Lipoarabinomannan-Induced Cell Signaling Involves Ceramide and Mitogen-Activated Protein Kinase

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Lipoarabinomannan (LAM) is a major cell wall-associated lipoglycan, produced in large amounts (15 mg/g of bacteria) in different species of mycobacteria. Our laboratory has previously reported that LAM from Mycobacterium smegmatis exerts its cytotoxic activity via inhibition of protein kinase C, a key signaling molecule inside the mononuclear cells (S. Ghosh, S. Pal, S. Das, S. K. Dasgupta, and S. Majumdar, FEMS Immunol. Med. Microbiol. 21:181–188, 1998). In this study we report that LAM from Mycobacterium tuberculosis induces a signal transduction pathway in favor of survivability of the host cells via the generation of ceramide, a novel second messenger. The endogenous ceramide level in mononuclear cells was found to be enhanced during LAM treatment. The effects of LAM on protein tyrosine phosphorylation in human peripheral blood mononuclear cells were examined. LAM enhanced the tyrosine phosphorylation of p42 mitogen-activated protein kinase and phosphoinositol 3-kinase (PI3 kinase) and dephosphorylation of stress-activated protein kinase. LAM-induced phosphorylation of p42 (extracellular signal-regulated kinase 2) was further enhanced by wortmannin, a PI3 kinase inhibitor. To examine whether these effects are due to elevation of endogenous ceramide, we exposed the cells to cell-permeative C2-ceramide exogenously and studied the activities of different protein kinases. Fluorescence-activated cell sorter analysis and morphological studies showed that LAM induces cell survival. Therefore, these results suggest the ability of LAM to induce ceramide in the altered signaling pathway and help in cell survival.

Archeological evidence indicates that tuberculosis has afflicted humans for thousands of years (9, 39). Mycobacterium tuberculosis, the causal organism of tuberculosis, now infects a large proportion of the population. Even now, 2 to 3 million people worldwide die each year from this disease. While tuberculosis is a preventable and largely curable disease, our understanding of the cellular and molecular interaction between mycobacteria and host immune cells is far from complete. For enhancing the antimycobacterial resistance of the host, immunization with Mycobacterium bovis (BCG) has been widely used for many years but has had little effect on reducing the incidence of tuberculosis. M. tuberculosis bacilli are noteworthy for having evolved mechanisms that allow them to survive and multiply in macrophages (49). It was observed that the macrophages, which should actively participate to overcome the infection, become nonfunctional during pathogenesis (41). Phagocytosis of the highly host-adapted intracellular pathogen M. tuberculosis by mononuclear phagocytes has been known as a multi-ligand-, multi-receptor-mediated event.

Lipoarabinomannan (LAM) is a cell wall-associated lipoglycan present in different species of mycobacteria, which, while remaining anchored to the mycobacterial cell membrane, is thought to extend all the way to the surface. It exhibits a wide range of immunomodulatory effects (8, 21, 23, 41). Vesicles containing LAM are released by phagosome containing ingested mycobacteria, suggesting active transport out of infected cells (40, 52). LAM released by infected macrophages may act in a paracrine manner to modulate the function of surrounding leukocytes. LAM alters the signaling processes in the macrophages (16), and it has been reported that LAM inhibits M. tuberculosis-induced apoptosis (38). Recently it has also been reported that LAM affects the signaling pathway involved in cell survival (26). In a previous paper, we showed that there was 60 to 90% inhibition of total protein kinase C (PKC) activity in cells treated with LAM isolated from an avirulent species, Mycobacterium smegmatis (16). However, PKC activity has also been found to be inhibited in Lam-treated cells of virulent species M. tuberculosis (8). The effect of LAM isolated from virulent as well as avirulent strains of mycobacteria on different isotypes of PKC (both Ca dependent and Ca independent) has been studied in our laboratory. The results obtained suggest that LAM from M. tuberculosis is more potent in inhibiting the β-1 isotype of PKC. Also, there was enhanced expression of atypical PKC-ζ in LAM-treated cells of M. tuberculosis, while there was inhibition in expression of PKC-ζ when cells of M. smegmatis were treated with LAM (D. K. Roy and S. K. Majumdar, Abstr. 12th Am. Soc. Cell Biol. Meet., abstr. 404a, 2001).

As it has been observed that the classical PKC signaling pathway is inhibited in cells treated with LAM, we tried to study a signal transduction pathway other than the classical one. Ceramide is known to be an important second messenger in the altered signaling pathway (19, 28, 44). Several components of the sphingomyelin pathway form a novel signaling pathway, which is conserved from yeast to humans (3, 12, 19, 33, 44). Ceramide, the central molecule in this pathway, serves as the second messenger for cellular functions ranging from proliferation and differentiation to growth arrest and apoptosis (28). Ceramide generation involves hydrolysis of sphingomyelin by sphingomyelinase or de novo synthesis by a synthase (25,
29, 44, 45). Both neutral and acidic sphingomyelinas are transiently activated by diverse exogenous stimuli such as tumor necrosis factor alpha, gamma interferon, interleukin-1, Fas ligands, ionizing radiation, or chemotherapeutic agents, leading to an increase in the endogenous ceramide level within seconds to minutes (18, 24). Prolonged and persistent accumulation of ceramide may also occur over a period of several hours (19). In this paper we have shown that the endogenous cellular level of ceramide in human peripheral blood mononuclear cells (PBMC) increased when they were treated with LAM isolated from the virulent species M. tuberculosis. Ceramide interacts with several signaling systems, involving mitogen-activated protein kinase (MAPK) (30, 35), stress activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK) (7, 48), PKC-ε (4, 31), and phosphatases (18). MAPKs are activated by extracellular stimuli in many types of cells via dual phosphorylation of tyrosine and threonine kinases and are thought to be involved in various cellular responses (5, 32). Triggering of the ceramide pathway has been implicated in the activation of SAPK/JNK, along with extracellular signal-regulated kinase (ERK) and p38 MAPK activities. We report here the LAM-induced activity of p42 MAPK (ERK 2) and the p46 JNK1 isoform of SAPK in human PBMC. However, wortmannin, an inhibitor of phosphatidylinositol 3-kinase (PI3 kinase) was also found to affect the activity of ERK 2, indicating involvement of p13 kinase in the regulation of ERK 2. Further, we found that LAM promotes cell survival. Hence, we report that LAM-induced promotion of cell survival involves ceramide, p13 kinase, and MAPK (ERK 2) activation.

MATERIALS AND METHODS

Purification of LAM. M. tuberculosis was isolated from the blood of affected patients at the Bengal Tuberculosis Association, Calcutta, India, and cultured on solid medium. After 3 days, it was transferred to a liquid medium named Kirschner medium and allowed to grow at 37°C under shaking condition (30 × g) for 14 days. For purification of the lipoglycan LAM from M. tuberculosis, the standard method of Hunter and Brennan (21) was used.

Blood donors. Peripheral blood was collected from adult healthy individuals of both sexes. All of them were from a middle socioeconomic background and were human immunodeficiency virus and hepatitis B virus negative.

Effector cells. PBMC were isolated by centrifugation (400 × g, 40 min, 22°C) on a Ficoll-Hyphaque (Sigma Diagnostics Inc.) density gradient. The cells at the interface were collected, washed, and resuspended in RPMI 1640 culture medium (Sigma) supplemented with 10% fetal bovine serum (Gibco BRL).

Preparation of macrophage monolayer and bacteria. PBMC were isolated from healthy, purified protein derivative-negative adult volunteers and cultured in polystyrene petri dishes (Tarsun) for 5 days. The macrophages were purified by adherence to these petri dishes. Effects of experimental manipulations on macrophage viability were assessed by exclusion of trypan blue. The strain of M. tuberculosis was cultured, and the concentration of bacteria was adjusted for use in the experiments after counting. Infections were at different ratios of bacteria to cells.

Preparation of whole-cell lysate of PBMC. Untreated (control) and LAM (from M. tuberculosis)-treated PBMC suspended in RPMI were cultured in 5% CO2 at 37°C for 18 h. Following incubation, the cells were sonicated in lysis buffer and whole-cell lysates were prepared.

The cells were pelleted by centrifugation at 320 × g for 10 min and resuspended in ice-cold extraction buffer containing 10 mM Tris-HCl (pH 7.5), 4.5 mM EGTA, 2.5 mM EDTA, 1.0 mM Na2VO4, and an antiprotease mixture consisting of 0.33 mM leupeptin, 0.2 mM phenylmethysulfonyl fluoride, 4.8 μg trypsin inhibitor units of aprotinin ml−1, and 50 mM 2-mercaptoethanol (Sigma). The mixture was sonicated for 5 to 10 s at 4°C. The postnuclear fraction was discarded by centrifugation at 2,800 × g for 20 min.

Assay for detection and quantification of ceramide. The ceramide assay was carried out by using radiolabeled stearic acid as described by Gamen et al. (15). Human PBMC (3 × 10⁶) were labeled for 30 h with 5 μCi of [1-14C]stearic acid (Amersham Pharmacia Biotech) bound to acid-free serum albumin (1:1 molar ratio) in complete medium. Cells were washed with sterile HEPES to remove the unbound radioactive stearic acid, resuspended in RPMI 1640 with 10% fetal calf serum, and infected with LAM from M. tuberculosis (virulent). The cells were then incubated for different time periods as required. Total cellular lipids were extracted at 4°C with chloroform-methanol (2:1, vol/vol). The radioactivities of aliquots from CHCl₃ phases were determined by liquid scintillation counting, and equal amounts of radioactivity in each sample were applied to thin-layer chromatography silica Gel G plates (Whatman) along with standard C₂₀ and C₁₅ ceramide (Sigma). The plates were air-dried, and lipid bands were identified in an iodine chamber. The bands corresponding to the standard were scraped, and radioactivity was counted in an L. S. Counter (Wallac) with 4 ml of Cocktail-O (Spectrochem, Bombay, India).

Gel electrophoresis and immunoblotting. Whole-cell lysates (2.5 × 10⁶ cells per set) were prepared in lysis buffer (10 mM Tris [pH 7.4], 4.5 mM EGTA, 2.5 mM EDTA, 0.2 mM phenylmethysulfonyl fluoride, 1.0 mM Na2VO4, 4.8 μg trypsin inhibitor units of aprotinin ml−1, and 0.33 mM leupeptin). The supernatants were assayed for protein with a Bio-Rad protein assay kit. Protein samples (100 μg) were analyzed by sodium dodecyl sulfate-7.5% polyacrylamide gel electrophoresis (SDS–7.5% PAGE) together with prestained molecular weight markers (Bio-Rad) and transferred to nitrocellulose as described by Majumder et al. (27). The blots were washed in Tris-buffered saline (TBS) (50 mM Tris containing 150 mM NaCl) and blocked overnight with 3% (wt/vol) bovine serum albumin dissolved in TBS. The blots were then probed with antiphosphothreonine antibody (1:500) (Santa Cruz Biotechnology, Santa Cruz, Calif.) according to the method of Towbin et al. (47). The blots were next washed three times with TBS supplemented with Polybrene T-20 (TBST) and TBS, sequentially, and incubated with anti-rabbit secondary antibody (Sigma) covalently linked to alkaline phosphatase. Nitroblue tetrazolium-bromochloroindolyl phosphate (Gene I) was used as the substrate for color development. The same procedure was used for anti-phospho-erK-ERK (1:1,000) (Calbiochem) and anti-phospho-SAPK (1:1,000) (Calbio-chem) immunoblotting. The blot was stripped off with a buffer containing 50 mM Tris-HCl (pH 6.7), 100 mM 2-mercaptoethanol (Sigma), and 2% SDS and incubated for 1 h at 50°C before reprobing with another type of antibody.

Staining with DAPI. Cells (3 × 10⁶), after treatment with 20 μg of LAM ml⁻¹ and a 10 μM concentration of the MEK 1 inhibitor PD 98059 (Sigma), were collected in phosphate-buffered saline (PBS), fixed with absolute alcohol, and stained with 4,6-diamidino-2-phenyl indole (DAPI), a DNA-specific dye, at a concentration of 1 μg ml⁻¹ for 1 h at room temperature. The cells were washed two times with PBS and mounted on the slides. The slides thus prepared were visualized under a microscope with a UV transmittance filter for illumination at 470 nm (50).

Analysis of DNA fragmentation. Cells (3 × 10⁶) were treated with 20 μg of LAM ml⁻¹ and a 10 μM concentration of the MEK 1 inhibitor PD 98059 and incubated at 37°C for various time periods. Chromosomal DNA was extracted from PBMC by incubating the cells in Tris-EDTA buffer containing NP-40, RNase, and proteinase K (Boehringer Mannheim) (16). DNA fragmentation was analyzed by 1.5% gel electrophoresis.

FACS analysis. Fluorescence-activated cell sorter (FACS) analysis of death of the mononuclear cells following different treatments was done with ethidium bromide staining. Briefly, 3 × 10⁶ cells per set, after the required treatment, were washed with PBS, fixed with 1% p-formaldehyde at 4°C for 30 min, and again washed with PBS twice. The cells were then incubated with 5 μl of RNase A (Boehringer) in 95 μl of PBS for 1 h and stained with ethidium bromide for 15 min, followed by dilution of the test solution by adding PBS buffer and evaluation by FACS (FACSCalibur; Becton Dickinson) (488-nm excitation and 515-nm long-pass filter for detection) (13).

RESULTS

Endogenous ceramide level in LAM-treated mononuclear cells. In order to investigate whether ceramide plays any role in the signal transduction mechanism during mycobacterial infections, we treated PBMC from healthy donors with different doses of LAM and assayed for the cellular ceramide level at different time intervals. Figure 1A demonstrates the effects of different doses of LAM on the endogenous ceramide level. There were increases in the endogenous ceramide level of 25, 56, 32, and 14% after treatment with 10, 20, 30, and 40 μg of LAM ml⁻¹ for 18 h, respectively. Taking 20 μg of LM ml⁻¹ as background, we have shown that ceramide level increased from 1 to 2.1 μg ml⁻¹. Figure 1B demonstrates the effects of LAM on neutral and acidic ceramides. There was an increase in the neutral ceramide level from 1.1 to 1.8 μg ml⁻¹, while the acidic ceramide level increased from 0.8 to 1.4 μg ml⁻¹. Figure 1C shows the changes in the levels of ceramide in different conditions. We observed a significant increase in the ceramide level in the presence of LAM. The results are summarized in Table 1.
as the optimal dose, treatment of cells with LAM resulted in a 23% increase in the ceramide level after 6 h of treatment and in 58, 32, and 25% increases at 18, 24, and 36 h, respectively (Fig. 1B). No significant change was observed at 48 h (data not shown). Therefore, there was a delayed and transient increase in the endogenous ceramide level after 18 h of treatment with 20 μg/LAM mL⁻¹.

**Effect of LAM on MAPK (ERK 1 and ERK 2).** Lysates of PBMC stimulated with different concentrations of LAM for 18 h were subjected to immunoblotting with antiphosphotyrosine antibody. Stimulation of human PBMC with LAM of *M. tuberculosis* resulted in a concentration-dependent increase in phosphorylation of p42 (ERK 2) protein. When the cells were treated with LAM for 18 h, there was about a 37% increase in the expression of ERK 2 with 20 μg/LAM mL⁻¹ and about a 60% increase with 40 μg/LAM mL⁻¹ (Fig. 2A). The detected p42 protein was found to be the ERK 2 isoform of MAPK after the blot was stripped off (46) and reprobed with anti-phospho-ERK antibody, which exhibited 40% increased activity of ERK 2 with 20 μg/LAM mL⁻¹ and 55% increased activity with 40 μg/LAM mL⁻¹ (Fig. 2B). However, p44 (ERK 1) was not detected at significant levels in the study.

The human macrophages were incubated with intact bacteria at different ratios. After 2 h of infection, macrophages were washed to remove the nonadherent bacilli and the cells were cultured overnight. It was observed that there was an increase in activity and expression of p42 (ERK 2) protein when immunoblottedting was with anti-phospho-ERK antibody. There was increased activity of p42 (ERK 2) of about 15, 38, and 42% in the macrophages infected at ratios of 1:10, 1:20, and 1:30, respectively (Fig. 3B).

**Effect of ceramide on MAPK activity.** From the experiments described above it has been found that LAM at different concentrations enhanced the endogenous ceramide level, and it had already been established that ceramide affects the other kinases in its downstream steps (7, 30, 35, 48). Since LAM enhances the expression and activity of ERK 2, it was essential
to study whether exogenous addition of ceramide mimics the same effect on ERK activity. Hence, we used cell-permeative C2-ceramide at different doses, exogenously. The cells were treated with 10 and 30 μg of C2-ceramide ml−1 for 18 h and then immunoblotted with antiphosphotyrosine (Fig. 4A) and anti-phospho-ERK (Fig. 4B) antibodies. The results showed increased ERK 2 activity in ceramide-treated cells at 18 h. At 6 h, no such phosphorylation was noted.

Effect of LAM and ceramide on SAPK/JNK activity. The results described above prompted us to study another protein kinase, SAPK/JNK, which is known to be activated in cells undergoing apoptosis (6, 43). Western blot analysis showed a 46-kDa band after immunoblotting with antiphosphotyrosine antibody (data not shown). We found that the p46 band coincided with the JNK1 isoform when reprobed with anti-phospho-SAPK antibody. This p46 (JNK1) isoform of SAPK/JNK was found to be inhibited when the cells were treated with 20 μg of LAM ml−1 for 18 h. There was about 10 and 30% inhibition of the activity of JNK1 with 10 and 20 μg of LAM ml−1, respectively (Fig. 5A). However, when the cells were treated with 10 and 30 μg of cell permeable C2-ceramide in order to obtain the SAPK/JNK activity, we found that ceramide treatment does not alter the activity of SAPK/JNK in the human mononuclear cells (Fig. 5B). Thus, inhibition of JNK due to treatment of LAM appeared to be independent of ceramide.

When the human macrophages were infected with intact M. tuberculosis, there was also inhibition of expression and activity of p46 SAPK/JNK (Fig. 3A).

Effect of wortmannin on the MAPK activity. In various cell types, PI3 kinase is implicated in regulating cell growth and inhibiting apoptosis (34, 36), in intracellular vesicle trafficking and secretion (10, 22), and in cytoskeletal organization (17, 37). To elucidate the role of PI3 kinase, we examined the effect of wortmannin, a fungal metabolite and a specific inhibitor of PI3 kinase, which irreversibly inhibits p110α by reacting covalently with lysine 802 (36, 37, 51). However, Western blot analysis with anti-phospho-ERK antibody demonstrated that pretreatment with 100 nM wortmannin further enhances LAM-induced p42 ERK activity in human PBMC (Fig. 6).
LAM induces the cells to survive. Similar results were observed with DAPI staining, where intact nuclei were seen in LAM-treated cells (Fig. 8B), similarly as seen in untreated cells (Fig. 8A). Many apoptotic nuclei were seen in cells treated with 10 μM PD 98059 for 2 h (Fig. 8C), while the percentage of apoptosis was less in LAM-treated cells pretreated with PD 98059 (Fig. 8D), showing that LAM induces the cells to survive via an ERK-dependent pathway. The numbers of live and apoptotic cells observed after the different treatments are shown in Table 1.

We also carried out a DNA degradation study with control and treated cells (Fig. 9). A trailing pattern of DNA was seen in the lane treated with a 10 μM concentration of the MEK 1 inhibitor PD 98059 (10 μM) for 2 h, with a distinct apoptotic peak. (E) Cells treated with MEK 1 inhibitor PD 98059 (10 μM) followed by LAM (20 μg ml⁻¹), with decreased amplitude of the apoptotic peak. The percentages of apoptotic nuclei were 4, 5, 4, 36, and 18% for panels A, B, C, D, and E, respectively.
untreated cells (lane 1) or in the cells treated with LAM (20 μg ml⁻¹) for 18 h (lane 2).

**DISCUSSION**

The pathogenic species *M. tuberculosis* has evolved strategies to undermine both acquired immune responses and innate host resistance in humans. The mechanism of pathogenesis involves the role of immunosuppressive molecules such as

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**TABLE 1.** Numbers of live and apoptotic cells observed by DAPI staining after different treatments

| Treatment                | No. of cells (mean ± SE) |
|--------------------------|--------------------------|
|                          | Total  | Live  | Apoptotic |
| None                     | 100    | 95 ± 2| 4 ± 1     |
| LAM (20 μg/ml)           | 100    | 96 ± 2| 3 ± 2     |
| PD 98059 (10 μM)         | 100    | 64 ± 4| 33 ± 3    |
| PD 98059 (10 μM) + LAM (20 μg/ml) | 100    | 82 ± 3| 16 ± 3    |

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*FIG. 8.* Staining of PBMC with UV-fluorescent DAPI dye. (A) Control cells, showing intact nuclei. (B) LAM (20 μg ml⁻¹)-treated cells, showing intact nuclei. (C) Cells treated with the MEK 1 inhibitor PD 98059 (10 μM) for 2 h, showing nuclei that have undergone fragmentation characteristic of apoptosis (arrows). (D) Cells pretreated with PD 98059 (10 μM) for 1 h and then treated with LAM (20 μg ml⁻¹), showing more viable nuclei and few apoptotic nuclei, observed under a microscope with a UV transmittance filter. Data for the numbers of cells observed in DAPI staining are shown in Table 1. Results are from a representative experiment of three.
LAM. Although LAM has been shown to be an immunosuppressive molecule (1, 8, 15, 42), its mode of action is still not clear. The present study examined the possibility that LAM may alter cell signaling mechanisms in human PBMC. An altered signaling pathway in response to various stimuli often involves the production of ceramide via activation of sphingomyelinase, followed by activation of several kinases, which may ultimately lead to an apoptotic pathway (3, 20). Therefore, our first step was to measure the endogenous intracellular level of ceramide. We found that the endogenous ceramide level is elevated in human PBMC treated with LAM of M. tuberculosis. At a concentration of 20 μg of LAM ml⁻¹, there was transient enhancement of the endogenous ceramide level at 18 h. However, at higher concentrations, the endogenous ceramide level decreased. This may be due to the toxic effect of LAM at higher concentration (16). Ceramide is believed to participate in signal transduction either by stimulating phosphoprotein phosphatases (18) or by activating specific serine/threonine kinases (30, 35). One such kinase is MAPK, whose activation is known to be involved in the regulation of broad range of cellular processes (11, 32). Our data suggest a stimulation of phosphorylation of p42 (ERK 2) MAPK in LAM-treated cells. In order to understand the role of ceramide in the phosphorylation of p42 (ERK 2), we studied the effect of exogenously added cell-permeable C2-ceramide at different doses on the phosphorylation of p42 and found that activity of p42 (ERK 2) increased with ceramide treatment, indicating that MAPK may be downstream of ceramide. It has been reported by a number of workers that LAM inhibits M. tuberculosis-induced apoptosis (26, 38). Treatment with 10 μM PD 98059, an inhibitor of MAPK, caused the cells to undergo apoptosis, indicating the essentiality of the ERK pathway for cell survival. The involvement and activation of SAPK/JNK in the signaling pathway has been implicated in the induction of apoptosis in a variety of systems (6, 43). Here we observed that the phosphorylation of the p46 isoform of SAPK/JNK decreased in cells treated with LAM of M. tuberculosis, while the activity of SAPK remained somewhat unaltered when PBMC were treated with cell-permeable C2-ceramide exogenously. This indicates that the inhibition of p46 JNK1 was not due to the enhanced level of ceramide after 18 h of LAM treatment but may be due to the expression of LAM-induced phosphatases in the cell, as we found that the inhibition of p46 JNK in LAM-treated cells can be withdrawn by addition of okadaic acid, an inhibitor of serine/threonine phosphatase (M. Sirkar et al., unpublished data). This inhibition of SAPK/JNK is another parameter to show that LAM-induced mononuclear cells are not undergoing apoptosis. Moreover, the atypical PKC-ζ has been shown to be expressed in LAM (virulent)-treated human mononuclear cells (Roy and Majumdar, Abstr. 12th Am. Soc. Cell Biol. Meet.). Thus, our data clearly indicate that both ceramide and LAM mediate cell proliferation, which proceeds via an ERK-dependent pathway.

PI3 kinase is implicated in the regulation of cellular growth, and its involvement in the inhibition of apoptosis is well established (14, 34, 36). It has been reported that PI3 kinase is inhibited at a Wortmannin concentration of 100 nM (2). Here we found that 100 nM Wortmannin enhanced the LAM-induced p42 (ERK 2) in human mononuclear cells. Therefore, we postulate that inhibition of LAM-induced PI3 kinase involves enhancement of phosphorylation of ERK 2. This result agrees with the data in other reports that LAM stimulates Bad phosphorylation in a PI3 kinase-dependent pathway (26).

All of these results strongly indicate that the delayed and transient increase in the endogenous ceramide level in LAM-treated PBMC modulates the protein kinases downstream. LAM of M. tuberculosis has characteristics to evoke the phosphorylation of p42 ERK, which might be regulated by PI3 kinase, while an inhibition in the activity of p46 JNK was noted. Exogenously added cell-permeative C2-ceramide also increased the activity of ERK, while the activity of SAPK/JNK remained unaltered at the same concentrations. Moreover, it was noted during FACS analysis and DAPI staining that PD 98059 inhibits the ERK pathway and takes the cells towards apoptosis, in contrast to the viability peak seen with LAM treatment alone. The percentage of apoptosis is considerably less in LAM-treated cells pretreated with PD 98059, a selective inhibitor of MEK 1 (upstream of ERK), in comparison to the large apoptotic nuclei seen in cells treated with PD 98059 alone. This suggests that LAM induces cell survival through the ERK pathway. Hence, LAM alters the signaling pathway and helps in increasing the survivability of the host cells, so that the pathogen could reside and multiply within the host cells.

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