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Lipoarabinomannan from *Mycobacterium tuberculosis* Promotes Macrophage Survival by Phosphorylating Bad through a Phosphatidylinositol 3-Kinase/Akt Pathway*

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Efforts in prevention and control of tuberculosis suffer from the lack of detailed knowledge of the mechanisms used by pathogenic mycobacteria for survival within host cell macrophages. The exploitation of host cell signaling pathways to the benefit of the pathogen is a phenomenon that deserves to be looked into in detail. We have tested the hypothesis that lipoarabinomannan (LAM) from the virulent species of *Mycobacterium tuberculosis* possesses the ability to modulate signaling pathways linked to cell survival. The Bcl-2 family member Bad is a proapoptotic protein. Phosphorylation of Bad promotes cell survival in many cell types. We demonstrate that man-LAM stimulates Bad phosphorylation in a phosphatidylinositol 3-kinase (PI-3K)-dependent pathway in THP-1 cells. Man-LAM activated PI-3K. LAM-stimulated phosphorylation of Bad was abrogated in cells transfected with a dominant-negative mutant of PI-3K (Δp85), indicating that activation of PI-3K is sufficient to trigger phosphorylation of Bad by LAM. Since phosphorylation of Bad occurred at serine 136, the target of the serine/threonine kinase Akt, the effect of LAM on Akt kinase activity was tested. Man-LAM could activate Akt as evidenced from phosphorylation of Akt at Thr^{308} and by the phosphorylation of the exogenous substrate histone 2B. Akt activation was abrogated in cells transfected with Δp85. The phosphorylation of Bad by man-LAM was abrogated in cells transfected with a kinase-dead mutant of Akt. These results establish that LAM-mediated Bad phosphorylation occurs in a PI-3K/Akt-dependent manner. It is therefore the first demonstration of the ability of a mycobacterial virulence factor to up-regulate a signaling pathway involved in cell survival. This is likely to be one of a number of virulence-associated mechanisms by which bacilli control host cell apoptosis.

Despite the potential role of the macrophage in the eradication of microbes, pathogenic *Mycobacterium* species have survived down the ages as some of the most successful in evading macrophage surveillance mechanisms in a manner that ensures their survival and replication inside the macrophage. A variety of mechanisms contribute to the survival of *Mycobacterium tuberculosis* within macrophages (1) including inhibition of phagosome-lysosome fusion (2), inhibition of the acidification of phagosomes (3), and resistance to killing by reactive oxygen (4) and reactive nitrogen intermediates (5, 6). *M. tuberculosis* produces large quantities of lipoarabinomannan (LAM).[1] LAM can inhibit macrophage activation and triggering and represents a virulence factor contributing to the persistence of mycobacteria within macrophages. LAM is a complex molecule consisting of a phosphatidylinositol (PI) moiety that anchors a large mannose core to the mycobacterial cell wall (7–9). The mannose core consists of multiple branched, arabinofuranosyl side chains. *M. tuberculosis* and *M. leprae* modify the nonreducing end of the arabinofuranosyl chains with mannose residues yielding man-LAM, whereas rapidly growing mycobacterial species have nonreducing termini of two types, the linear Ara_4 and the branched Ara_6 motifs, thereby giving rise to ara-LAM (7, 10). LAM exhibits a wide array of immunomodulatory functions including inhibition of interferon-γ-induced functions such as macrophage microbicidal and tumoricidal activity (11), scavenging of potentially cytotoxic oxygen free radicals (12), inhibition of protein kinase C activity (13), and evocation of a number of cytokines such as tumor necrosis factor-α (14). The early response genes c-*fos*, *KC*, and *JE* are induced by ara-LAM but not by man-LAM (15). The ability of man-LAM to impair responsiveness to interferon-γ and to attenuate tumor necrosis factor-α and interleukin-12 mRNA production through effects on the protein phosphatase SHP-1 has been suggested to be a major mechanism by which man-LAM promotes intracellular survival (16).

Despite the recent advances, the intracellular signaling following interaction of the mycobacterium with host cells and the role of LAM in these processes is incompletely understood. The survival of *M. tuberculosis* in macrophages probably involves more than one mechanism. In this study, we have tested whether LAM plays a role in triggering a signaling pathway that suppresses the intrinsic cell death machinery of phagocytic cells.

The regulation of programmed cell death, apoptosis, is an exceptionally complicated process that involves a myriad of proteins. The family of proteins that includes Bcl-2 comprises members that are both antiapoptotic and those that are proapoptotic such as Bax and Bad. Bad interacts with Bcl-2 and $Bcl\text{-}X_L$, sequestering these proteins, and thus promotes apoptosis (17). Phosphorylation of Bad at either of two sites, serine residues 112 and 136 (numbering based on the sequence of murine Bad) creates consensus sites for interaction with the 14-3-3 protein. Bad is then bound to 14-3-3 instead of Bcl-2 or $Bcl\text{-}X_L$, resulting in the liberation of the antiapoptotic proteins and promotion of cell survival. Interleukin-3 (18) and other survival factors promote cell survival through their ability to

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[1] The abbreviations used are: LAM, lipoarabinomannan; PI, phosphatidylinositol; PI-3K, phosphatidylinositol 3-kinase.

stimulate phosphatidylinositol 3-kinase (PI-3K) (19) and inactivate the apoptotic factor Bad (20, 21). The PI-3K-sensitive pathway involves the activation of the serine/threonine kinase Akt or protein kinase B (22–25) and direct phosphorylation of Bad at serine 136 (26–29). Akt/protein kinase B is the major downstream target of receptor tyrosine kinases that signal via the PI-3K. Activated protein kinase B has been implicated in glucose metabolism, transcriptional control, and the regulation of apoptosis in many cell types (30, 31). In addition to Bad, Akt also phosphorylates caspase 9 (32), forkhead transcription factor (33, 34), and the IκB kinase, thereby activating NF-κB (35, 36).

In addition to phosphorylation at serine 136 mediated by protein kinase B, Bad undergoes protein kinase A- (37) and $p90^{RSK}$- (38) mediated phosphorylation on serine 112. The Ca^{2+}-activated protein phosphatase calcineurin can dephosphorylate Bad, reversing the phosphorylation at both serine 112 and serine 136 (39). A third phosphorylation site at serine 155 has recently been identified (40–43). When Bad is bound to prosurvival Bcl-2 family members, Bad serine 155 phosphorylation requires the prior phosphorylation at serine 136, which recruits 14-3-3 proteins that then function to increase the accessibility of serine 155 to survival-promoting kinases (43). Bad is not a ubiquitously expressed protein. Nevertheless, several major signaling pathways influence cell survival through their effects on the phosphorylation state of Bad (44). We demonstrate that man-LAM from the virulent Erdman strain of *M. tuberculosis* promotes phosphorylation of Bad at serine 136 through a PI-3K/Akt pathway in the human cell line THP-1 and hypothesize that this probably represents one of the mechanisms by which man-LAM promotes cell survival to the benefit of the pathogen.

EXPERIMENTAL PROCEDURES

Reagents—-Histone 2B was purchased from Roche Molecular Biochemicals. Phorbol 12-myristate 13-acetate was purchased from Sigma. Protein A/G Plus-agarose, rabbit anti-p85 phosphoinositide 3-kinase, goat anti-Akt, and rabbit anti-Bad antibody were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Anti-phophotyrosine antibody was from Life Technologies, Inc. Wortmannin, LY294002, PD98059, and SB203580 were from Calbiochem. Phosphorylation state-specific antibodies Bad (Ser^{112}) and (Ser^{136}) and Akt (Thr^{308}) antibodies and the Phototope-HRP Chemiluminescent Western Detection Kit were from New England Biolabs, Inc. (Beverly, MA). All other chemicals were from Sigma.

Cell Culture and Transfection—THP-1 cells (derived from a patient with acute monocytic leukemia) are mature cells from the monocyte/macrophage lineage. These were obtained from the National Center for Cell Science (Pune, India). Media and supplements were obtained from Life Technologies, Inc. The cell line was maintained in RPMI 1640 containing 10% heat-inactivated fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, 2 mM glutamine, and 20 mM sodium bicarbonate. The cells were incubated at 5% CO_2 and 95% humidity in a 37 °C chamber. THP-1 cells were treated with phorbol 12-myristate 13-acetate to induce maturation of the monocytes to a macrophage-like, adherent phenotype. The cells were washed three times with culture medium without fetal bovine serum and resuspended to a concentration of 2×10^6 cells/ml. Cell viability was determined to be >95% by the trypan blue dye exclusion method. 2×10^6 cells were plated in each well of six-well plates. Cells were deprived of serum by culturing in RPMI without fetal bovine serum for 12–16 h, before treatment with LAM. Transfections were carried out on adherent THP-1 cells (2×10^6 cells/well in six-well plates). Cells were transfected with 2 μg of plasmid (recombinants or empty vectors) using the Effectene Reagent (Qiagen) in RPMI with 10% fetal bovine serum according to the manufacturer's instructions. The dominant-negative mutant of p85 was deleted in the inter-SH2 region of wild type p85α (Δp85), which abolishes binding to the p110 subunit of PI 3-kinase. The kinase-deficient mutant of Akt (Akt.KD) carried the mutation K179M.

Mycobacterial Lipids—Endotoxin-free man-LAM and ara-LAM were kindly provided by Dr. John Belisle (Colorado State University, Ft. Collins, CO, through NIAID, National Institutes of Health, Contract NO1-AI-75320). Man-LAM was derived from the virulent Erdman strain of *M. tuberculosis*. LAM was dissolved at a concentration of 1 mg/ml in pyrogen-free water and diluted with medium before each experiment.

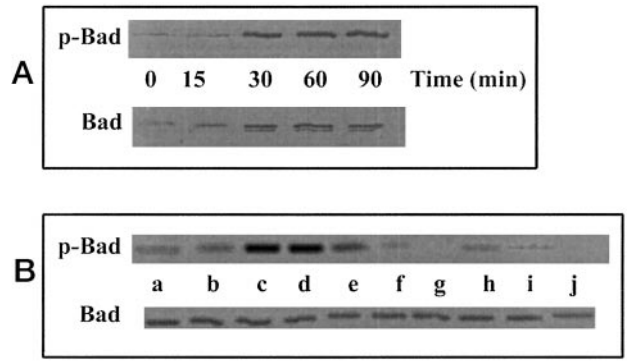

FIG. 1. **Man-LAM-induced phosphorylation of Bad in THP-1 cells.** Differentiated THP-1 cells in serum-free medium were incubated with man-LAM (2 μg/ml) for different periods of time, followed by immunoprecipitation of lysates with anti-Bad antibody, SDS-polyacrylamide gel electrophoresis, and immunoblotting and development by enhanced chemiluminescence as described under "Experimental Procedures." *A*, a representative Western blot using anti-phospho-Bad Ser^{136}. Total Bad was immunoblotted to confirm equal loading. *B*, cells were treated with either wortmannin (*lanes e*, *f*, and *g*, representing concentrations of 1.5, 15, and 150 nM, respectively) for 45 min or LY294002 (*lanes h*, *i*, and *j*, representing concentrations of 2, 20, and 200 μM, respectively) for 4 h or vehicle (*DMSO*) (*lanes a* and *c*, representing treatment for 45 min, and *lanes b* and *d*, representing treatment for 4 h, respectively). Cells were then either not treated (*lanes a* and *b*) or treated (*lanes c–j*) with 2 μg/ml man-LAM for 60 min. Bad phosphorylation was then visualized using phospho-Bad antibodies after lysing the cells. Total Bad was immunoblotted to confirm equal loading.

Immunocomplex Akt Kinase Assay—To assay for Akt protein kinase activity, cells were lysed in lysis buffer (45); lysates were incubated with anti-Akt antibody, and kinase assays were carried out with the immunoprecipitates using histone 2B as substrate (45). After incubation at room temperature for 30 min, the reaction was stopped by adding protein gel denaturing buffer, and the mixture was separated by SDS-polyacrylamide gel electrophoresis, followed by autoradiography.

Immunocomplex PI 3-kinase Assay—Following immunoprecipitation of cell lysates with anti-p85 PI-3K antibody, immunoprecipitates were washed, and PI-3K assays were performed (46). Reactions were carried out for 15 min at room temperature, phosphorylated lipid products were extracted and separated on TLC plates (47), and incorporated radioactivity was measured by liquid scintillation counting.

Preparation of Cell Membranes—Cells after treatment without or with LAM were washed, resuspended in buffer A (137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 2.5 mM EDTA, 1 mM EDTA, 1 mM dithiothreitol, and 0.1 mM pefabloc), sonicated twice for 10 s each, and centrifuged for 5 min at $800 \times g$. After discarding the nuclei and unbroken cells, membranes were prepared by ultracentrifugation at $250{,}000 \times g$ for 60 min. The pellets (membranes) were washed and suspended in buffer A.

Immunoblotting—Cell lysates were prepared as described above and immunoprecipitated using anti-Akt, anti-Bad, or the respective phosphospecific antibodies. The immunoprecipitates were fractionated on SDS-polyacrylamide (10% for Akt and 12% for Bad) gels and transferred to nitrocellulose membranes. Membranes were blocked with 5% nonfat dry milk, incubated with primary antibody overnight at 4 °C, followed by goat anti-rabbit secondary antibody conjugated to horseradish peroxidase for 1 h at room temperature, and finally visualized using the Phototope-HRP Western detection kit.

RESULTS

Phosphorylation of Bad—Following treatment of THP-1 cells with man-LAM, the phosphorylation status of Bad was examined using phosphospecific antibodies. Man-LAM from the virulent Erdman species of *M. tuberculosis* promoted the phosphorylation of Bad at Ser^{136} in a time-dependent manner (Fig. 1*A*). LAM from a fast growing species (ara-LAM) did not exhibit an effect equivalent to that of man-LAM (data not shown). Phos-

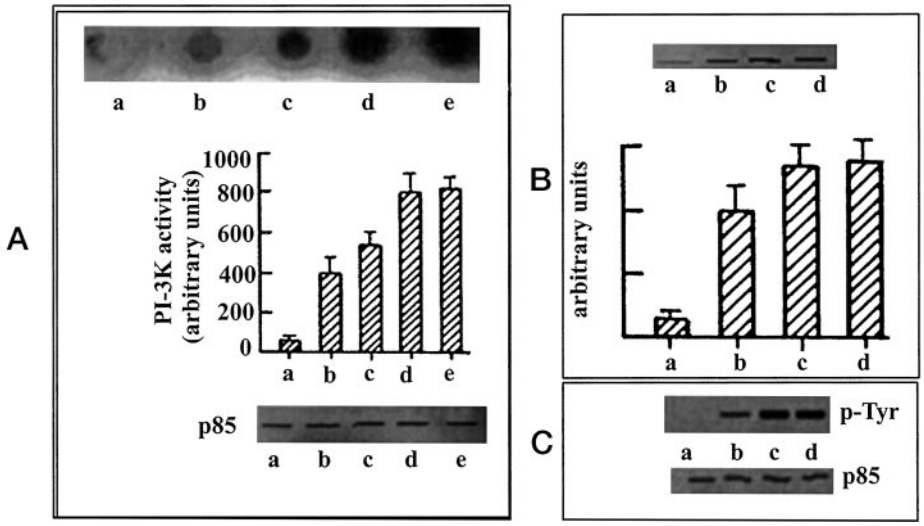

FIG. 2. **Man-LAM-induced activation of PI 3-kinase in THP-1 cells.** *A*, serum-starved cells were exposed to man-LAM(2 μg/ml) for different time periods followed by immunoprecipitation of lysates with anti-p85 PI 3-kinase antibody. PI 3-kinase activity was assayed using PI as substrate as described under "Experimental Procedures," and PIP was detected by autoradiography after separation by TLC (*top panel*). The *middle panel* represents a densitometric analysis of the autoradiograms. Data represent the mean ± S.E. of three independent experiments. The *bottom panel* is a Western blot of anti-p85 in the immunoprecipitate. *Lanes a*, *b*, *c*, *d*, and *e* represent the time points 0, 5, 10, 30, and 60 min, respectively. *B*, translocation of PI-3K to the membrane. The *top panel* shows the effect of man-LAM (2 μg/ml) treatment for different time periods on the translocation of p85α to the membrane fraction as measured by Western blotting using anti-p85α antibody. *Lanes a*, *b*, *c*, and *d* represent time points 0, 10, 30, and 60 min, respectively. The *bottom panel* is a densitometric analysis of the blot. *Error bars* illustrate S.E. from three independent experiments. *C*, tyrosine phosphorylation of PI-3K by LAM. Cells after treatment were lysed and immunoprecipitated with anti-p85α, followed by immunoblotting with anti-phosphotyrosine antibody as described under "Experimental Procedures." The *top panel* shows the effect of man-LAM (2 μg/ml) treatment for different time periods on the tyrosine phosphorylation of p85α. The *bottom panel* is a representative Western blot of anti-p85α to show that the same amount of p85α was present in each sample. *Lanes a*, *b*, *c*, and *d* represent the time points 0, 10, 30, and 60 min.

phorylation of Bad at serine 112 was not observed.

PI-3K Inhibitors Block Bad Phosphorylation—The phosphorylation of Bad at serine 136 occurs through a PI-3K/Akt-dependent pathway (26–29). Cells were pretreated with selective inhibitors prior to stimulation with man-LAM in order to test their ability to block Bad phosphorylation. The PI-3K inhibitors wortmannin and LY294002 inhibited Bad phosphorylation at serine 136 in a dose-dependent manner (Fig. 1*B*). The MEKK1 inhibitors PD90859 and SB20358 did not influence Bad phosphorylation (data not shown). It was inferred that man-LAM signals through PI-3K to phosphorylate Bad.

LAM Activates PI-3K—Since man-LAM-induced Bad phosphorylation was abrogated by PI-3K inhibitors, *in vitro* PI-3K activity was tested after treatment with man-LAM. Man-LAM was found to stimulate PI-3K activity (Fig. 2*A*) in a time-dependent manner. Control experiments showed that equal amounts of precipitated PI-3K were used to assay kinase activity.

Activation of heterodimeric (p85-p110) PI-3K is often promoted by recruitment to the plasma membrane through interaction of SH2 domains in p85 with tyrosine-phosphorylated proteins. Treatment with man-LAM caused a time-dependent increase in the amount of the p85 subunit detected in the membrane fraction, indicating translocation from the cytosol (Fig. 2*B*). Tyrosine phosphorylation of p85 might represent another mechanism of PI-3K activation (48). Cell lysates were immunoprecipitated with anti-p85 and analyzed by Western blotting with anti-phosphotyrosine antibody. man-LAM treatment increased the tyrosine phosphorylation of the p85α subunit of PI-3K in a time-dependent manner (Fig. 2*C*).

Akt Activation by LAM—The PI-3K-sensitive pathway of Bad phosphorylation involves the activation of the protein kinase Akt (or protein kinase B) and the phosphorylation of Bad at serine 136. The interaction of the amino-terminal pleckstrin homology domain of Akt with the phospholipid product of PI-3K induces a conformational change in Akt, making it a more efficient substrate for the phosphatidylinositide-dependent kinase 1 (49–51). Stimulus-induced Akt phosphorylation on Thr^{308} by phosphatidylinositide-dependent kinase 1 and on Ser^{473} is required for maximal activity. To determine whether Akt is a downstream effector of man-LAM-induced PI-3K signaling in THP-1 cells, Akt phosphorylation on Thr^{308} was measured using immunoblotting with phosphospecific Thr^{308} anti-Akt antibody, followed by densitometric analysis of the autoradiograms. Man-LAM-stimulated phosphorylation of Akt at Thr^{308} in a dose-dependent (Fig. 3*A*) and time-dependent (Fig. 3*B*) manner.

Stimulation of Akt Kinase Activity by Man-LAM—LAM stimulated histone 2B phosphorylation in a time-dependent manner (Fig. 3*C*), indicating LAM-induced stimulation of Akt kinase activity. Histone phosphorylation stimulated by man-LAM was inhibited by the PI-3K inhibitors wortmannin and LY294002 (Fig. 3*D*). Unlike man-LAM, exposure of cells to ara-LAM for 60 min did not result in stimulation of Akt kinase activity (Fig. 3*D*, *lanes g–i*).

Expression of a Dominant Negative Mutant of p85α Abolishes Akt Kinase Activation—To establish whether Akt is the serine/threonine kinase that is downstream of PI-3K in the man-LAM signaling pathway, the effect of expression of the dominant negative mutant of p85α on LAM-induced Akt kinase activation was assessed. Thr^{308} phosphorylation of Akt was abrogated by expression of the Δp85 mutant of PI-3K but not by the control empty vector (Fig. 3*E*). In harmony with this, Bad phosphorylation at serine 136 was also abrogated in cells expressing Δp85 (data not shown).

Kinase-deficient Akt Mutant (Akt.KD) Blocks Man-LAM-induced Phosphorylation of Bad—In order to establish a connection between Akt kinase activation and the phosphorylation of Bad at serine 136 induced by LAM, we transfected THP-1 cells with a kinase-dead mutant (K179M) of Akt and assessed the phosphorylation status of Bad. Akt.KD abrogated the phosphorylation of Bad following stimulation by LAM, whereas control empty vector had no such effect (Fig. 3*F*).

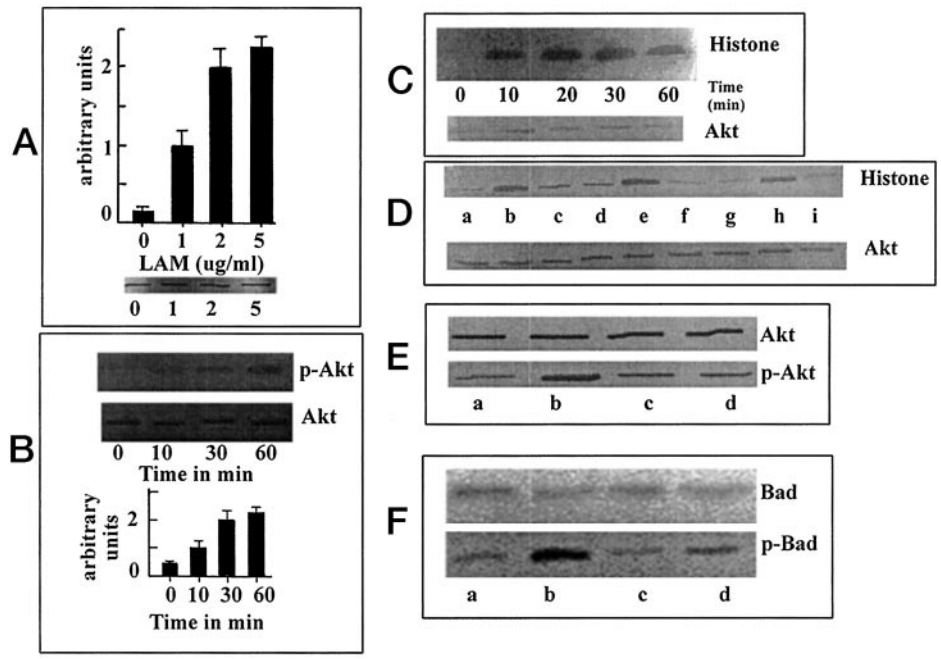

FIG. 3. **Effect of man-LAM on Akt and Bad phosphorylation.** *A*, serum-starved cells were exposed to man-LAM at different concentrations for 30 min. Lysates were immunoprecipitated with anti-Akt antibody followed by immunoprecipitation with phospho-Akt Thr^{308} antibody. Immunoprecipitates were separated on SDS-polyacrylamide gels, followed by Western blotting with phospho-Akt Thr^{308}, development by enhanced chemiluminescence, and densitometric analysis (*top panel*). Data represent the mean ± S.E. of three independent experiments. The *bottom panel* is a representative Western blot showing that equal amounts of immunoprecipitated Akt protein were detected by immunoblotting in each sample. *B*, cells were exposed to man-LAM for different time periods. Lysates were immunoprecipitated with anti-Akt antibody followed by Western blotting with control Akt or phospho-Akt Thr^{308}. The *bottom panel* is a densitometric analysis of the autoradiograms. *Error bars* represent S.E. of three independent determinations. *C*, cells were treated with man-LAM for different time periods, and Akt kinase activity was measured following immunoprecipitation with anti-Akt antibody using histone 2B as substrate (*top panel*). The *bottom panel* shows the amount of immunoprecipitated Akt protein in each sample. *D*, cells were treated with either 100 nM wortmannin (*lane c*) for 45 min or 50 μM LY294002 (*lane f*) for 4 h or vehicle (*DMSO*) (*lanes a* and *b* representing treatment for 45 min and *lanes d* and *e* representing treatment for 4 h, respectively). Cells were then either not treated (*lanes a* and *d*) or treated (*lanes b*, *c*, *e*, and *f*) with 2 μg/ml man-LAM for 60 min. Cells were treated either with medium (*lane g*) or with 2 μg/ml man-LAM (*lane h*) or with 2 μg/ml ara-LAM (*lane i*) for 60 min. Akt kinase activity was measured using histone 2B as substrate. *E*, cells were transfected with a dominant negative mutant of p85α (*lanes c* and *d*) or the control empty vector (*lanes a* and *b*) using Effectene as described. After transfection, cells were serum-starved and stimulated without (*lanes a* and *c*) or with (*lanes b* and *d*) man-LAM (2 μg/ml) for 60 min. Akt phosphorylation was then visualized (*lower blot*) after lysing the cells. The *upper blot* shows that the same amount of Akt protein was present in each sample. *F*, cells were transfected with a kinase-deficient mutant of Akt (Akt.KD) (*lanes c* and *d*) or with control empty vector (*lanes a* and *b*). After transfection, cells were serum-starved and stimulated without (*lanes a* and *c*) or with (*lanes b* and *d*) man-LAM (2 μg/ml) for 60 min. Bad phosphorylation was then visualized (*lower blot*) after lysing the cells. The *upper blot* shows that the same amount of Bad protein was present in each sample.

DISCUSSION

The molecular basis of the pathogenicity of *M. tuberculosis* is poorly understood. *M. tuberculosis* is a facultative intracellular pathogen. Whereas the normal function of macrophages is to engulf and destroy microorganisms, mycobacteria have evolved ways to circumvent the defense mechanisms of macrophages. Central to the ability of *M. tuberculosis* to infect the host and cause active or latent disease is the propensity of the tubercle bacillus to enter the host mononuclear phagocyte and survive and multiply within macrophages. Macrophage apoptosis contributes to host defense against *M. tuberculosis* infection. Human alveolar macrophages undergo apoptosis in response to *M. tuberculosis* infection (52). Very recently it has been demonstrated that bacillary control of host cell apoptosis is a virulence-associated phenotype of *M. tuberculosis*, with virulent strains having the ability to evade apoptosis of infected macrophages (53). In light of these observations, understanding the virulence factors that may modulate host cell apoptosis is necessary. We chose to study whether man-LAM from a virulent strain of *M. tuberculosis* could modulate cell signaling pathways known to control cell survival.

The phosphorylation of Bad is one of the mechanisms of protection of cells from programmed cell death. We have tested the hypothesis that man-LAM from the virulent species of *M. tuberculosis* protects cells from apoptosis at least partly through phosphorylation of Bad. Man-LAM was found to stimulate Bad phosphorylation on serine 136 in THP-1 cells. Bad phosphorylation at serine 136 is believed to occur via a PI-3K/Akt signaling pathway. Consistent with this, the PI-3K inhibitors wortmannin and LY294002 inhibited the LAM-stimulated phosphorylation of Bad. Activation of PI-3K was observed after stimulation of cells with LAM. Furthermore, LAM caused p85 to translocate to the membrane, a phenomenon associated with enhanced lipid kinase activity of this enzyme. Tyrosine phosphorylation of p85 may represent another mechanism of PI-3K activation. LAM stimulated tyrosine phosphorylation of p85. The fact that PI-3K activation is sufficient for Bad phosphorylation upon treatment of cells with LAM was demonstrated by the fact that transfection of THP-1 cells with the dominant negative mutant (Δp85) of PI-3K abrogated LAM-mediated Bad phosphorylation. One of the major functions of Akt is protection of cells from programmed cell death. This protection has been demonstrated for several cell types including COS cells (23), fibroblasts (24), and neuronal cells (55). This is at least in part due to the ability of Akt to phosphorylate Bad at serine 136. We attempted to establish a connection between PI-3K, Akt, and the phosphorylation of Bad. Akt phosphorylation at Thr^{308} was stimulated by LAM in a PI-3K-sensitive manner. Akt kinase activity assessed by using histone 2B as exogenous substrate was also stimulated by LAM. Transfection of THP-1 with the

Δp85α mutant of PI-3K abolished the LAM-stimulated phosphorylation of Akt at Thr^{308} as well as the ability of Akt to phosphorylate histone 2B, establishing that Akt is a downstream effector of LAM-mediated PI-3K signaling. The connection between Akt and Bad was similarly established by transfecting cells with a kinase-deficient mutant of Akt (Akt.KD) and assessing the phosphorylation of Bad after stimulation with LAM. LAM-mediated Bad phosphorylation was abolished in cells transfected with Akt.KD. In summary, we demonstrate that man-LAM from the virulent Erdman strain of *M. tuberculosis* activates Akt in a PI-3K-sensitive manner, leading to the phosphorylation of Bad at serine 136, suggesting that this may be one of the mechanisms by which LAM directly promotes macrophage cell survival. Inhibition of macrophage apoptosis would allow the mycobacteria to escape from being packaged into apoptotic bodies. Uptake of bacilli packaged in this way is suggested to result in more effective microbicidal processing (54).

This is the first demonstration of a mycobacterial virulence factor having the capability of up-regulating a macrophage survival signaling pathway, thereby creating an environment favorable for the survival of the pathogen.

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