Interferon-γ (IFN-γ) is crucial for protection against Mycobacterium tuberculosis, and the transcription factor cAMP response element binding protein (CREB) increases IFN-γ transcription. We determined whether the transmembrane receptor signaling lymphocyte activation molecule (SLAM) and interleukin-17 (IL-17) affect CREB phosphorylation and IFN-γ production in persons with tuberculosis. When T cells from patients with tuberculosis were activated with M. tuberculosis, 80% of SLAM+ T cells expressed phosphorylated CREB, and SLAM activation increased CREB phosphorylation and IFN-γ production. In contrast, IL-17 down-regulated SLAM expression, CREB phosphorylation, and IFN-γ production. Therefore, IL-17 and SLAM have opposing effects on IFN-γ production through CREB activation in persons with tuberculosis.

Tuberculosis remains an enormous global health problem despite currently available drug treatments. There are nearly 9 million new cases of and 1.7 million deaths due to tuberculosis annually, and it is one of the most common causes of morbidity and mortality among patients with HIV infection. BCG, the only available vaccine, is of variable efficacy, especially in tuberculosis-endemic regions. Development of a more effective vaccine depends on a better understanding of the human immune response to this pathogen.

Protective immunity against mycobacterial infection requires the generation of IFN-γ, a macrophage-activating cytokine produced by T cells that is crucial in immunity to Mycobacterium tuberculosis infection [1]. The degree of reduction in IFN-γ production by peripheral blood mononuclear cells (PBMCs) is a marker of disease severity in patients with tuberculosis [2]. Thus, elucidation of the mechanisms for reduced IFN-γ production in individuals that develop the disease will enhance our knowledge of the pathogenesis of tuberculosis. 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 [3]. Therefore, to gain insight into the mechanisms that enhance cell-mediated immune responses to M. tuberculosis, we studied proteins that might regulate transcription of IFN-γ. Previously, we showed that the transcription factor cAMP response element binding protein (CREB) increased M. tuberculosis–stimulated IFN-γ secretion by binding to the IFN-γ proximal promoter [4]. Furthermore, we demonstrated that T cell expression of signaling lymphocytic activation molecule (SLAM) was directly correlated with responsiveness to M. tuberculosis antigen and that SLAM engagement up-regulated IFN-γ production in patients with tuberculosis [5]. In addition, IFN-γ inhibits IL-17 expression, but the effects of IL-17 on IFN-γ production are unclear [6]. Thus, here we analyzed the role of SLAM, CREB, and IL-17 as regulators of the molecular pathway that leads to IFN-γ production during active tuberculosis.

Subjects, materials, and methods. We evaluated 35 patients with tuberculosis diagnosed at Hospital Muñiz (Buenos Aires, Argentina) on the basis of clinical and radiological data, together with identification of acid-fast bacilli in sputum. All patients had received antituberculosis therapy for <1 week. We also studied 30 BCG-vaccinated healthy donors. After informed consent was obtained, we collected peripheral blood in heparinized tubes. The local ethics committee approved all studies.

Cells were stimulated in vitro with an extract from the virulent M. tuberculosis strain H37Rv, generously provided by Mycobacteria Research Laboratories (Colorado State University [Fort Collins, CO]), that was prepared by probe sonication. PBMCs were isolated by density gradient centrifugation with Ficoll-Paque (Amersham Biosciences) and were cultured in RPMI
were analyzed using pCREB and efficiency of iRNA inhibition [5]. Equivalent amounts of protein tis). Western blotting for SLAM was performed to determine the expression of SLAM and pCREB in T cells. Using SLAM siRNA, we blocked the expression of SLAM and measured pCREB expression by Western blotting. Using SLAM siRNA, we efficiently inhibited expression of SLAM, as measured by Western blot (data not shown). Furthermore, SLAM siRNA strongly inhibited the M. tuberculosis–induced expression of pCREB and reduced IFN-γ production by T cells from patients with tuberculosis (figure 1B).

Because our present and published results indicated that both SLAM and CREB contribute to M. tuberculosis–induced IFN-γ production, we next determined whether the effects of SLAM ligation might be mediated in part through CREB. Thus, we evaluated the effect of SLAM activation on phosphorylation of CREB. PBMCs from patients with tuberculosis were stimulated for 24 h with M. tuberculosis antigen in the presence or absence of an agonistic SLAM antibody, and phosphorylation of CREB was determined by Western blotting. SLAM activation enhanced phosphorylation of CREB (figure 1C), and this was confirmed in a larger number of patients (figure 1C), indicating that it is a potential mechanism by which SLAM induces IFN-γ secretion. Increased pCREB expression induced after SLAM ligation directly correlated with higher levels of IFN-γ produced against the antigen by PBMCs from patients with tuberculosis (figure 1C). Together, our data provide the first evidence suggesting that SLAM ligation activates CREB, which in turn induces IFN-γ secretion by T cells during a physiologic response to M. tuberculosis.

IL-17 has recently been shown to contribute to immune responses to M. tuberculosis in mice [9] and humans [10]. Although IFN-γ inhibits development of Th17 cells [13], the effects of IL-17 on IFN-γ production are unclear. To investigate this question, we added rhIL-17 to M. tuberculosis–stimulated PBMCs from patients with tuberculosis. As shown in figure 2A, IL-17 markedly reduced the levels of SLAM on T cells from patients with tuberculosis that were stimulated in vitro with M. tuberculosis. Because SLAM expression is directly correlated with IFN-γ production in tuberculosis [5] and our present data indicated that IL-17 diminished the levels of SLAM (figure 2A and 2B), we next investigated the effect of IL-17 on IFN-γ production. We observed that IL-17 inhibited IFN-γ secretion by antigen-stimulated cells (figure 2B). Because M. tuberculosis stimulation induced 80% of SLAM+ T cells to express pCREB (figure 1A), we then investigated whether IL-17 would affect this population. IL-17 significantly decreased the number of SLAM-pCREB+ T cells by >50% (figure 2C).

To determine whether our findings were relevant in vivo, we compared IL-17 and IFN-γ production by PBMCs in healthy donors and patients with tuberculosis. After antigen stimulation, the percentage of IL-17+ T cells was 10% ± 3% in 10 patients with tuberculosis, compared with 4.0% ± 0.7% in 10 healthy donors (P < .05 by the Mann-Whitney U test). These results correlated with levels of IFN-γ produced by M. tubercu-
Ions–stimulated PBMCs (13.8 ± 3.7 ng/mL in patients with tuberculosis, compared with 54 ± 13 ng/mL in healthy donors; \( P < .05 \) by the Mann-Whitney \( U \) test). Together, our data indicate that IL-17 inhibits IFN-\( \gamma \) production induced by \( M. \) tuberculosis during active tuberculosis, in part through down-regulation of SLAM and pCREB expression.

**Discussion.** IFN-\( \gamma \) is pivotal for human defenses against \( M. \) tuberculosis [11]. In this work, we demonstrated that down-
regulation of SLAM and pCREB expression participated in IFN-\(\gamma\) inhibition in persons with tuberculosis. However, IFN-\(\gamma\) gene regulation is complex, and production of IFN-\(\gamma\) is controlled by several signaling proteins, as well as by multiple transcription factors that bind to different promoter elements upon antigen stimulation, indicating that additional factors besides SLAM and CREB control IFN-\(\gamma\) transcription. For example, we recently demonstrated that ATF-2 binds as part of a complex with CREB to the IFN-\(\gamma\) proximal promoter during T cell activation and positively regulates IFN-\(\gamma\) production [12]. Stimulation of PBMCs with \(M. tuberculosis\) also increases production of IL-12 and IL-18. The former signals through STAT4, which binds to a site 236 bp upstream of the IFN-\(\gamma\) start codon [13], and the combination of IL-12 and IL-18 up-regulates binding of AP-1 transcription factors to the \(-196\) to \(-183\) bp region [13]. In addition, we previously reported that SLAM, a transmembrane signaling receptor that influences cytokine production by activated T cells, enhanced IFN-\(\gamma\) secretion in response to my-
cobacteria through a signaling cascade that increased activation of NFκB and T-bet [14]. In the current study, we demonstrated that ~80% of antigen-induced SLAM⁺ T cells expressed pCREB, that blocking SLAM with siRNA markedly reduced pCREB, and that engaging SLAM with agonistic monoclonal antibody stimulated pCREB expression. In both cases, pCREB expression paralleled IFN-γ production by patients with tuberculosis. Moreover, we showed that pCREB⁺SLAM⁺ T cells were markedly decreased in patients with tuberculosis, compared with healthy donors, which correlated with the reduction in IFN-γ production by PBMCs from individuals with active tuberculosis. Together, these findings demonstrate for the first time that the physiologic mechanism by which SLAM activation leads to the production of IFN-γ against M. tuberculosis involves activation of the transcription factor CREB.

T cells that produce IL-17 have recently been found to contribute to pulmonary immune responses to M. tuberculosis and M. bovis BCG, in part by inducing chemokines that recruit CD4⁺ cells that produce IFN-γ [9, 15]. IFN-γ inhibits the expansion of IL-17–producing cells during murine mycobacterial infection by reducing IL-23 production [13], and IL-17–producing T cells constitute a significant proportion of M. tuberculosis–activated cells in PBMCs of healthy tuberculin reactors [10]. However, the effect of IL-17 on IFN-γ production in persons with tuberculosis remains unclear. In the current study, we observed that IL-17 decreased SLAM expression and the frequency of SLAM⁺pCREB⁺ cells in M. tuberculosis–activated T cells from patients with tuberculosis, and these decreases directly correlated with reduced IFN-γ production (figure 2). These findings demonstrate that IL-17 inhibits IFN-γ production during tuberculosis by decreasing the expression and function of stimulatory signaling proteins in this pathway. We also found that the numbers of Th17 cells were significantly increased in patients with tuberculosis, compared with BCG-vaccinated healthy donors. We speculate that increased IL-17 production may regulate the capacity of T cells from patients with tuberculosis to produce IFN-γ in response to mycobacterial antigens, consistent with the known antagonistic effects of IFN-γ and Th17 cells [13].

In summary, our results provide new information linking the costimulatory molecule SLAM to the transcription factor CREB and the proinflammatory cytokine IL-17. Additional work is needed to elucidate the pathways that participate in the control of IFN-γ secretion in persons with tuberculosis.

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References