Phosphorylation of Mitogen-Activated Protein Kinases Contributes to Interferon γ Production in Response to Mycobacterium tuberculosis

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Immune control of Mycobacterium tuberculosis depends on interferon γ (IFN-γ)-producing CD4+ lymphocytes. Previous studies have shown that T cells from patients with tuberculosis produce less IFN-γ, compared with healthy donors, in response to mycobacterial antigens, although IFN-γ responses to mitogens are preserved. In this work, we found that M. tuberculosis–induced IFN-γ production by human T cells correlated with phosphorylation of the mitogen-activated protein kinases (MAPKs), extracellular signal-regulated kinase (ERK), and p38. Moreover, the majority of IFN-γ–producing T cells expressed signaling lymphocyte activation molecule (SLAM), and SLAM activation further increased ERK phosphorylation. Interestingly, patients with tuberculosis had delayed activation of ERK and p38, and this was most marked in patients with the poorest IFN-γ responses (ie, low responders). Besides, SLAM signaling failed to phosphorylate ERK in low responders. Our findings suggest that activation of p38 and ERK, in part through SLAM, mediates T-cell IFN-γ production in response to M. tuberculosis, a pathway that is defective in patients with tuberculosis.

Keywords. Tuberculosis; signaling; CREB; MAPK; IFN-γ.

Tuberculosis remains a substantial global health problem, its resurgence fueled by a large population of immunocompromised human immunodeficiency virus (HIV)–infected persons and by an alarming increase in the prevalence of drug-resistant organisms. BCG vaccine, the only available vaccine against tuberculosis, is of variable efficacy, and development of a more effective vaccine hinges on a better understanding of the human immune response to this pathogen. During infection with Mycobacterium tuberculosis, innate mechanisms help to control bacillary spread, but T-lymphocyte recruitment to the lung is required to contain the infection in granulomas [1]. Protective immunity requires the generation of T-helper 1 (Th1) cytokine responses [2] and interferon γ (IFN-γ), which activates macrophages to inhibit mycobacterial growth [1]. Persons with mutations linked to IFN-γ signaling have increased susceptibility to mycobacterial infection and disseminated infection after BCG vaccination [3, 4]. Besides, T cells from patients with tuberculosis produce less IFN-γ than those from persons with latent M. tuberculosis infection [5], and IFN-γ production is lowest in patients with the most severe manifestation of tuberculosis [6].

Thus, elucidation of the mechanisms for reduced IFN-γ production in individuals who develop tuberculosis will enhance our knowledge of disease pathogenesis, contributing to a better understanding of the immune response to this intracellular pathogen.
To understand these mechanisms, it is important to delineate how T-helper precursor cells activate the gene encoding IFN-γ and become committed to the Th1 phenotype [7].

Mitogen-activated protein kinases (MAPKs) are involved in many aspects of immune responses, including initiation of innate immunity, activation of adaptive immunity, and termination of immune responses through cell death and regulatory T cells [8, 9]. MAPKs are essential for macrophage activation, and the extent of MAPK phosphorylation in human monocyte-derived macrophages controls growth of intracellular Mycobacterium avium [10]. Moreover, MAPKs phosphorylate and activate downstream molecules, resulting in T-cell activation, proliferation, and differentiation into T-helper phenotypes [8, 11, 12]. Mycobacterium-induced production of proinflammatory cytokines, such as TNF-α and interleukin 1, depends on MAPK activation [10, 13, 14]. However, although there is compelling evidence for the role of extracellular signal-regulated kinase (ERK) and p38 protein kinases in the antimonyocellular activity of antigen-presenting cells, little is known about the contribution of these kinases to human T-lymphocyte responses to mycobacteria.

Regulation of the IFN-γ gene is complex, involving multiple transcription factors that bind to the proximal and distal promoter elements [15–17], including members of the cyclic adenosine monophosphate response element binding protein (CREB)/activating transcription factor family. We previously demonstrated that CREB increased IFN-γ secretion to M. tuberculosis by binding to the IFN-γ proximal promoter [5]. We also showed that activation of signaling lymphocyte activation molecule (SLAM) in T cells from patients with tuberculosis increased CREB phosphorylation and IFN-γ production [18]. CREB is regulated by several MAPKs [19], but it is unknown whether these MAPKs contribute to the signaling pathways that enhance transcription of IFN-γ by human T cells in response to M. tuberculosis. In this report, we found that M. tuberculosis–induced IFN-γ production correlated with phosphorylation of the MAPKs ERK and p38. The majority of IFN-γ–producing T cells expressed SLAM, and SLAM activation further increased ERK phosphorylation. Patients with tuberculosis had delayed activation of ERK and p38, and SLAM signaling failed to phosphorylate ERK in patients whose T cells produced little M. tuberculosis–induced IFN-γ. Therefore, our findings suggest that activation of p38 and ERK mediates T-cell IFN-γ production in response to M. tuberculosis and that this pathway is defective in patients with tuberculosis.

METHODS

Patients
We studied HIV-seronegative patients with tuberculosis diagnosed at the Hospital Muñiz (Buenos Aires, Argentina) on the basis of clinical and radiological data, identification of acid-fast bacilli in sputum, and isolation of M. tuberculosis in culture. All patients had received antituberculosis therapy for <1 week and were classified on the basis of lymphocyte responses to M. tuberculosis antigen, as previously reported [20]. High responders were defined as patients who showed significant lymphocyte proliferation, a high level of IFN-γ production, and a high level of SLAM expression in response to the antigen. Low responders were defined as patients who exhibited low proliferative responses, a low level of IFN-γ secretion, and a reduced level of SLAM expression [20]. We also studied healthy adults with no history of tuberculosis who received BCG vaccine at birth and had negative results of Quantiferon tuberculosis Gold tests, indicating the absence of latent M. tuberculosis infection. Two subjects with X-linked lymphoproliferative disease (XLP), confirmed at the International XLP Registry Headquarters, University of Nebraska Medical Center (Omaha, NE) [20, 21], were also studied. Both patients display defective expression of the SLAM-associated protein. All participants provided written informed consent for sample collection and subsequent analysis.

Antigen
Cells were stimulated in vitro with a cell lysate from the virulent M. tuberculosis H37Rv strain (NR-14822), prepared by probe sonication (M. tuberculosis-Ag), and obtained through the Biodefense and Emerging Infections Research Resources Repository, housed within the National Institute of Allergy and Infectious Diseases of the National Institute of Health.

Cell Preparation and Reagents
Peripheral blood mononuclear cells (PBMCs) were isolated by centrifugation over Ficoll-Hyphaque (Amersham Biosciences) and cultured (1 × 10⁶ cells/mL), with or without M. tuberculosis-Ag (10 μg/mL) with Roswell Park Memorial Institute 1640 medium (Gibco) supplemented with l-glutamine, gentamicin, and 10% human serum (Sigma-Aldrich). CD3⁺ T cells were purified by immunomagnetic negative selection, with a purity of 95%–98%, as confirmed by flow cytometry.

In some experiments, cells were cultured with or without ERK (PD98059, 50 μM, Calbiochem) or p38 (SB220025, 10 μM, Calbiochem) inhibitors for 1 hour and incubated with M. tuberculosis-Ag for 48 hours, after which IFN-γ production was determined by an enzyme-linked immunosorbent assay (eBioscience). PD98059 specifically inhibits activation of ERK but does not affect activation of other related dual-specificity protein kinases or that of 18 other serine/threonine protein kinases. SB220025 is a specific inhibitor of human p38 MAPK, which binds to an extended pocket in the active site of the enzyme. It has a selectivity for p38 MAPK that is 2000-fold greater than that for ERK and 500-fold greater than that for protein kinase A. In some experiments, agonistic anti-SLAM monoclonal antibody (10 μg/mL, A12, eBioscience)
was used as described below (ie, in the Western blot subsection).

Flow Cytometry
PBMCs were incubated with or without ERK or p38 inhibitors for 1 hour. They were then stimulated with *M. tuberculosis*-Ag for 5 days, with Golgi Stop reagent containing monensin (1 μL/mL, BD Biosciences) added for the final 5 hours of culture. Cells were subsequently stained with antibodies to CD3 and SLAM (both from BD Biosciences), and intracellular staining was performed to detect IFN-γ and phospho-CREB (p-CREB, BD Biosciences), as previously described [18]. Negative control samples were incubated with irrelevant isotype-matched monoclonal antibodies, and all samples were analyzed on a FACS ARIA II flow cytometer (BD Biosciences).

Western Blot
Total cell protein extracts were prepared from PBMCs stimulated with *M. tuberculosis*-Ag for 24 or 48 hours or from purified CD3+ lymphocytes obtained from *M. tuberculosis*-Ag–stimulated PBMCs after 48 hours.

In different experiments, PBMCs were stimulated with *M. tuberculosis*-Ag for 5 days. Afterwards, the cells were harvested, rested for 5 hours in low (1%) serum medium, and restimulated with anti-SLAM agonistic antibody for different periods. Western blotting was performed by standard methods. Each nitrocellulose membrane was blotted, stripped, and reblotted with rabbit monoclonal antibodies to phospho-ERK (p-ERK, Thr-202/Tyr-204), total ERK, phospho-p38 (p-p38, Thr-180/Tyr-182), and total p38 (all from Cell Signaling). Bound antibodies were revealed with horseradish peroxidase–conjugated anti-rabbit antibody (1:3000; Bio-Rad, Hercules, CA), using ECL PLUS (Amersham Biosciences). Images were obtained with an Intelligent Dark Box (Fuji film LAS1000) and analyzed with ImageJ Analysis software. The intensity of each band was expressed in arbitrary units.

Transfection of SLAM Small-Interfering RNA (siRNA)
SLAM siRNA was synthesized as previously described [18]. Briefly, the DNA template for transcription was prepared by published methods [22]: forward T7-tagged primer, 5′-GGCTAATACGAGCTCAGCTATTGGAGAGGAGCATTAGAAGACACAC-3′; reverse T7-tagged primer, 5′-GGCTAATACGAGCTCAGCTATTGGAGAGGAGCATTAGAAGACACAC-3′.
TTC-3′ (Integrated DNA Technologies). The polymerase chain reaction product was sequenced (Northwood DNA), and DNA purification was performed using the QIAquick Gel Extraction Kit Protocol (Qiagen). siRNA was generated using the Dicer siRNA generation kit (Genlantis). PBMCs were transfected (GeneSilencer siRNA Transfection Reagent, Genlantis) with 500 ng of SLAM or control (GFP) siRNA for 48 hours. Then cells were washed and incubated for 72 hours, with or without M. tuberculosis-Ag. Protein kinase expression was determined by Western blot, as described above.

Statistical Analysis
We used GraphPad Prism v5.0 for statistical analysis. The Mann–Whitney U and the Wilcoxon rank sum tests were used to analyze differences between unpaired samples and paired samples, respectively. P values of < .05 were considered statistically significant.

RESULTS
ERK and p38 MAPKs Regulate IFN-γ Production Against M. tuberculosis Through CREB
M. tuberculosis–stimulated PBMCs from patients with tuberculosis produced less IFN-γ than those from healthy donors (P = .04; Figure 1A). Pretreatment of PBMCs with ERK (PD98059) and p38 (SB220025) inhibitors significantly reduced M. tuberculosis–induced IFN-γ levels by both groups (Figure 1A), and the effect of PD98059 was greater in healthy donors than in patients with tuberculosis (P = .02). Pretreatment of PBMCs with the ERK and p38 MAPK inhibitors did not affect cell viability, as determined by an MTT assay (data not shown). Both kinase inhibitors also reduced the percentages of IFN-γ–producing T lymphocytes expanded by M. tuberculosis-Ag (Figure 1B and 1C).

On the basis of our current results (Figure 1) and our previous demonstration that CREB phosphorylation contributes to M. tuberculosis–stimulated production of IFN-γ [23], we next investigated whether ERK and p38 controlled CREB phosphorylation. M. tuberculosis-Ag induced expression of both IFN-γ and p-CREB in T lymphocytes from patients with tuberculosis and those from healthy donors, with the latter having higher percentages of IFN-γ–producing p-CREB+ T cells (Figure 2). Both MAPK inhibitors significantly decreased the number of these lymphocytes (Figure 2), suggesting that ERK and p38 MAPKs regulate IFN-γ production against M. tuberculosis by a mechanism that involves CREB.

Mtb-Ag Induces ERK and p38 Phosphorylation in T Cells From Patients With Tuberculosis
To determine whether M. tuberculosis-Ag induces MAPK activation, we measured phosphorylation of ERK and p38 by Western blot. Although there was notable individual variation, M. tuberculosis stimulation markedly induced ERK and p38 phosphorylation, with maximal induction after 48 hours (Figure 3). Phosphorylation of ERK and p38 occur very rapidly in response to stimulation through the T-cell receptor, but phosphorylation of these enzymes was not significantly increased until 24 hours after exposure to M. tuberculosis (data not shown), perhaps because of delays related to antigen processing and presentation.

Patients with XLP have uncontrollable Th1 responses and high basal levels of IFN-γ [20]. Therefore, to determine whether ERK and p38 MAPKs regulate IFN-γ production in response to M. tuberculosis specifically or to other stimuli, we evaluated MAPK activation in PBMCs from 2 BCG-vaccinated patients with XLP that were stimulated with M. tuberculosis-Ag. PBMCs from patients with XLP produced high concentrations of IFN-γ without stimulation, but there was no significant MAPK phosphorylation (Figure 4A–C). However, ERK and p38 phosphorylation were greatly increased in Ag-stimulated cells from patients with XLP. In addition, in patients with tuberculosis, ERK activation was minimal at 24 hours and modest at 48 hours, whereas ERK phosphorylation was maximal at 24 hours in healthy donors and individuals with XLP (Figure 4D). Similar findings were noted for phosphorylation of p38 (data not shown). Thus, patients with tuberculosis who have diminished IFN-γ production in response to M. tuberculosis and ineffective immunity [23] have delayed kinetics of MAPK activation.

Because the experiments described above were performed with PBMCs that included different cell types, we wished to confirm that M. tuberculosis activates ERK and p38 in T cells. After stimulation of PBMCs with M. tuberculosis-Ag, we purified CD3+ cells by negative selection and found that M. tuberculosis stimulation augmented expression of p-ERK and p-p38 (Figure 5A).

Reduced Production of IFN-γ in Response to M. tuberculosis in Patients With Active Disease is Associated With Impaired MAPK Activation
During tuberculosis, low responders have reduced IFN-γ production and more severe disease, compared with high responders [20]. Mean M. tuberculosis–induced IFN-γ production (±SD) was 0.49 ± 0.17 ng/mL among low responders and 11.95 ± 3.89 ng/mL among high responders (P = .0007). IFN-γ response status (ie, low or high) correlated with M. tuberculosis–induced phosphorylation of ERK and p38, as low responders had impaired activation of ERK and p38, both in PBMCs and purified T cells, compared with high responders (Figure 5B and 5C).

Costimulation Through SLAM Increases Phosphorylation of ERK but Not p38 in Patients With Tuberculosis
We previously demonstrated that SLAM, a protein expressed on activated T cells, induces Th1 responses in patients with tuberculosis. In the current study, we investigated whether costimulation through SLAM increases ERK and p38 phosphorylation in patients with tuberculosis. Pretreatment of PBMCs with SLAM siRNA, but not control (GFP) siRNA, significantly reduced the phosphorylation of both ERK and p38 in M. tuberculosis–stimulated PBMCs (Figure 6). The addition of SLAM to PBMCs from patients with tuberculosis and XLP increased phosphorylation of both these enzymes (Figure 6B). This suggests that SLAM costimulation potentiates phosphorylation of both MAPKs in PBMCs from patients with tuberculosis.
tuberculosis through a mechanism that involves CREB phosphorylation [18, 20]. We extended these studies to show that nearly 60% of IFN-γ–producing T cells coexpressed SLAM and p-CREB after M. tuberculosis stimulation (Figure 6A).

Unlike high responders, low responders do not upregulate SLAM expression after M. tuberculosis stimulation (Supplementary Figure 1), a defect that has been found to contribute to reduced IFN-γ production [20]. Engagement through SLAM significantly increased M. tuberculosis–induced IFN-γ production from patients with tuberculosis, and this increase was much higher in high responders than in low responders (Figure 6B). Furthermore, SLAM siRNA reduced ERK phosphorylation (Figure 6C). In contrast, activation of SLAM did not increase p38 expression, either in high responders or low responders (Figure 6B), and SLAM siRNA did not affect p38 phosphorylation (Figure 6C).

**DISCUSSION**

Because MAPK pathways regulate Th1 development [24], we hypothesized that certain MAPKs could mediate M. tuberculosis–induced IFN-γ production. We found that stimulation of T cells from healthy donors and patients with tuberculosis with M. tuberculosis activates ERK and p38 MAPK and that inhibition of these 2 signaling molecules reduces the number of

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**Figure 2.** Peripheral blood mononuclear cells (PBMCs) from 9 healthy donors (HDs) and 7 high responders (tuberculosis; defined in Methods) were incubated with or without extracellular signal-regulated kinase (PD98059 [PD]) or p38 mitogen-activated protein kinase (SB220025 [SB]) inhibitors for 1 hour. Cells were then stimulated with Mycobacterium tuberculosis antigen for 5 days, and cyclic adenosine monophosphate response element binding protein phosphorylation (p-CREB) and interferon γ (IFN-γ) production were analyzed in CD3+ cells by flow cytometry. A, Results for a representative HD are shown. IgG1, immunoglobulin G1. B, The bars show the mean ± standard error of the mean. The percentages were calculated by first gating on lymphocytes by light scatter and then gating on CD3+ T cells. *P < .05, **P < .01, by the Wilcoxon rank sum test.
IFN-γ–producing T cells, as well as total T-cell production of IFN-γ. On exposure to mycobacterial antigens, T cells from patients with tuberculosis showed delayed activation of ERK and p38, and patients whose T cells produced the lowest IFN-γ levels had the greatest reductions in phosphorylation of these signaling molecules. Inhibition of ERK and p38 MAPKs reduced the number of IFN-γ–producing p-CREB+ T cells. Costimulation through SLAM increased IFN-γ production and ERK phosphorylation by high responders, and inhibition with SLAM siRNA abrogated these effects. The sum of these data demonstrates that ERK and p38 MAPK pathways contribute to M. tuberculosis–induced IFN-γ production by primary human T cells through mechanisms that increase phosphorylation of CREB. In addition, SLAM contributes to ERK-mediated IFN-γ production.

We found that human T cells phosphorylate p38 MAPK and ERK when stimulated with M. tuberculosis antigens and that inhibition of these kinases reduces M. tuberculosis–induced IFN-γ production (Figure 1). Patients with tuberculosis, whose T cells produce less IFN-γ than those of healthy donors, displayed delayed activation of ERK and p38 (Figure 4D), with the most marked changes in low responders (Figure 5B and 5C), with poor immunity and the most severe disease [20, 25]. These findings suggest that ERK and p38 contribute to IFN-γ production in individuals with M. tuberculosis infection and that the activity of these pathways correlates with the clinical manifestations of infection. Chemical inhibition of both ERK and p38 reduced M. tuberculosis–stimulated IFN-γ production to a greater extent than either inhibitor alone but did not completely abolish IFN-γ production (V. Pasquinelli, unpublished data), indicating that other signaling molecules also control IFN-γ production in response to M. tuberculosis. Phosphorylation of p38 and ERK were not observed in patients with XLP whose PBMCs produce IFN-γ without antigen stimulation (Figure 4C), indicating that these kinases do not mediate all mechanisms of IFN-γ production. ERK and p38 also contribute to IFN-γ production in response to polyclonal activation of T cells with antibodies to CD3 and CD28 (V. Pasquinelli, unpublished data), showing that stimuli other than M. tuberculosis also act through these kinases.

The role of p38 MAPK in T-cell production of IFN-γ depends on the mode of T-cell stimulation and may differ in mice and humans. Elegant studies in mice with a p38 MAPK gene deletion in T cells showed that p38 is not required for IFN-γ production in response to antigen but is central for eliciting IFN-γ secretion in response to interleukin 12 (IL-12) and interleukin 18 [26], p38 MAPK phosphorylates STAT4, a transcription factor through which IL-12 upregulates IFN-γ transcription in T cells [27, 28]. In humans, some investigators found that T-cell receptor (TCR) stimulation induces p38 MAPK phosphorylation and IFN-γ production by antigen-experienced T cells [29], whereas others reported that p38 MAPK does not affect T cells activated through the TCR [30]. In the current report, we found that primary human T cells phosphorylate p38 MAPK when stimulated with M. tuberculosis antigens and that inhibition of p38 MAPK reduces M. tuberculosis–induced IFN-γ production. Mycobacteria elicit IL-12 production by mononuclear phagocytes, and T-cell IFN-γ production in response to M. tuberculosis antigens is inhibited by anti–IL-12 [31]. In combination with prior publications, our current results suggest that M. tuberculosis stimulates IFN-γ production by stimulation of antigen-experienced T cells through the TCR and through IL-12, with at least the latter process dependent on p38 MAPK. The signaling pathways that control IFN-γ production are likely to depend on the pathogen or other T-cell stimulus. For example, ERK but not p38 MAPK plays a dominant role in IL-12–mediated restoration of T-cell IFN-γ production following burn injury [32].

Most studies of p38 MAPK signaling in the setting of mycobacterial infection have evaluated the effects on mononuclear phagocytes. Accordingly, MAPKs contribute to production of TNF-α and interleukin 10 by macrophages in response to M. tuberculosis [33–37], and M. tuberculosis delays phagosomal maturation by reducing recruitment of early endosome autoantigen 1 to the phagosomal membrane through p38 MAPK [38] and inhibits release of TNF-α by macrophages during murine infection with M. tuberculosis [39]. Furthermore, pathogenic M. avium complex strains reduce p38 MAPK activity in macrophages, whereas less virulent...
mycobacteria do not [13, 35], suggesting that inhibition of p38 MAPK activity constitutes a mycobacterial virulence mechanism. Early secreted antigenic target of 6 kDa (ESAT-6) is a secreted protein of *M. tuberculosis* that confers virulence in animal models [40, 41]. We recently found that high concentrations of ESAT-6 inhibit T-cell IFN-γ production by activating p38 MAPK [42], contrasting with our current findings, in which ERK and p38 pathways enhanced *M. tuberculosis*-induced IFN-γ production by T cells from healthy donors. *M. tuberculosis* produces many proteins, lipids, and lipoglycans, which have differential effects on cell signaling pathways and on the human immune response. We speculate that mycobacterial components other than ESAT-6 induce IFN-γ production through p38 MAPK pathways and that these factors dominate when T cells are exposed to *M. tuberculosis* cell lysates used in the current study, which contain limited...
amounts of ESAT-6, as this protein is secreted and is present primarily in the culture filtrate. Further studies are needed to determine whether p38 MAPK–dependent IFN-γ production occurs when T cells encounter live M. tuberculosis.

CREB is a transcription factor that controls genes that mediate immune responses, including inhibition of nuclear factor κB activation, induction of macrophage survival, and promotion of proliferation, survival, and regulation of lymphocytes [43]. We previously demonstrated that CREB binds to the IFN-γ promoter and upregulates the production of IFN-γ induced by M. tuberculosis [23]. Protein kinase A is the classic activator of CREB, but other signaling pathways can also phosphorylate CREB in T cells, including protein kinase C, Ras, ERK1/2, and MAPKs [44-46]. Two of the substrates of p38 MAPK are mitogen- and stress-activated kinases 1 and 2, both of which can phosphorylate CREB in T cells [47]. In line with these reports, we found that inhibition of the ERK and p38 MAPK pathways reduced the number of T cells expressing p-CREB and IFN-γ in response to M. tuberculosis (Figure 2). These findings suggest that ERK and p38 contribute to CREB phosphorylation and IFN-γ production, but it is uncertain whether this is mediated directly through these kinases or indirectly through other linked cell-signaling pathways.

Costimulation lowers the threshold for TCR signaling but also increases the probability that activated cells will undergo proliferation and subsequent differentiation [48]. We have previously shown that T cells responding to M. tuberculosis antigens rapidly upregulate the costimulator SLAM and that these
Peripheral blood mononuclear cells (PBMCs) from 8 high responders (HRs; defined in Methods) were stimulated with Mycobacterium tuberculosis antigen for 5 days, and expression of signaling lymphocyte activation molecule (SLAM), phosphorylated cyclic adenosine monophosphate response element binding protein phosphorylation (p-CREB), and interferon-γ (IFN-γ) was measured by flow cytometry. The percentages of p-CREB+ and SLAM+ T cells were determined by gating on IFN-γ–producing CD3+ cells. B, PBMCs from 6 HRs and 5 low responders (HRs and LRs, respectively; defined in Methods) were stimulated with M. tuberculosis. After 5 days, cells were harvested, rested for 5 hours in low (1%) serum media, and restimulated with an agonistic anti-SLAM monoclonal antibody (α-SLAM) for 30 minutes. Phosphorylated and total extracellular signal-regulated kinase (p-ERK and tERK, respectively) and p38 (p-p38 and t-p38, respectively) expression was then measured by Western blot. Densitometry was performed, and the fold-increase (relative to time 0) is shown. **P < .01, by the Mann–Whitney U test. C, SLAM or GFP small-interfering RNA (siRNA) were incubated with PBMCs from 5 HRs for 48 hours. Cells were collected, washed, and stimulated with M. tuberculosis for another 72 hours. p-ERK, tERK, p-p38, and t-p38 expression was then measured by Western blot. Densitometry was performed, and the ratios of phosphorylated to total protein levels were expressed as arbitrary units. *P < .05, by the Wilcoxon rank sum test.

In summary, we found that IFN-γ production in patients with tuberculosis is regulated by ERK and p38 MAPK signaling pathways that involve CREB activation. Costimulation through SLAM augmented phosphorylation of ERK and IFN-γ production, adding another layer of complexity to MAPK activation in T cells and cytokine production. We also showed that patients with tuberculosis whose T cells secrete little IFN-γ in response to M. tuberculosis have impaired activation of ERK and p38 MAPK. Further study of the dysfunctional activation of MAPKs during tuberculosis could identify new targets to increase IFN-γ production and improve patient outcome, particularly in those with drug-resistant disease.

### Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

### Notes

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