Production of Interleukin-18 in Human Tuberculosis

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To investigate the role of interleukin (IL)–18 in human tuberculosis, IL-18 production was evaluated in blood and at the site of disease in patients with tuberculosis. Mycobacterium tuberculosis–stimulated peripheral blood mononuclear cells (PBMC) from tuberculosis patients secreted less IL-18 and interferon-γ (IFN-γ) than did PBMC from healthy persons reactive to tuberculin. M. tuberculosis–induced IFN-γ production was inhibited by anti–IL-18 and enhanced by recombinant IL-18. Alveolar macrophages secreted IL-18 in response to M. tuberculosis, and IL-18 and IFN-γ concentrations were higher in pleural fluid of patients with tuberculosis than in pleural fluid of patients with nontuberculous diseases. These findings demonstrate that IL-18 production by PBMC correlates with IFN-γ production and effective immunity to tuberculosis, suggesting that IL-18 contributes to a protective type 1 cytokine response in persons with mycobacterial infection.

Tuberculosis causes a staggering burden of mortality worldwide, killing 1.9 million persons each year [1]. Effective treatment of tuberculosis in developing countries is hampered by the high cost of antituberculosis drugs, inability to ensure completion of prolonged therapy, and rising rates of drug resistance. Vaccination provides an alternative cost-effective strategy that would contribute greatly to tuberculosis control. Development of an effective vaccine hinges on an improved understanding of the immune response to Mycobacterium tuberculosis.

Interferon-γ (IFN-γ) plays a pivotal role in immune defenses against M. tuberculosis. Mice lacking IFN-γ because of a targeted gene deletion have markedly increased susceptibility to tuberculosis [2, 3], and humans with a defective IFN-γ receptor have severe mycobacterial disease [4, 5]. IFN-γ production by peripheral blood mononuclear cells (PBMC) provides an immunologic marker that correlates with the clinical manifestations of infection with M. tuberculosis. Healthy persons reactive to tuberculin develop protective immunity against exogenous infection with M. tuberculosis, and M. tuberculosis–stimulated PBMC from these persons produce high concentrations of IFN-γ. On the other hand, in tuberculosis patients with severe disease who do not generate effective immunity, M. tuberculosis–stimulated PBMC produce reduced concentrations of IFN-γ [6–8].

Elucidation of the mechanism for reduced IFN-γ production in tuberculosis will enhance our understanding of the immune response to mycobacterial infection. Interleukin (IL)–18 contributes to generation of type 1 T cells from naïve T cells and stimulates production of IFN-γ by T cells and NK cells [9]. Studies in animal models and in cells cultured in vitro have demonstrated that IL-18 contributes to production of IFN-γ and to protection against mycobacteria and other intracellular pathogens [10–17]. However, there is limited information on the contribution of IL-18 to immune defenses in patients with infectious disease. To evaluate the role of IL-18 in human tuberculosis, we studied IL-18 production in blood and at the site of disease in patients with tuberculosis.

Materials and Methods

Patient population. Blood was obtained from 15 healthy persons reactive to tuberculin, 3 healthy tuberculin-negative persons, and 15 human immunodeficiency virus (HIV)–seronegative patients with culture-proven pulmonary tuberculosis who had received anti-tuberculosis therapy for <4 weeks. Acid-fast stains of sputum were positive for 13 patients and negative for 2.

Pleural fluid was obtained from 11 patients with tuberculous pleuritis who had received anti-tuberculosis therapy for <5 days. All patients had unilateral exudative effusions. The diagnosis was confirmed for 8 patients by culture of M. tuberculosis from pleural fluid or tissue and for 3 patients by histologic demonstration of granulomatous pleuritis in combination with a response to antituberculosis therapy. Pleural fluid was obtained from 8 patients
with nontuberculous pleural effusions (cancer in 5 cases, trauma in 2, bacterial infection in 1). None of these patients had clinical or laboratory evidence of HIV infection.

Bronchoalveolar lavage fluid was obtained from 11 patients who were undergoing bronchoscopy for clinical indications. Bronchoscopy was done to evaluate the possibility of cancer for 9 patients and to evaluate pneumonia that was not resolving for 2 patients. None of the patients had clinical or laboratory evidence of tuberculosis or HIV infection. In all cases, the pathologic process was unilateral, and bronchoalveolar lavage was done in the lung in which there was no clinical, bronchoscopic, or radiographic evidence of cancer or pneumonia.

Isolation of PBMC and CD14+ monocytes. PBMC were isolated from blood by differential centrifugation over Ficoll-Paque (Pharmacia, Piscataway, NJ). PBMC were centrifuged on a Percoll gradient (Amersham Pharmacia Biotech, Uppsala, Sweden), and purified CD14+ cells were isolated from the monocyte fraction by positive selection with magnetic conjugated to anti-CD14 (Miltenyi Biotech, Auburn, CA). The positive cells were 94% CD14+, as measured by flow cytometry.

Cell culture conditions. PBMC (2 × 10^5) or CD14+ monocytes (5 × 10^5) were plated in flat-bottomed 96-well plates (Becton Dickinson Labware, Franklin Lake, NJ) in 200 μL of RPMI 1640 (Life Technologies, Grand Island, NY) containing penicillin-streptomycin (Life Technologies) and 10% heat-inactivated human serum, in RPMI, and 1.2 × 10^5 cells were allowed to adhere to flat-bottomed 96-well plates. About 90% of the bronchoalveolar lavage cells were alveolar macrophages, as judged by Giemsa staining. Nonadherent cells were removed, and the adherent cells were >98% alveolar macrophages. These adherent cells were cultured in the presence or absence of 10 μg/mL M. tuberculosis Erdman strain (provided by P. Brennan, Colorado State University, Fort Collins). In some experiments, recombinant IL-18 (MBL International, Nagoya, Japan), recombinant IL-12 (gift of M. Gately, Hoffman–La Roche, Nutley, NJ), neutralizing antibodies to IL-18 (MBL International), or isotype control mouse IgG1 (PharMingen, San Diego) were also added to the cells.

Bronchoalveolar lavage fluid was passed through sterile gauze and centrifuged at 834 g for 10 min to pellet the cells minus that in supernatants from unstimulated cells. Supernatants from cultured CD14+ monocytes or alveolar macrophages were collected after 24 h. For measurement of IFN-γ levels were measured by ELISA. Data are mean ± SE. ND, not done. For data that were not normally distributed, the Wilcoxon rank sum test was used. P < .05 was considered statistically significant.

Results

IL-18 production by M. tuberculosis-stimulated PBMC. In preliminary experiments, PBMC were obtained from 2 healthy persons reactive to tuberculin and from 2 tuberculosis patients. Supernatants were harvested from PBMC cultured with M. tuberculosis for 24–120 h. IL-18 levels were highest after 24 h, so this time point was chosen for subsequent experiments.

PBMC from 13 healthy persons reactive to tuberculin and 12 tuberculosis patients were cultured in the presence or absence of M. tuberculosis for 24 h. IL-18 levels in M. tuberculosis-stimulated culture supernatants from tuberculosis patients were reduced compared with those from healthy tuberculin reactors (50 ± 21 vs. 153 ± 21 pg/mL; P = .001; figure 1). IL-18 production by M. tuberculosis-stimulated PBMC from healthy tuberculin-negative donors was minimal (figure 1).

Monocytes, macrophages, and dendritic cells are believed to be the major source of IL-18 [9]. To determine if reduced IL-18 secretion by PBMC in tuberculosis patients was due to decreased IL-18 production by monocytes, we isolated CD14+ monocytes from PBMC of 10 healthy persons reactive to tuberculin and 10 tuberculosis patients and cultured them in the presence or absence of M. tuberculosis for 24 h. Parallel to the changes with PBMC, IL-18 production by monocytes was reduced in tuberculosis patients compared with healthy tuberculin reactors (3 ± 2 vs. 97 ± 21 pg/mL; P = .001; figure 1).

Effect of endogenous IL-18 on M. tuberculosis–induced IFN-γ production. Prior studies have shown that M. tuberculosis...
Tuberculosis patients from to pg/mL (endogenous IL-18 contributes to antibodies (data not shown). These findings demonstrate that IL-18 to IFN-γ production, PBMC from 6 tuberculosis patients (figure 3). Compared with mean IFN-γ concentrations of 465 ± 174 pg/mL on stimulation with M. tuberculosis alone, mean IFN-γ concentrations were significantly higher with addition of optimal concentrations of IL-18 (1201 ± 128 pg/mL; P = .01) or IL-12 (1405 ± 205 pg/mL; P = .006). However, addition of both IL-12 and IL-18 did not further increase IFN-γ production (1450 ± 197 pg/mL [P > .2] compared with addition of IL-18 or IL-12 alone). In 3 healthy persons reactive to tuberculin, IL-18 and IL-12 did not significantly increase M. tuberculosis–induced IFN-γ concentrations (figure 4B).

**Production of IL-18 by alveolar macrophages.** Because tuberculosis is a disease that affects the lungs in most patients, we evaluated the capacity of alveolar macrophages to secrete IL-18 in response to M. tuberculosis. Macrophages were isolated from bronchoalveolar lavage fluid from 11 patients without tuberculosis. Heat-killed M. tuberculosis elicited IL-18 production by macrophages from 10 of 11 patients (188 ± 120 vs. 9 ± 3 pg/mL; P < .001; figure 5).

**IL-18 at the site of disease.** Previous studies have shown that IFN-γ concentrations are markedly increased at the site of disease in patients with pleural effusions due to tuberculosis but not in those with effusions due to other diseases [21]. To

**Figure 2.** Effect of anti-interleukin (IL)-18 on Mycobacterium tuberculosis–induced interferon-γ (IFN-γ) production. Peripheral blood mononuclear cells were isolated from 6 healthy tuberculin reactors and were cultured with 10 µg/mL heat-killed M. tuberculosis in the absence or presence of 10 µg/mL neutralizing antibodies to IL-18 (a-IL-18). IFN-γ concentrations were measured by ELISA.

**Figure 3.** Effect of recombinant interleukin (IL)-18 on Mycobacterium tuberculosis–induced interferon-γ (IFN-γ) production. Peripheral blood mononuclear cells isolated from 6 tuberculosis patients and from 3 healthy persons reactive to tuberculin were cultured with 10 µg/mL heat-killed M. tuberculosis, M. tuberculosis and 10 ng/mL recombinant IL-18, or IL-18 alone. IFN-γ concentrations were measured by ELISA. Data are mean ± SE.
Figure 4. Effects of recombinant interleukin (IL)-18 and IL-12 on Mycobacterium tuberculosis–induced interferon-γ (IFN-γ) production by 6 tuberculosis patients (left panel) and 3 healthy persons reactive to tuberculin (right panel). Peripheral blood mononuclear cells were cultured with 1 μg/mL heat-killed M. tuberculosis only (none) or with M. tuberculosis and optimal concentrations of recombinant IL-18 or IL-12 or both. IFN-γ concentrations were measured by ELISA. Data are mean ± SE.

Figure 5. Production of interleukin (IL)-18 by alveolar macrophages in response to Mycobacterium tuberculosis. Macrophages were isolated from bronchoalveolar lavage fluid obtained from 11 patients who did not have tuberculosis. Macrophages were cultured in medium alone or with 10 μg/mL heat-killed M. tuberculosis. IL-18 concentrations were measured by ELISA.

investigate the potential contribution of IL-18 to IFN-γ production in vivo, we measured cytokine concentrations in pleural fluid of 11 tuberculosis patients and in 8 nontuberculous pleural effusions. IFN-γ concentrations were significantly higher in tuberculous effusions (1573 ± 414 pg/mL; P = .002). Parallel changes were observed in IL-18 concentrations (278 ± 53 vs. 106 ± 17 pg/mL; P = .009).

Discussion

The current study demonstrates that IL-18 contributes to IFN-γ production in human tuberculosis. M. tuberculosis–stimulated PBMC from tuberculosis patients secreted less IL-18 and IFN-γ than did PBMC from healthy tuberculin reactors.

M. tuberculosis–induced IFN-γ production was inhibited by anti–IL-18 and enhanced by recombinant IL-18. Alveolar macrophages secreted IL-18 in response to M. tuberculosis, and IL-18 and IFN-γ concentrations were higher in pleural fluid of tuberculosis patients than in pleural fluid of patients with nontuberculous diseases. These findings provide evidence that production of IL-18 and IFN-γ are tightly correlated and that IL-18 may mediate a protective type 1 cytokine response against mycobacteria in peripheral blood and at the site of disease.

The most distinctive feature of IL-18 is its capacity to enhance IFN-γ production and cytolytic activity by T cells and NK cells [9]. IL-18 does not induce IFN-γ production by naive T cells or by unstimulated Th1 clones, which do not express IL-18 receptors [11, 12, 15, 22]. However, IL-18 up-regulates secretion of IFN-γ by activated T cells stimulated with anti-CD3 [11] and by Th1 clones stimulated with antigen [12]. In contrast, IL-18 does not augment IL-4 production by activated Th2 clones [12].

In animal models, neutralization of IL-18 with antibodies markedly enhanced susceptibility to disease from several intracellular organisms, including Cryptococcus neoformans [13], Salmonella species [23, 24], and Yersinia enterocolitica [25]. Complete abrogation of IL-18 production by a targeted gene deletion resulted in strikingly reduced IFN-γ secretion and impaired NK cell activity in response to bacterial infection [14], as well as increased susceptibility to Leishmania species [16] and M. tuberculosis [17]. These findings demonstrate that other cytokines cannot compensate for the pivotal contribution of IL-18 to host defenses against intracellular pathogens.

The vast majority of studies of IL-18 have evaluated its effects in animal models or on cultured cells in vitro, and the study by Garcia et al. [20] of leprosy patients represents the only published information on IL-18 expression in humans with infectious disease. Several findings in tuberculosis and leprosy strongly suggest that IL-18 is an important stimulus for IFN-γ production in peripheral blood and at the site of disease in mycobacterial infection. First, in healthy tuberculin reactors
with an effective immune response to tuberculosis and increased *M. tuberculosis*-induced IFN-γ production, IL-18 levels were significantly higher than in tuberculosis patients with ineffective immunity and reduced IFN-γ production. Similarly, IFN-γ and IL-18 concentrations were greater in patients with tuberculous leprosy and limited disease from *M. leprae* than in lepromatous leprosy patients with extensive disease [20]. Second, mycobacterium-induced IFN-γ production by PBMC from patients infected with *M. tuberculosis* or *M. leprae* was inhibited by anti–IL-18 and augmented by recombinant IL-18. Third, IL-18 concentrations and IFN-γ concentrations were elevated specifically in pleural fluid of patients with tuberculosis, paralleling the increased IFN-γ mRNA expression in skin of tuberculous leprosy patients [20]. Finally, normal alveolar macrophages secreted IL-18 in response to *M. tuberculosis*, suggesting that IL-18 is produced in the lung during tuberculosis infection.

An interesting difference between tuberculosis and leprosy is that *M. leprae* elicited production of only 30–35 pg/mL IL-18 by blood monocytes and PBMC [20]. In contrast, *M. tuberculosis* induced blood monocytes, PBMC, and alveolar macrophages to secrete 100–200 pg/mL IL-18, with a maximum level of almost 500 pg/mL. Comparable concentrations of IL-18 were present at the site of disease in tuberculous pleuritis. These findings suggest that *M. tuberculosis* is a more potent stimulus for IL-18 production than is *M. leprae*.

PBMC produced more IL-18 in response to *M. tuberculosis* than did monocytes, particularly in tuberculosis patients (figure 1). This suggests that cells other than monocytes secrete IL-18 or up-regulate IL-18 production by monocytes. IL-18 mRNA is present in CD4+ and CD8+ T cells [26], suggesting that they can produce IL-18. PBMC from healthy persons reactive to tuberculosis produced more IL-18 in response to *M. tuberculosis* than did PBMC from tuberculin-negative donors (figure 1), suggesting that *M. tuberculosis*-reactive T cells enhance IL-18 production. Addition of purified CD3+ cells to macrophages augments *M. tuberculosis*-induced IL-18 secretion (R.V., unpublished data), and the mechanism for this effect is under investigation.

Alveolar macrophages produced higher IL-18 concentrations in response to *M. tuberculosis* than did monocytes from healthy tuberculosis reactors (figures 1 and 5), and similar findings have been observed for tumor necrosis factor-α [27]. Because alveolar macrophages have undergone differentiation in vivo, they may be primed to generate higher concentrations of proinflammatory cytokines in response to microbial stimuli. Alternatively, because we obtained alveolar cells from patients with cancer or pneumonia, the alveolar macrophages may have been activated in vivo. Studies of alveolar macrophages from normal donors are needed to resolve this question.

Reduced *M. tuberculosis*-induced IFN-γ secretion by peripheral blood T cells in tuberculosis patients may be mediated in part by abnormalities in cytokine production by monocytes. Compared with *M. tuberculosis*-stimulated monocytes from healthy tuberculin reactors, those from tuberculosis patients produce comparable concentrations of IL-10 and IL-12 [7, 8, 28, 29] but higher levels of transforming growth factor-β [30], and neutralization of transforming growth factor-β up-regulates IFN-γ production [8, 31]. Our current findings demonstrate that depressed IL-18 production by monocytes also contributes to reduced IFN-γ production in tuberculosis patients. Recombinant IL-18 increased *M. tuberculosis*-induced IFN-γ production in tuberculosis patients but not in healthy tuberculin reactors. In the latter case, IL-18 production may already be optimal, and addition of supraphysiologic concentrations does not further enhance IFN-γ production.

Th1 cells but not Th2 cells express receptors for IL-12 and IL-18 [22, 32], and both cytokines act to increase IFN-γ secretion. When Th1 clones and T cells are stimulated with anti-CD3 and anti-CD28, IL-12 up-regulates IL-18 receptor expression [10, 12, 18, 19]. As a result, IL-12 and IL-18 have synergistic effects on production of IFN-γ. However, we observed no additive or synergistic effects of IL-12 and IL-18 on *M. tuberculosis*-induced IFN-γ production. The reasons for our findings remain speculative.

In conclusion, we demonstrated that IL-18 production by PBMC correlated with IFN-γ production and effective immunity to tuberculosis. In addition, IL-18 was present at the site of disease in tuberculosis patients, and alveolar macrophages produced IL-18 in response to *M. tuberculosis*. These findings confirm and extend the results of previous studies suggesting that IL-18 contributes to the type 1 cytokine response in mycobacterial infection.

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References


