Induction of p38 Mitogen-activated Protein Kinase Reduces Early Endosome Autoantigen 1 (EEA1) Recruitment to Phagosomal Membranes*

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Mycobacterium tuberculosis survives in the infected host by parasitizing macrophages in which the bacillus resides in a specialized phagosome sequestered from the phagolysosomal degradative pathway. Here we report a role of the stress-induced p38 mitogen-activated protein kinase (p38 MAPK) in the component of M. tuberculosis phagosome maturation arrest that has been linked previously to the reduced recruitment of the endosomal and phagosomal membrane-tethering molecule called early endosome autoantigen 1 (EEA1; Fratti, R. A., Backer, J. M., Gruenberg, J., Corvera, S., and Deretic, V. (2001) J. Cell Biol. 154, 631–644). A pharmacological inhibition of M. tuberculosis var. bovis Bacillus Calmette-Guérin-induced p38 MAPK activity caused a marked increase in EEA1 colocalization with mycobacterial phagosomes. Consistent with the increase in EEA1 association and its role in phagosomal maturation, the pharmacological block of p38 activity caused phagosomal acidification and enrichment of the late endocytic markers lysobisphosphatidic acid and CD63 (lysosomal integral membrane protein 1) on mycobacterial phagosomes. A negative regulatory role of p38 MAPK activation in phagosome maturation was further demonstrated by converse experiments with latex bead phagosomes. Artificial activation of p38 MAPK caused a decrease in EEA1 colocalization with model latex bead phagosomes, which normally acquire EEA1 and subsequently mature into the phagolysosome. These findings show that p38 MAPK activity contributes to the arrest of M. tuberculosis phagosomal maturation and demonstrate a negative regulatory role of p38 in phagolysosomal biogenesis.

The regulatory events governing phagosomal maturation represent prime targets for manipulation by intracellular microbes parasitizing host phagocytic cells (1, 2). One prominent model system in this context is the Mycobacterium tuberculosis phagosome (3, 4). An essential aspect of the infection cycle of M. tuberculosis is its macrophage parasitism. The notorious property of M. tuberculosis to persist in the host macrophages rests upon the ability of this pathogen to inhibit the default pathway of phagosome maturation (5–12), a phenomenon referred to in the early literature as the inhibition of phagosome-lysosome fusion (13). It has been established that M. tuberculosis, the vaccine strain M. tuberculosis var. bovis BCG,1 and an opportunistic mycobacterial species, Mycobacterium avium, reside in privileged phagosomal compartments partitioned away from the terminal endocytic organelles (6, 14–16). Mycobacterial phagosomal compartments (MPCs) display fewer or less active H⁺ ATPase molecules (7, 17) as well as a lack of cathepsin D or the presence of its immature form (15, 18, 19), the absence of mannose 6-phosphate receptors (6), and the reduced clearance of plasma membrane markers (15) and an early phagosomal protein termed “taco” (20), which has been shown to be identical to the generic phagocytosis protein coronin (21).

Phagosomes are dynamic organelles that interact with the major sorting and biosynthetic pathways in the cell (11, 12, 21, 22) in a series of processes resulting in acquisition and removal of membrane and luminal content during phagolysosome biogenesis. The trafficking events within the endosomal and phagosomal network are controlled by a subset of Rab GTPases from the Ras superfamily. The small GTPases Rab5 and Rab7 have been implicated in the maturation processes of phagosomes containing intracellular pathogens (11, 23–26). However, the roles of Rab5 and Rab7 during phagosome biogenesis and maturation remain to be fully delineated. Recent studies have implicated two Rab5 effectors, phosphatidylinositol 3-OH kinase (PI3K) hVPS34 (11, 27) and the endosomal tethering molecule EEA1 (11), in the maturation of phagosomes into organelles having late endocytic characteristics (11, 12). Mycobacterial phagosomes exclude or alter the dynamics of EEA1 acquisition and thus avoid further maturation into the phagolysosome.

Recent studies by Gruenberg and co-workers have demonstrated that endocytic pathway and p38 mitogen-activated protein kinase (MAPK) stress signaling are interconnected (28). These researchers reported an effect of p38 on the association of EEA1 with endosomes. Upon activation of p38, EEA1 is displaced from endocytic membranes (28). Interestingly, p38 MAPK is activated in cells infected with M. tuberculosis (29). In this work we examined whether the activation of p38 plays a role in EEA1 exclusion from M. tuberculosis phagosomes and in inhibiting the maturation of mycobacterial phagosomes.

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1 The abbreviations used are: BCG, Bacillus Calmette-Guérin; ANOVA, analysis of variance; EEA1, early endosome autoantigen; GDI, GDP dissociation inhibitor; LBPA, lysobisphosphatidic acid; LBC, latex bead phagosomal compartment; ManLAM, mannose-capped lipooligosaccharide; MPC, Mycobacterium tuberculosis phagosomal compartment; SNARE, SNAP receptors; TGN, trans-Golgi network.
EXPERIMENTAL PROCEDURES

Cell Line and Bacterial Culture Conditions—The murine macrophage-like cell line, J774, was maintained in Dulbecco’s modified Eagle’s medium supplemented with L-glutamine and 5% fetal bovine serum. M. tuberculosis var. bovis BCG harboring pshp60-gfp (30) was grown in Middlebrook 7H9 broth. Single cell suspensions were generated using a Tenbroek tissue grinder, followed by a 5-min pulse in a water bath sonicator. Remaining bacterial aggregates were removed by centrifugation.

Phagosome Purification—Synchronization of latex beads and M. tuberculosis var. bovis BCG phagocytosis was achieved by allowing latex beads or M. tuberculosis var. bovis BCG to attach to macrophages cooled to 4 °C without allowing uptake. Samples were shifted to 37 °C for the required periods of time, and mycobacterial or latex bead phagosomes were isolated and characterized as described previously (10). Briefly, for mycobacterial phagosomes, macrophages were infected with a multiplicity of infection of 25 M. tuberculosis var. bovis BCG input per macrophage. Cells were mechanically lysed by passage through 22-gauge needles connected to a syringe apparatus. Cellular debris and nuclei were removed by centrifugation at 200 × g to generate post-nuclear supernatants. Post-nuclear supernatants from different samples were sedimented by centrifugation through a sucrose step gradient of 8.5, 15, and 50% sucrose at 1,000 × g for 45 min. The sediment from the 15 and 50% sucrose samples was collected and loaded onto a linear 32–53% sucrose gradient. Organelles were separated by isopycnic centrifugation (100,000 × g for 15 h). Membranes were pelleted from each fraction by centrifugation at 250,000 × g for 40 min and resuspended in Hepes-KCl. For latex bead phagosome purification, J774 cells were infected with latex beads and incubated as indicated above. Cells were treated with media alone, 1 mM H2O2, or allowed to phagocytose latex beads or M. tuberculosis var. bovis BCG.

Fig. 1. Activation status of p38 MAPK in cells infected with M. tuberculosis var. bovis BCG and latex beads. Western blot analysis of whole cell lysates. Cells were pre-treated with media alone (lanes 1, 2, 5, and 6), 10 μM SB203580 (SB; lanes 3 and 7), and 10 μM PD98059 (P; lanes 4 and 8) as described under “Experimental Procedures.” Cells were then treated with 1 mM H2O2 (lanes 6–8) or allowed to phagocytose latex beads (LB; lane 1) or M. tuberculosis var. bovis BCG (lanes 2–4). C, control. Inhibitors remained for the entirety of the experiments. Whole cell lysates (20 μg of protein) were separated by 12% SDS-PAGE and probed with antibodies against p38 MAPK and phospho-p38 MAPK.

Fig. 2. Inhibition of p38 MAPK activity enhances EEA1 recruitment and maturation of mycobacterial phagosomes. Shown are epifluorescence or confocal microscopy images of mycobacterial phagosomes in J774 cells. A, D, G, J, and M, green fluorescence protein (GFP) fluorescence of M. tuberculosis var. bovis BCG. Upon phagocytosis, cells were fixed, permeabilized, and incubated with anti-EEA1 (B, E, and N) or anti-LBPA (M and K) antibodies and washed and incubated with secondary Alexa 568-conjugated antibody. C, F, I, L, and O, merged green and red channels. Insets contain magnified views of the areas encased by dashed boxes in the main panels. Cells were treated with media alone (A–C and G–I) or 10 μM SB203580 (D–F and J–O) as described under “Experimental Procedures.”
phagosomal compartments (LBCs) were isolated from post-nuclear supernatants by flotation as described (22).

Treatment with Inhibitors—When required, cells were pre-treated with SB203580 (10 mM) or PD98059 (10 mM) (Calbiochem) at 37 °C for 1 h prior to infection. Some cells were also treated with 1 mM H₂O₂ during the infection period. All treatments remained throughout the experiment.

Epifluorescence and Confocal Fluorescence Microscopy—Macrophages grown on glass coverslips were infected with latex beads or M. tuberculosis var. bovis BCG. Synchronization of infection was achieved by centrifugation of particles onto macrophages at 107 × g for 5 min. Macrophages were fixed with 3.7% paraformaldehyde, and permeabilization was achieved with 0.2% saponin. After blocking, cells were incubated with primary antibody followed by secondary Alexa 568-conjugated antibody (Molecular Probes). LysoTracker Red DND-99 staining of acidic organelles was carried out as described previously (31). Epifluorescence microscopy images were collected and processed using LSR Esprit software. Confocal fluorescence microscopy images were collected and processed using the PerkinElmer Life Sciences UltraView confocal microscopy system. Colocalization of endocytic markers with phagosomes were determined using the previously described methods of unbiased counting (11, 32).

**Western Blot Analysis**—For immunoblots of phagosomes, equal amounts of MPCs and LBCs (by protein) were separated by 12.5% SDS-PAGE and transferred to polyvinylidene difluoride membranes. Membranes were probed with primary antibodies followed by horseradish peroxidase-conjugated secondary antibodies. When comparing MBCs and LBCs on discontinuous membranes, antibody incubations as well as ECL reactions were performed on these membranes simultaneously. When examining the phosphorylation of p38 MAPK, cells were lysed with ice-cold lysis buffer (1% Nonidet P-40, 25 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 μM okadaic acid, 0.1 mM Na₃VO₄, and protease inhibitors) on ice for 30 min while shaking. Bound antibodies were visualized using the ECL Western blotting system. Membranes were exposed to film simultaneously, and identical exposure times were used.

**Antibodies**—For Western blotting and immunofluorescence experiments, we used antibodies against EEA1 (from S. Corvera, University of Massachusetts, Worcester, MA), GDP dissociation inhibitor (GDI; from A. Sheshiva, Wayne State University, Detroit, MI), lysobisphosphatidic acid (LBPA) (from J. Gruenberg, University of Geneva, Geneva, Switzerland), p38 MAPK (Transduction Labs), phospho-p38 MAPK (Cell Signaling Technology), Rab5 (from L. Huber, Research Institute of Molecular Pathology, Vienna, Austria), syntaxin 8 (provided by W. Hong, Institute of Molecular and Cell Biology, Singapore), and CD63 (Santa Cruz Biotechnology).

**Statistical Analysis**—All statistical analyses were calculated using Fisher’s protected least squares difference (LSD) post hoc test (ANOVA) (SuperANOVA 1.1.1, Abacus Concepts, Inc.). p values of 0.05 were considered significant.

**RESULTS**

p38 MAPK Activation during Mycobacterial Infection—To determine the activation state of p38 MAPK after the uptake of *M. tuberculosis* var. *bovis* BCG, we monitored the phosphorylation of p38 MAPK by Western blot using a phospho-specific antibody (Fig. 1). Cells were allowed to phagocytose latex beads or *M. tuberculosis* var. *bovis* BCG for 15 min prior to lysis and Western blot analysis. Although both latex beads and *M. tuberculosis* var. *bovis* BCG caused an increase in p38 phosphorylation, *M. tuberculosis* var. *bovis* BCG caused a significantly higher level of p38 activation (Fig. 1, lane 2) relative to latex beads (Fig. 1, lane 1). To determine whether the phosphorylation of p38 was due to autophosphorylation or to cross-talk between the p38 pathway and the extracellular signal-regulated kinase pathway, we used specific inhibitors to monitor p38 phosphorylation. Treating cells with the p38-specific inhibitor SB203580 completely inhibited *M. tuberculosis* var. *bovis* BCG-mediated p38 activation (Fig. 1, lane 3), whereas the extracellular signal-regulated kinase-specific inhibitor PD98059 had no effect (Fig. 1, lane 4). As a positive control for p38 MAPK activation, cells were treated with 1 mM H₂O₂ for 15 min in the presence or absence of SB203580 or PD98059. As expected, exposing macrophages to H₂O₂ caused a marked increase in the phosphorylation of p38 (Fig. 1, lane 6) relative to untreated cells (Fig. 1, lane 5). This was inhibited when cells were treated with SB203580 (Fig. 1, lane 7) but not when they were treated with PD98059 (Fig. 1, lane 8).

**Inhibition of p38 MAPK Activity Enhances EEA1 Recruitment to Mycobacterial Phagosomes**—Previously, we have reported that the exclusion of EEA1 from *M. tuberculosis* var. *bovis* BCG phagosomes is a critical event in mycobacterial phagosome maturation arrest (11). Because EEA1 can be excluded from endosomes upon activation of p38 MAPK (28) and *M. tuberculosis* BCG is known to stimulate p38 MAPK activation (29), we tested whether inhibiting p38 MAPK activity could restore the maturation of mycobacterial phagosomes. Cells were treated with the p38 MAPK inhibitor SB203580 or carrier (MeSO₄) alone and infected with *M. tuberculosis* var. *bovis* BCG. Mycobacterial phagosomes were examined for EEA1 recruitment. We found that inhibiting p38 MAPK with SB203580 caused a 250% increase in mycobacterial phagosome colocalization with EEA1 in SB203580-treated cells relative to control (Figs. 2, A–F, and 3) (p = 0.0001; ANOVA). The colocalization of EEA1 and mycobacteria was confirmed by confocal microscopy (Fig. 2, M–O). These findings suggest that the exclusion of EEA1 from mycobacterial phagosomes relative to model latex bead phagosomes is, in part, due to p38 MAPK-mediated EEA1 displacement from phagosomal membranes (28).

**Inhibition of p38 MAPK Activation Enhances Acidification of Mycobacterial Phagosomes and Their Acquisition of the Late Endosomal/Lysosomal Markers LBPA and CD63**—The exclusion of EEA1 from mycobacterial phagosome has been associated with its arrested maturation into terminal phagosomes (11). To test whether inhibiting *M. tuberculosis*-mediated p38 MAPK activation causes the acquisition of late endocytic traits, we examined the accumulation of the late endosome-specific marker lysobisphosphatidic acid (33). LBPA, which is excluded from mycobacterial phagosomes, is acquired by model phagosomes in an EEA1-dependent manner (11). Thus, treatments that cause increases in EEA1 acquisition are expected to correspond with an accelerated maturation rate that can be measured by the acquisition of LBPA. When cells were treated with SB203580, we found the LBPA localization...
on mycobacterial phagosomes was increased by nearly 300% relative to untreated macrophages (Figs. 2, G–I, and 3) (p = 0.0001; ANOVA). Additional markers of phagosomal maturation, LysoTracker Red DND-99 (LT) and the lysosomal integral membrane protein CD63 were tested by confocal fluorescence microscopy (Fig. 4, A–L). A 2-fold increase in the accumulation of LysoTracker Red DND-99 (LT) and CD63 on mycobacterial phagosomes was associated with SB203580 treatment (Fig. 4, M and N). These findings show that mycobacteria-induced p38 MAPK activity plays a role in the arrest of phagosomes containing Mycobacterium tuberculosis var. bovis BCG by affecting EEA1 recruitment and subsequent trafficking events as reported previously (11).

Mycobacterial Phagosomes Retain GDI—Gruenberg and colleagues (28) have found that, during the activation of p38 MAPK, Rab GDI is phosphorylated, resulting in its higher state of activation and affinity for Rab5. Consequently, GDI activation causes a net displacement of EEA1 from endocytic membranes (28). When we examined phagosomal membranes for the presence of GDI, we found that purified MPCs consistently contained GDI (Fig. 5, A and B), whereas GDI was not detected on isolated LBCs (Fig. 5, A and B). The persistence of GDI on mycobacterial phagosomes is consistent with a higher state of GDI activity, resulting in an increased steady state level of membrane-associated GDI. This correlates with the higher activation state of p38 MAPK in cells infected with Mycobacterium tuberculosis var. bovis BCG relative to those that have ingested latex beads. To test whether p38 activation causes GDI accumulation on phagosomes, we isolated LBCs from cells

**Fig. 4.** Inhibition of p38 MAPK enhances CD63 recruitment to mycobacterial phagosomes and their acidification. Shown are confocal fluorescence microscopy images of mycobacterial phagosomes in infected macrophages. A, D, G, and J, green fluorescence protein fluorescence of M. tuberculosis var. bovis BCG. B and E, fluorescence images of the acidotropic dye LysoTracker Red DND-99 (LT) trapped in acidic compartments. H and K, immunofluorescence (Alexa 568) of the major lysosomal membrane protein CD63 (LIMP-1). C, F, I, and L, merged green and red channels. Cells were treated with media alone (A–C, G–I), or 10 μM SB 203580 (D–F, J–L). Quantitation of LysoTracker Red DND-99 (n = 424) (M) and CD63 (n = 827) (N) colocalization with the phagosomes took place 30 min post-infection for LysoTracker Red DND-99 (LT) and 30, 60, and 120 min post infection for CD63 in SB 203580 (SB)-treated and control cells.
treated with \( \text{H}_2\text{O}_2 \) in the presence or absence of SB203580 (Fig. 5C). We found that activating p38 resulted in the retention of GDI on LBCs (Fig. 5C). The effect of p38 activity on GDI retention by latex bead phagosomes was reversed when cells were treated with SB203580. The link between p38 activity and GDI retention is specific, because other agents that arrest phagosome maturation, such as wortmannin and brefeldin A, did not cause GDI association with LBCs.

**Inhibition of p38 Enhances EEA1 Association with Isolated LBCs**—To confirm that activation of p38 MAPK can alter phagosome maturation, J774 cells were allowed to phagocytize latex beads in the presence of \( \text{H}_2\text{O}_2 \) to activate p38 MAPK using the previously reported concentrations of hydrogen peroxide (28). \( \text{H}_2\text{O}_2 \) activation of p38 reduced EEA1 recruitment to latex bead phagosomes (Fig. 6). Only 16.8% of latex bead phagosomes in \( \text{H}_2\text{O}_2 \)-treated cells acquired EEA1 (Fig. 6, F and K) relative to 31.6% colocalization in untreated cells (Fig. 6, B and K) \((p < 0.01; \text{ANOVA})\). To determine whether p38 MAPK specifically blocked EEA1 acquisition, we also examined latex bead phagosomes in the presence of \( \text{H}_2\text{O}_2 \) and SB203580. SB203580 treatment of cells prior to \( \text{H}_2\text{O}_2 \) treatment fully restored EEA1 recruitment to latex bead phagosomes (Fig. 6, J and K). This demonstrates that the activation of p38 MAPK can significantly alter phagosome maturation by displacing the critical tethering molecule EEA1. In contrast, the endosomal soluble NSF attachment protein receptor (SNARE) syntaxin 8 localization was not altered on phagosomes in \( \text{H}_2\text{O}_2 \)-treated cells (Fig. 6, D, H, and K). Because EEA1 recruitment to phagosomes is required for phagosome maturation (11), these results indicate that stress-induced p38 MAPK activity has a negative regulatory role on the phagosome maturation stages controlled by EEA1.

To further confirm the effect of p38 activity on EEA1 recruitment, we complemented the morphological tests by biochemical studies using purified LBCs. Cells were incubated with latex beads for 1 h, a point when EEA1 is normally absent from LBCs (11). Cells were either treated with \( \text{H}_2\text{O}_2 \), SB203580, or both as described under “Experimental Procedures.” EEA1 was absent from LBCs isolated from untreated cells as well as from those treated with \( \text{H}_2\text{O}_2 \) (Fig. 7, lanes 1 and 2, respectively). However, EEA1 was present on LBCs isolated from \( \text{H}_2\text{O}_2 \)-treated cells that received \( \text{H}_2\text{O}_2 \) and SB203580 (C, third lane) as described under “Experimental Procedures.” Equivalent amounts of MPC and LBC (5 \( \mu \text{g of protein} \)) were separated by 12% SDS-PAGE, transferred to Immobilon-P, and probed with antibodies against GDI and Rab5.

Inert particles, reduces the recruitment of EEA1 to model latex bead phagosomes. This is purely dependent on the activation of p38 MAPK, because the specific inhibitor SB203580 restored EEA1 levels on latex bead phagosomes.

The role of p38 MAPK signaling in membrane trafficking is only beginning to be explored. Gruenberg and co-workers (28) have discovered that GDI removal of Rab5 from membranes is augmented upon GDI phosphorylation by the activated phospho-p38. They also reported that phospho-GDI caused a displacement of EEA1 from endosomal membranes. This was ascribed specifically to the phosphorylation of GDI, because mutations at the sites of phosphorylation abrogated the effect of phospho-p38 on EEA1 distribution (28). The effect of phospho-p38 on EEA1 association with membranes is likely due to a block in binding of EEA1 to Rab5. In addition to this process, by using pharmacological agents or mycobacterial products we have recently observed that interference with \( \text{Ca}^{2+} \)/calmodulin-dependent pathway of phosphatidylinositol 3-kinase hVPS34 recruitment and phosphatidylinositol 3-phosphate (PtdIns3-P) formation on target membranes (35) also obstructs recruitment of EEA1 to organellar membranes. It is likely that both of these processes conspire to bring about the mycobacterial phagosome maturation arrest.

In the course of our studies of phagosomal maturation, we have found that Rab5 is present on the arrested mycobacterial phagosomes in quantities similar to those on model latex bead phagosomes, which later progress into the phagolysosomal
stage (10). Surprisingly, Rab5 levels on mycobacterial phagosomes did not diminish when p38 MAPK was activated (Fig. 7). This suggests that, although GDI was present on membranes in our experiments, it had not yet extracted Rab5 from mycobacterial phagosomes. This notion is consistent with our finding that GDI is retained on mycobacterial phagosomes but not on latex bead phagosomes. The presence of low amounts of GDI on latex bead phagosomes isolated from H2O2-treated cells probably reflects low steady state levels of GDI and not the presence of fixed Rab5/GDI complexes. This suggests the possibility that another factor (or its absence) on mycobacterial phagosomes, independent of p38 activation, prevents GDI from dissociating from membranes once it is complexed with Rab5.

These considerations suggest that mycobacteria-containing phagosomes are altered in a way that blocks phospho-GDI removal of Rab5 and suggest that Rab5-GDI complexes are fixed to mycobacterial phagosomes. Nevertheless, it is possible to link EEA1 exclusion with the increased presence of GDI on phagosomal membranes. For example, the formation of Rab5/GDI complexes may block Rab5 interactions with its downstream effectors or its activation by preventing the loading of Rab5 with GTP.

M. tuberculosis has been reported to activate the stress-induced p38 MAPK (29). One mechanism associated with the activation of this signaling pathway is mediated by the major cell wall component mannose-capped lipoarabinomannan (Man-LAM). M. tuberculosis produces a wide variety of bioactive lipids that have been implicated in the pathogenesis of tuberculosis.

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**Fig. 6.** H2O2-induced p38 MAPK activity inhibits EEA1 recruitment to latex bead phagosomes. Shown is epifluorescence microscopy of latex bead phagosomes in J774 cells (A, C, E, G, and I) with green fluorescence of the latex beads. Upon phagocytosis, cells were fixed, permeabilized, incubated with anti-EEA1 antibody (B, F, and J) or anti-syntaxin 8 antibody (D and H), and washed and incubated with secondary Alexa 568-conjugated antibody. Cells were treated with media alone (A–D), 1 mM H2O2 (E–H), or 10 μM SB203580 followed by 1 mM H2O2 (I–J) as described under “Experimental Procedures.” K, quantitation of EEA1 colocalization with phagosomes (30 min post-infection) in cells treated with media, H2O2, or SB203580 followed by H2O2 (n = 2,659 phagosomes). Arrowheads indicate co-localization of latex beads with EEA1. Insets contain magnified views of the areas enclosed by boxes in the main panels. The data are means ± S.E. of three separate experiments. *, p < 0.01.

**Fig. 7.** H2O2-induced p38 MAPK activity inhibits phagosome maturation of latex bead phagosomes. Western blot analysis of latex bead phagosomes in J774 cells. Cells were treated with media alone (first and fourth lanes from the left), 1 mM H2O2 (second and fifth lanes), or 10 μM SB203580 followed by 1 mM H2O2 (third and sixth lanes) as described under “Experimental Procedures.” Equivalent amounts of phagosomes (5 μg of protein) and postnuclear supernatants (20 μg of protein) were separated by 12% SDS-PAGE, transferred to Immobilon-P, and probed with antibodies against EEA1 and Rab5.
Convergence between the signaling and membrane trafficking that with the Rab5/EEA1 pathway of phagosome maturation and illustrate that the p38 MAPK signaling pathway can intersectosomes. The data presented here, along with our previous work, classical traits associated with arrested mycobacterial phagosome (12) and is responsible for some of the subunits of the V0 ATPase complex from the subsequently inhibits the trafficking of immature cathepsin D and curs through EEA1-syntaxin 6 interactions (40). This conse-

cular exclusion of EEA1 leads to a block in trafficking from the phagosome maturation caused by mycobacteria

Because M. tuberculosis exclusion and the inhibition of phagosome maturation (11, 12). ManLAM also inhibits the Ca2+/calmodulin-hVPS34-phosphatidylinositol 3-phosphate pathway leading to EEA1 recruitment (38). Incidentally, ManLAM has also been linked to EEA1–dylinositol 3-phosphate pathway leading to EEA1 recruitment—interactions (38). Incidentally, ManLAM has also been linked to EEA1

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