein MyD88, the IL-1 receptor kinases (IRAK), TNF receptor-associated factor-6 (TRAF6) and eventually the I*k*B kinase complex[40]. Recent studies have demonstrated that the novel proteins (Tollip)[41] and TIRAP[42,43] are likely to associate with the intracellular domains on the IL-1 receptor and/or TLR proteins. Additionally, TLR2 and TLR4 activate the signalling molecule Akt[44]. Arabinose-capped LAM is a TLR2 agonist[45]. CD14 is likely to be necessary for activation of TLR2 by arabinose-capped LAM. The 19 kDa lipoprotein antigen of *M. tuberculosis*[46] and the heat-stable protease-resistant soluble tuberculosis factor (STF)[47] are both TLR2 agonists. *M. tuberculosis* also demonstrates a cell-associated, heat labile TLR4 agonist activity which contrasts with the secreted heat-stable TLR2 agonist STF. In contrast to *M. tuberculosis*, *M. avium* does not activate cells in a TLR4-dependent manner[48]. The 19 kDa lipoprotein may play a role in NO-dependent antimicrobial activity in mouse macrophages[49]. However, how it functions in human macrophages remains largely unknown.

TLR proteins are also linked to apoptosis. Whereas TLR2 activation provides a proapoptotic signal[50], blockade of TLR 4 eliminates almost all *M. tuberculosis*-induced human alveolar macrophage apoptosis[51]. Paradoxically, TLR proteins mediate both the suppression of antigen presentation and the induction of antimicrobial responses by macrophages. Stimulation of macrophages with *M. tuberculosis* 19 kDa antigen reduces MHC class II mRNA expression and surface expression in macrophages[52,53]. This altered expression of class II molecules correlates with decreased presentation of peptide antigens to T-lymphocyte hybridomas. Mycobacteria also have the ability to regulate TLR gene expression. *M. avium* infection of murine macrophages leads to increased TLR2 mRNA and to the expression of TLR2 on the cell surface[54]. The question regarding the relative contribution of these receptors for containing *M. tuberculosis* infection *in vivo* has recently been addressed subjecting mice defective in CD14, TLR2 or TLR4 to low (100 CFU) or high (2000 CFU) dose aerosol infection with *M. tuberculosis*. Granuloma formation, macrophage activation and secretion of proinflammatory cytokines in response to low-dose aerosol infection were identical in mutant and control mice[55]. However, high-dose aerosol challenge with 2000 CFU *M. tuberculosis* revealed TLR2 but not TLR4-defective mice to be more susceptible than normal mice. The function of the TLRs and CD14 in initiating protective responses against naturally low-dose airborne infection is therefore redundant. A single deficiency in a pattern recognition receptor does not impair innate resistance to *M. tuberculosis*. However, macrophages from TLR2$^{-/-}$ show significantly decreased IL-12 p 40 levels.

## Mitogen activated protein kinase signalling

The mitogen activated-protein (MAP) kinases are dual specificity kinases. In macrophages these are activated upon the binding of growth factors and inflammatory cytokines to specific receptors, and are important in the activation of cytokine gene trascription. The MAP kinase family is composed of the ERK1/2, p38 and SAPK/JNK pathways. There is considerable co-operation between these kinases[56]. The MAP kinases are activated by upstream MAPK kinases through a Thr-x-x-x-Tyr phosphorylation motif[57]. The activated MAPKs are responsible for phosphorylating and activating numerous transcription factors which function to stimulate the synthesis of various proinflammatory cytokines such as TNF-*a*, IL-1*b* and IL-6. The MAPKs are also involved in transcriptional regulation of NOS2 and cyclooxygenase[58] 2. Recent studies have thrown light on the link between macrophage MAPK activation and mycobacterial infection. ERK1/2 activation induces induction of the *NOS2* gene following macrophage cell line stimulation with both Man-LAM and IFN-*g*[59]. Work by Reiner and colleagues with the human monocytic cell line THP-1 suggests that Man-LAM limits macrophage activation by reducing ERK1/2 phosphorylation[21]. Work with both human[60] and murine[61] macrophages supports the view that TNF-*a* secretion from macrophages challenged with *M. avium* is dependent on MEK1 and ERK1/2 activation. Activation of MAPK pathways in both human and murine macrophages suggests that these pathways contribute to the virulence of the mycobacterial pathogen. Murine macrophages infected with virulent *M. avium* exhibit early activation of p38 MAPK and ERK1/2 but this activation is quickly lost. In contrast, macrophages infected with non-pathogenic mycobacteria show sustained activation of MAP kinases[62]. There appears to be a reverse correlation between the pathogenicity of a mycobacterial species or strain and its ability to induce sustained activation of MAP kinases. Tse *et al.*[63] have shown that synthesis of the macrophage deactivating agent prostaglandin E2 by *M. avium*-infected macrophages is dependent on p38 MAPK and its production results in increased virulence of the Sm T morphotype. Much still needs to be elucidated regarding the upstream events regulating MAPK activation by pathogenic mycobacteria. Ca^{2+} is a potential upstream modulator. Infection with dead but not live *M. tuberculosis* results in a transient Ca^{2+} flux within macrophages[64]. Calcium efflux triggered by *M. bovis* induces interleukin 8 secretion from human monocytes[65]. The relationship between Ca^{2+} flux and MAPK signalling

has not yet been studied. There is still little information available on the transcription factors activated downstream of the MAP kinases. NF-IL-6 has been reported to control aerosolized *M. tuberculosis* infection in mice[66]. AP-1 is activated in macrophages treated with mannosylated phosphatidylinositol (PIM) from *M. tuberculosis* through TLR2 signalling[67].

Macrophage apoptosis triggered by mycobacteria

Macrophages infected with mycobacteria undergo apoptosis. Host macrophage apoptosis represents an innate defense mechanism linked to killing of intracellular mycobacteria[68,69]. Macrophages infected either with an attenuated (H37Ra) or with a virulent strain of *M. tuberculosis* undergo apoptotic death when exposed to TNF-*a* or FasL, accompanied by a reduction in bacillary viability[70]. Monocytic THP-1 cell apoptosis in response to *M. tuberculosis* infection involves caspases[71] 9 and 3. *M. avium*-mediated apoptosis of murine J774.1 macrophages involves caspases 8, 9 and 3 and is critically dependent on apopotosis signal-regulating kinase (ASK)1 and p38 MAPK[72]. This signalling pathway is summarized in Figure 2.
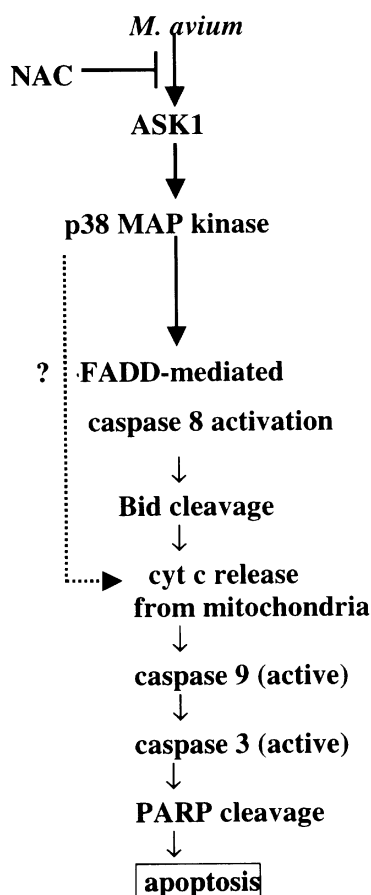
Figure 2. Schematic representation of mycobacteria-induced ASK 1- and caspase 8-dependent cell death in macrophages.

Evasion of apoptosis by virulent mycobacteria has been demonstrated[73]. Duan *et al.*[74] suggest that increased Ca^{2+} flux into *M. tuberculosis*-infected macrophages leads to low levels of caspase activation and diminished mitochondrial cytochrome c release, hallmarks of apoptosis[74].

It appears possible that virulent mycobacteria may avoid apoptosis by regulating the multimeric <u>d</u>eath <u>i</u>nducing <u>s</u>ignal <u>c</u>omplex (DISC). The possible mechanisms of death signalling in mycobacteria-infected macrophages and the mechanisms whereby death receptor signals are blocked will be the obvious focus of future studies.

Manipulation of phagosome maturation by pathogenic mycobacteria

Mycobacteria successfully parasitize the macrophage using a number of strategies to manipulate the phagosome (where they reside) and prevent its maturation. Bacteria-containing vacuoles interact with endosomes and remain arrested in what could be described as a transitional state in phagosome biogenesis[75,76]. Bacteria-containing vacuoles are less acidic than their neighbouring lysosomes[77] probably due to the paucity of vacuolar ATPases[78]. During phagosome maturation, phagosomes interact with the endosomal network and acquire markers found on early endosomes. Markers such as early endosomal antigen 1 (EEA1) and the small GTPases of the Rab family are present on certain organelles and take part in specific fusion events. Rab5 dissociates from these vesicles as they acquire Rab7, another GTPase that function during fusion of the endosomes and lysosomes. On the other hand Rab5 is associated with, and retained by, phagosomes containing live *M. bovis* BCG or *M. tuberculosis*[79,80]. Recent reports have shown the association of the macrophage 32–35 kDa galactose-binding lectin galectin (gal-3) with live *Mycobacterium*-containing phagosomes[81]. Live mycobacteria also have the ability to inhibit the increase in cytosolic Ca^{2+} concentration normally caused by phagocytosis[64,82]. Treatment of infected cells with the calcium ionophore A23178 causes an increase in $[Ca^{2+}]_c$ reducing bacterial viability[64], and correlating with an increase in calcium/calmodulin-dependent protein kinase (CaMKII) localization to the cytosolic face of these vacuoles. Recent studies suggest that *M. tuberculosis* blocks Ca^{2+} signalling and phagosome maturation in human macrophages via inhibition of sphingosine kinase[83].

Work from the group of Jan Pieters has argued in favour of a role of TACO or coronin 1 which is associated with the mycobacterial phagosome in prevention of vacuole maturation[84] and suggests that sequestration of cholesterol in *Mycobacterium*-containing vacuoles could be associated with retention of coronin 1 on the vacuolar membrane[85]. The specificity of the binding of *Mycobacterium* to cholesterol is still a matter of debate. Mycobacterial vacuoles are dynamic, fusion-competent vesicles[86].

As of now how bacilli-containing vacuoles are retained in the early endosomal network remains incompletely understood, particularly in terms of the critical bacterial effector molecules involved in this process.

Antigen processing and presentation

Infected macrophages or bystander antigen-presenting cells signal the presence of the pathogen through display of the pathogen-derived molecules in the context of MHC (mouse) or HLA (human) antigens. Class II antigens present peptides generated within the endosomal-lysosomal continuum of the host cell for T cell recognition. These are relevant for vacuolar pathogens such as *M. tuberculosis*. Newly synthesized MHC-II molecules associate with invariant chain in the endoplasmic reticulum and are transported via the trans-Golgi network to the endocytic pathway. Invariant chain is degraded by endosomal/lysosomal proteases, leaving the MHC–II molecules associated with class-II associated invariant chain peptide (CLIP). The MHC class II molecule is primed for antigen sampling by another chaperone, H2-M (mouse) or HLA-DM (human) which removes CLIP and renders the antigen-combining cleft accessible. Once the MHC class II molecule is loaded, it is transported to the cell surface where the class II molecule, together with other co-stimulatory molecules, is free to combine with the T cell receptor. Mycobacteria in resting macrophages sequester their vacuoles away from the normal antigen processing and presentation machinery of their host cell[87]. Infected macrophages also downregulate expression of both class II and co-stimulatory molecules[88–91]. Whether inhibition of JAK-mediated phosphorylation of STATI is involved in this or not, is still a matter of debate[92].

Differential receptor usage by *M. tuberculosis* on DCs and macrophages may account for the different survival ability and trafficking pattern of mycobacteria in the two cell types[93,94]. DC-SIGN is present in *M. tuberculosis* vacuoles during the early steps of bacterial uptake, and is then rapidly expelled from the phagosome possibly as a result of recycling to the plasma membrane. DC-SIGN could potentially carry mycobacterial glycolipids from the bacterial vacuole to the cell plasma membrane and/or to various subcellular compartments where glycolipids could be loaded onto CD1 molecules for presentation to CD1-resistant lymphocytes[95].

In spite of recent advances in our understanding of mycobacteria–host interactions, there are many areas in which knowledge needs to be generated. The critical mycobacterial components that are important in virulence are not yet understood. The macrophage receptors that initiate the intracellular signalling pathways are not known. Finally, studies carried out with *M. avium* need to be extended to *M. tuberculosis*. How mycobacteria signal the expression of molecules which influence survival or

clearance has been most elegantly studied in the case of macrophages exposed to *M. avium* or its cell wall LAM. The early innate response was analysed in cDNA expression arrays. Within 2 h the proinflammatory response included expression of genes encoding cytokines TNF-*a*, IL-8, IL-1 and adhesion molecules[96]. The proinflammatory response subsided within 24 h. After four days, when some bacteria were being degraded, others escaped destruction to replicate within intracellular vacuoles. Inducible NOS was not up-regulated and increased transferrin receptors could possibly facilitate mycobacterial growth. Sustained adhesion molecule and chemokine expression along with the formation of multinucleated giant cells appeared consistent with *in vivo* events. Thus macrophages appeared to be insufficiently microbicidal and to provide a non-hostile environment. Similar studies need to be performed in detail with *M. tuberculosis*.

Despite the knowledge generated in recent years, the control of mycobacterial infection through development of innovative therapies requires a more detailed understanding of the cellular processes controlling the survival of the bacterium within its host, particularly in relation to the key pathways linked to persistence. Development of suitable models of persistence should aid in this endeavour. As powerful techniques of proteomics emerge in which increasingly smaller amount of proteins can be separated and analysed, these tools should provide a better understanding of the life of the mycobacterium within its intracellular niche, particularly in relation to pathogen-containing vacuole biogenesis and its arrest.

1. Flynn, J. L. and Chan, J., Immunology of tuberculosis. *Annu. Rev. Immunol.*, 2001, **19**, 93–129.
2. Bancherau, J. and Steinman, R. M., Dendritic cells and the control of immunity. *Nature*, 1998, **392**, 245–252.
3. Ting, L. M., Kim, A. C., Cattamanchi, A. and Ernst, J. D., *Mycobacterium tuberculosis* inhibits IFN-*g* transcriptional responses without inhibiting activation of STAT1. *J. Immunol.*, 1999, **163**, 3898–3906.
4. Trinchieri, G., Interleukin-12: a cytokine at the interface of inflammation and immunity. *Adv. Immunol.*, 1998, **70**, 83–243.
5. Cooper, A. M., Magram, J. Ferrante, J. and Orme, I. M., Interleukin 12 (IL-12) is crucial to the development of protective immunity in mice intravenously infected with *Mycobacterium tuberculosis*. *J. Exp. Med.*, 1997, **186**, 39–45.
6. Doffinger, R., Altare, F. and Casanova, J. L., Genetic heterogeneity of Mendelian susceptibility to mycobacterial infection. *Microbes Infect.*, 2000, **2**, 1553–1557.
7. Altare, F., Jouanguy, E., Lamhamedi, S., Doffinger, R., Fischer, A. and Casanova, J. L., Mendelian susceptibility to mycobacterial infection in man. *Curr. Opin. Immunol.*, 1998, **10**, 413–417.
8. Nau, G. J., Richmond, J. F. L., Schlesinger, A., Jennings, E. G., Lander, E. S. and Young, R. A., Human macrophage activation programs induced by bacterial pathogens. *Proc. Natl. Acad. Sci. USA*, 2002, **99**, 1503–1508.
9. Tailleux, *et al.*, DC-SIGN is the major *Mycobacterium tuberculosis* receptor on human dendritic cells. *J. Exp. Med.*, 2003, **197**, 121–127.
10. Schlesinger, L. Z., Macrophage phagocytosis of virulent but not attenuated strains of *Mycobacterium tuberculosis* is mediated by

mannose receptors in addition to complement receptors. *J. Immunol.*, 1993, **150**, 2920–2930.

11. Maeda, N. *et al.*, The cell surface receptor DC-SIGN discriminates between *Mycobacterium* species through selective recognition of the mannose caps on lipoarabinomannan. *J. Biol. Chem.*, 2003, **278**, 5513–5516.

12. Chatterjee, D. and Khoo, K. H., Structural features of lipoarabinomannan from *Mycobacterium bovis* BCG: Determination of molecular mass by laser desorption mass spectrometry. *Glycobiology*, 1998, **8**, 113–120.

13. Venisse, A., Berjeaud, J. M., Chaurand, P., Gilleron, M. and Puzo, G., Structural features of lipoarabinomannan from *Mycobacterium bovis* BCG: Determination of molecular mass by laser desorption mass spectrometry. *J. Biol. Chem.*, 1993, **268**, 12401–12411.

14. Chatterjee, D., Khoo, K. H., McNeil, M. R., Dell, A., Morris, H. R. and Brennan, P. J., Structural definition of the non-reducing termini of mannose-capped LAM from *Mycobacterium tuberculosis* through selective enzymatic degradation and fast atom bombardment-mass spectrometry. *Glycobiology*, 1993, **3**, 497–500.

15. Prinzis, S., Chatterjee, D. and Brennan, P. J., Structure and antigenicity of lipoarabinomannan from *Mycobacterium bovis* BCG. *Gen. Microbiol.*, 1993, **139**, 2649–2658.

16. Gilleron, M., Himoudi, N., Adam, O., Constant, P., Venisse, A., Riviere, M. and Puzo, G., *Mycobacterium smegmatis* phosphoinositols-glyceroarabinomannans. Structure and localization of alkali-labile and alkali-stable phosphoinositides. *J. Biol. Chem.*, 1997, **272**, 117–124.

17. Khoo, K. H., Dell, A., Morris, H. R., Brennan, P. J. and Chatterjee, D., Inositol phosphate capping of the nonreducing termini of lipoarabinomannan from rapidly growing strains of *Mycobacterium*. *J. Biol. Chem.*, 1995, **270**, 12380–12389.

18. Nigou, J., Zelle, Rieser, C., Gilleron, M., Thurnher, M. and Puzo, G., Mannosylated lipoarabinomannans inhibit IL-12 production by human dendritic cells: evidence for a negative signal delivered through the mannose receptor. *J. Immunol.*, 2001, **166**, 7477–7485.

19. Geijtenbeek, T. B., Van Vliet, S. J., Koppel, E. A., Sanchez-Hernandez, M., Vanden-Broucke-Grauls, C. M., Appelmelk, B. and van Kooyk, Y., Mycobacteria target DC-SIGN to suppress dendritic cell function. *J. Exp. Med.*, 2003, **197**, 7–17.

20. van Kooyk, Y., Appelmelk, B. and Geijtenbeek, T. B. H., A fatal attraction: *Mycobacterium tuberculosis* and HIV-1 target DC-SIGN to escape immune surveillance. *Trends Mol. Med.*, 2003, **9**, 153–159.

21. Knutson, K. L., Hmama, Z., Herrea-Velit, P., Rochford, R. and Reiner, N. E., Lipoarabinomannan of *Mycobacterium tuberculosis* promotes protein tyrosine dephosphorylation and inhibition of mitogen-activated protein kinase in human mononuclear phagocytes. Role of the Src homology 2 containing tyrosine phosphatase 1. *J. Biol. Chem.*, 1998, **273**, 645–652.

22. Johansson, U., Ivanyi, J. and Londei, M., Inhibition of IL-12 production in human dendritic cells matured in the presence of Bacillus Calmette-Guerin or lipoarabinomannan. *Immunol. Lett.*, 2001, **77**, 63–66.

23. Nigou, J., Gilleron, M., Brando, T., Vercellone, A. and Puzo, G., Structural definition of arabinomannans from *Mycobacterium bovis* BCG. *Glycoconj. J.*, 1999, **16**, 257–264.

24. Rojas, M., Barrera, L. F. and Garcia, L. F., Induction of apoptosis in murine macrophages by *Mycobacterium tuberculosis* is reactive oxygen intermediates-independent. *Biochem. Biophys. Res. Commun.*, 1998, **247**, 436–442.

25. Rojas, M., Olivier, M., Gras, P., Barrera, L. F., Puzo, G. and Garcia, L. F., TNF-*a* and IL-10 modulate the induction of apoptosis by virulent *Mycobacterium tuberculosis* in murine macrophages. *J. Immunol.*, 1999, **162**, 6122–6131.

26. Rojas, M., Barrera, L. F., Puzo, G. and Garcia, L. F., Differential induction of apoptosis by virulent *Mycobacterium tuberculosis* in resistant and susceptible murine macrophages: role of nitric oxide and mycobacterial products. *J. Immunol.*, 1997, **159**, 1352–1361.

27. Maiti, D., Bhattacharyya, A. and Basu, J., Lipoarabinomannan from *Mycobacterium tuberculosis* promotes macrophage survival by phosphorylating Bad through a phosphatidylinositol 3-kinase/Akt pathway. *J. Biol. Chem.*, 2001, **276**, 329–333.

28. Datta, S. R., Brunet, A. and Greenberg, M. E., Cellular survival: a play in three Akts. *Genes Dev.*, 1999, **13**, 2905–2927.

29. Rojas, M., Garcia, L. F., Nigou, J., Puzo, G. and Oliver, M., Mannosylated lipoarabinomannan antagonizes *Mycobacterium tuberculosis*-induced macrophage apoptosis by altering Ca^{2+}-dependent cell signaling. *J. Infect. Dis.*, 2000, **181**, 240–251.

30. Poser, S. and Storm, D. R., Role of Ca^{2+}-stimulated adenylyl cyclases in LTP and memory formation. *Int. J. Dev. Eurosci.*, 2001, **19**, 387–394.

31. Horvai, A. E. *et al.*, Nuclear integration of JAK/STAT and Ras/AP-1 signaling by CBP and p300. *Proc. Natl. Acad. Sci. USA*, 1997, **94**, 1074–1079.

32. Chin, Y. E., Kitagawa, M., Kuida, K., Flavell, R. A. and Fu, X. Y., Activation of the STAT signaling pathway can cause expression of caspase 1 and apoptosis. *Mol. Cell. Biol.*, 1997, **17**, 5328–5337.

33. Fratti, R. A., Chaua, J., Vergne, I. and Deretic, V., *Mycobacterium tuberculosis* glycosylated phosphatidylinositol causes phagosome maturation arrest. *Proc. Natl. Acad. Sci. USA*, 2003, **100**, 5437–5442.

34. Rhoades, E., Hsu, F.-F., Torrelles, J. B., Turk, J., Chatterjee, D. and Russell, D. G., Identification and macrophage-activating activity of glycolipids released from intracellular *Mycobacterium bovis* BCG. *Mol. Microbiol.*, 2003, **48**, 875–888.

35. Camacho, L. R., Ensergueix, D., Perez, E., Gicquel, B. and Guilhot, C., Identification of a virulence gene cluster of *Mycobacterium tuberculosis* by signature-tagged transposon mutagenesis. *Mol. Microbiol.*, 1999, **34**, 257–267.

36. Cox, J. S., Chen, B., McNeil, M. and Jacobs, W. R., Complex lipid determines tissue-specific replication of *Mycobacterium tuberculosis* in mice. *Nature*, 1999, **402**, 79–83.

37. Beatty, W. B., Rhoades, E. R., Ullrich, H. J., Chatterjee, D. and Russell, D. G., Trafficking and release of mycobacterial lipids from infected macrophages. *Traffic*, 2000, **1**, 235–247.

38. Beatty, W. L., Ullrich, H. J. and Russell, D. G., Mycobacterial surface moieties are released from infected macrophages by a constitutive exocytic event. *Eur. J. Cell Biol.*, 2001, **80**, 31–40.

39. Heldwein, K. A. and Fenton, M., The role of Toll-like receptors in immunity against mycobacterial infection. *Microbes. Infect.*, 2002, **4**, 937–944.

40. Takeda, K. and Akira, S., Toll receptors and pathogen resistance. *Cell. Microbiol.*, 2003, **5**, 143–153.

41. Bulut, Y., Faure, E., Thomas, L., Equils, O. and Arditi, M., Cooperation of Toll-like receptor 2 and 6 for cellular activation by soluble tuberculosis factor and *Borrelia burgdorferi* outer surface protein A lipoprotein: role of Toll-interacting protein and IL-1 receptor signaling molecules in Toll-like receptor 2 signaling. *J. Immunol.*, 2001, **167**, 987–994.

42. Fitzgerald, K. A. *et al.*, Mal (MyD88-adapter-like) is required for Toll-like receptor-4 signal transduction. *Nature*, 2001, **413**, 78–83.

43. Horng, T., Barton, G. M. and Medzhitov, R., TIRAP: an adapter molecule in the Toll signaling pathway. *Nature Immunol.*, 2001, **2**, 835–841.

44. Jones, B. W., Heldwein, K. A., Means, T. K., Saukoven, S. and Fenton, M. J., Differential roles of Toll-like receptors in the elicitation of proinflammatory responses by macrophages. *Ann. Rheum. Dis.*, 2001, **60**, Suppl. 3: iii, 6–12.

45. Means, T. K., Lien, E., Yoshimura, A., Wang, S., Golenbock, D. T. and Fenton, M. J., The CD14 ligands lipoarabinomannan and lipopolysaccharide differ in their requirement for Toll-like receptors. *J. Immunol.*, 1999a, **163**, 6748–6755.

46. Brightbill, H. D. *et al.*, Host defense mechanisms triggered by microbial lipoproteins through toll-like receptors. *Science*, 1999, **285**, 732–736.

47. Means, T. K., Wang, S., Lien, E., Yoshimura, A., Golenbock, D. T. and Fenton, M. J., Human toll-like receptors mediate cellular activation by *Mycobacterium tuberculosis*. *J. Immunol.*, 1999b, **163**, 3920–3927.

48. Lien, E. *et al.*, Toll-like receptor 2 functions as a pattern recognition receptor for diverse bacterial products. *J. Biol. Chem.*, 1999, **274**, 33419–33425.

49. Thoma-Uszynki, S. *et al.*, Induction of direct antimicrobial activity through mammalian Toll-like receptors. *Science*, 2001, **292**, 1544–1547.

50. Aliprantis, A. O. *et al.*, Cell activation and apoptosis by bacterial lipoproteins through toll-like receptor-2. *Science*, 1999, **285**, 736–739.

51. Means, T. K. *et al.*, Differential effects of a Toll-like receptor antagonist on *Mycobacterium tuberculosis*-induced macrophage responses. *J. Immunol.*, 2001, **166**, 4074–4082.

52. Noss, E. H., Rai, R. K., Sellati, T. J., Radolf, J. D. B., Golenbock, D. T., Boom, W. H. and Harding, C. V., Toll-like receptor 2-dependent inhibition of macrophage class II MHC expression and antigen processing by 19-kDa lipoprotein of *Mycobacterium tuberculosis*. *J. Immunol.*, 2001, **176**, 910–918.

53. Pai, R. K., Convery, M., Hamilton, T. A., Boom, W. H. and Harding, C. V., Inhibition of IFN-*g*-induced class II transactivator expression by a 19-kDa lipoprotein from *Mycobacterium tuberculosis*: a potential mechanism for immune evasion. *J. Immunol.*, **171**, 175–184.

54. Wang, T., Lafuse, W. and Zwilling, B. S., Regulation of Toll-like receptor 2 expression by macrophages following *Mycobacterium avium* infection. *J. Immunol.*, 2000, **165**, 6308–6313.

55. Reiling, R., Holscher, C., Fehrenbach, A., Kroger, S., Kirsching, C. J., Goyert, S. and Ehlers, S., Toll like receptor 2 (TLR2)- and TLR4-mediated pathogen recognition in resistance to airborne infection with *Mycobacterium tuberculosis*. *J. Immunol.*, 2002, **169**, 3480–3484.

56. Cobb, M. H., MAP kinase pathways. *Progr. Biophys. Mol. Biol.*, 1999, **71**, 479–500.

57. Martin-Blanco, E., p38 MAPK signalling cascades: ancient roles and new functions. *Bioessays*, 2000, **22**, 637–645.

58. Chen, C., Chen, Y. H. and Lin, W. W., Involvement of p38 mitogen-activated protein kinase in lipopolysaccharide-induced iNOS and COX-2 expression in J774 macrophages. *Immunology*, 1999, **97**, 124–129.

59. Chan, E. D., Morris, K. R., Belisle, J. T., Hill, P., Remigio, L. K., Brennan, P. J. and Riches, D. W., Induction of inducible nitric oxide synthase-NO* by lipoarabinomannan of *Mycobacterium tuberculosis* is mediated by MEK1-ERK, MKK7-JNK, and NF-*k*B signaling pathways. *Infect. Immun.*, 2001, **69**, 2001–2010.

60. Reiling, N., Blumenthal, A., Flad, H. D., Ernst, M. and Ehlers, S., Mycobacteria-induced TNF-*a* and IL-10 formation by human macrophages is differentially regulated at the level of mitogen-activated protein kinase activity. *J. Immunol.*, 2001, **167**, 3339–3345.

61. Bhattacharyya, A., Pathak, S., Kundu, M. and Basu, J., Mitogen-activated protein kinases regulate *Mycobacterium avium*-induced tumor necrosis factor-*a* release from macrophages. *FEMS Immunol. Med. Microbiol.*, 2002, **34**, 73–80.

62. Roach, S. K. and Schorej, J. S., Differential regulation of the mitogen-activated protein kinases by pathogenic and nonpathogenic mycobacteria. *Infect. Immun.*, 2002, **70**, 3040–3052.

63. Tse, H. M., Josephy, S. I., Chan, E. D., Fouts, D. and Cooper, A. M., Activation of the mitogen-activated protein kinase signaling pathway is instrumental in determining the ability of *Mycobacterium avium* to grow in murine macrophages. *J. Immunol.*, 2002, **168**, 825–833.

64. Malik, Z. A., Denning, G. M. and Kusner, D. J., Inhibition of Ca^{2+} signaling by *Mycobacterium tuberculosis* is associated with reduced phagosome-lysosome fusion and increased survival within human macrophages. *J. Exp. Med.*, 2000, **191**, 287–302.

65. Mendez-Samperio, P., Plama, J. and Vazquez, A., Roles of intracellular calcium and NF-*k*B in the Bacillus Calmette-Guerin-induced secretion of interleukin-8 from human monocytes. *Cell. Immunol.*, 2001, **211**, 113–122.

66. Sugawara, I., Mizuno, S., Yamada, H., Matsumoto, M. and Akira, S., Disruption of nuclear factor-interleukin-6, a transcription factor, results in severe mycobacterial infection. *Am. J. Pathol.*, 2001, **158**, 361–366.

67. Jones, B. W., Means, T. K., Heldwein, K. A., Keen, M. A., Holl, P. J., Belisel, J. T. and Fenton, M. J., Different Toll-like receptor agonists induce distinct macrophage responses. *J. Leukoc. Biol.*, 2001, **69**, 1036–1044.

68. Fratazzi, C., Arbeit, R. D., Carini, C. and Remold, H. G., Programmed cell death of *Mycobacterium avium* serovar 4-infected human macrophages prevents the mycobacteria from spreading and induces mycobacterial growth inhibition by freshly added, uninfected macrophages. *J. Immunol.*, 1997, **158**, 4320–4327.

69. Molloy, A., Laochumroonvorapong, P. and Kaplan, G., Apoptosis, but not necrosis, of infected monocytes is coupled with killing of intracellular bacillus Calmette-Guerin. *J. Exp. Med.*, 1994, **180**, 1499–1509.

70. Oddo, M., Renno, T., Attinger, A., Bakker, T., MacDonald, H. R. and Meylan, P. R. A., Fas ligand-induced apoptosis of infected human macrophages reduces the viability of intracellular *Mycobacterium tuberculosis*. *J. Immunol.*, 1998, **160**, 5448–5454.

71. Riendeau, C. J. and Kornfeld, H., THP-1 cell apoptosis in response to mycobacterial infection. *Infect. Immun.*, 2003, **71**, 254–259

72. Bhattacharyya, A., Pathak, S., Basak, C., Law, S., Kundu, M. and Basu, J., Execution of macrophage apoptosis by *Mycobacterium avium* through apoptosis signal-regulating kinase 1/p38 mitogen-activated protein kinase signaling and caspase 8 activation. *J. Biol. Chem.*, 2003, **278**, 26517–26525.

73. Balcewicz-Sablinska, M. K., Keane, J., Kornfeld, H. and Remold, H. G., Pathogenic *Mycobacterium tuberculosis* evades apoptosis of host macrophages by release of TNF-R2, resulting in inactivation of TNF-*a*. *J. Immunol.*, 1998, **161**, 2636–2641.

74. Duan, L., Gan, H., Golan, D. E. and Remold, H. G., Critical role of mitochondrial damage in determining outcome of macrophage infection with *Mycobacterium tuberculosis*. *J. Immunol.*, 2002, **169**, 5181–5187.

75. Clemens, D. L., Lee, B. Y. and Horwitz, M. A., *Mycobacterium tuberculosis* and *Legionella pneumophila* phagosomes exhibit arrested maturation despite acquisition of Rab7. *Infect. Immun.*, 2000, **68**, 5154–5166.

76. Sturgill-Koszycki, S., Schaible, U. E. and Russell, D. G., *Mycobacterium*-containing phagosomes are accessible to early endosomes and reflect a transitional state in normal phagosome biogenesis. *EMBO J.*, 1996, **15**, 6960–6968.

77. Crowle, A. J., Dahl, R., Ross, E. and May, M. H., Evidence that vesicles containing living virulent *M. tuberculosis* or *M. avium* in cultured human macrophages are not acidic. *Infect. Immun.*, 1991, **59**, 1823–1831.

78. Sturgill-Koszycki, S. *et al.*, Lack of acidification in *Mycobacterium* phagosomes produced by exclusion of the vesicular proton-ATPase. *Science*, 1994, **263**, 678–681.

79. Via, L. E., Deretic, D., Ulmer, R. J., Hibler, N. S., Huber, L. A. and Deretic, V., Arrest of mycobacterial phagosome maturation is caused by a block in vesicle fusion between stages controlled by rab5 and rab7. *J. Biol. Chem.*, 1997, **272**, 13326–13331.

80. Clemens, D. L., Lee, B. Y. and Horwitz, M. A., Deviant expression of Rab5 on phagosomes containing the intracellular pathogens *Mycobacterium tuberculosis* and *Legionella pneumophila* is associated with altered phagosomal fate. *Infect. Immun.*, 2000, **68**, 2671–2684.

81. Beatty, W. L., Rhoades, E. R., Hsu, D. K., Liu, F.-T. and Russell, D. G., Association of a macrophage galactoside-binding protein

with *Mycobacterium*-containing phagosomes. *Cell. Microbiol.*, 2002, **4**, 167–176.

82. Malik, Z. A., Iyer, S. S. and Kusner, D. J., *Mycobacterium tuberculosis* phagosomes exhibit altered calmodulin-dependent signal transduction: contribution to inhibition of phagosome-lysosome fusion and intracellular survival in human macrophages. *J. Immunol.*, 2001, **166**, 3392–3401.

83. Malik, Z. A., Thompson, C. R., Hashimi, S., Porter, B., Iyer, S. S. and Kusner, D. J., Cutting edge: *Mycobacterium tuberculosis* blocks Ca^{2+} signaling and phagosome maturation in human macrophages via specific inhibition of sphingosine kinase. *J. Immunol.*, 2003, **170**, 2811–2815.

84. Ferrari, G., Langen, H., Naito, M. and Pieters, J., A coat protein on phagosomes involved in the intracellular survival of mycobacteria. *Cell*, 1999, **97**, 435–447.

85. Gatfield, J. and Pieters, J., Essential role for cholesterol in entry of mycobacteria into macrophages. *Science*, 2000, **288**, 1647–1650.

86. Russell, D. G., Dant, J. and Sturgill-Koszycki, S., *Mycobacterium avium-* and *Mycobacterium tuberculosis*-containing vacuoles are dynamic, fusion-competent vesicles that are accessible to glycosphingolipids from the host cell plasmalemma. *J. Immunol.*, 1996, **156**, 4764–4773.

87. Pancholi, P., Mirza, A., Bhardwaj, N. and Steinman, R. M., Sequestration from immune CD4+ T cells of mycobacteria growing in human macrophages. *Science*, 1993, **260**, 984–986.

88. Wadee, A. A., Kuschke, R. H. and Dooms, T. G., The inhibitory effects of *Mycobacterium tuberculosis* on MHC class II expression by monocytes activated with riminophenazines and phagocyte stimulants. *Clin. Exp. Immunol.*, 1995, **100**, 434–439.

89. Wojciechowski, C. W., DeSanctis, J., Skamene, E. and Radzioch, D., Attenuation of MHC class II expression in macrophages infected with *Mycobacterium bovis* bacillus Calmette-Guerin involves class II transactivator and depends on the *Nramp1* gene. *J. Immunol.*, 1999, **163**, 2688–2696.

90. Stenger, S., Niazi, K. R. and Modlin, R. L., Down-regulation of CD1 on antigen-presenting cells by infection with *Mycobacterium tuberculosis*. *J. Immunol.*, 1998, **161**, 3582–3588.

91. Noss, E. H., Harding, C. V. and Boom, W. H., *Mycobacterium tuberculosis* inhibits MHC class II antigen processing in murine bone marrow macrophages. *Cell Immunol.*, 2000, **201**, 63–74.

92. DeLerma Barbaro, A. *et al.*, Block of Stat-1 activation in macrophages phagocytosing bacteria causes reduced transcription of CIITA and consequent impaired antigen presentation. *Eur. J. Immunol.*, 2002, **32**, 1309–1318.

93. Jiao, X. *et al.*, Dendritic cells are host cells for mycobacteria *in vivo* that trigger innate and acquired immunity. *J. Immunol.*, 2002, **168**, 1294–1301.

94. Bodnar, K. A., Serbina, N. V. and Flynn, J. L., Fate of *Mycobacterium tuberculosis* within murine dendritic cells. *Infect. Immun.*, 2001, **69**, 800–809.

95. Schaible, U. E., Agens, K., Fischer, K., Collins, H. L. and Kaufmann, S. H., Intersection of group I CD1 molecules and mycobacteria in different intracellular compartments of dendritic cells. *J. Immunol.*, 2000, **164**, 4843–4852.

96. Greenwell-Wild, T., Vazquez, N., Sim, D., Schito, M., Chatterjee, D., Orenstein, J. M. and Wahl, S. M., *Mycobacterium avium* infection and modulation of human macrophage gene expression. *J. Immunol.*, 2002, **169**, 6286–6297.