CONCISE COMMUNICATION

Increased Production of Interleukin 4 by CD4⁺ and CD8⁺ T Cells from Patients with Tuberculosis Is Related to the Presence of Pulmonary Cavities

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In tuberculosis, cellular immunity is considered to be responsible for the eradication of infection but also for damage of host tissues. In animal models, the balance between Th1-type cytokines, especially interferon (IFN)-γ, and Th2-type cytokines, primarily interleukin (IL)-4, seems crucial for these effects. Reports on Th1-type and Th2-type cytokines in human tuberculosis are conflicting, and little is known about their role in tissue damage. Flow-cytometric assessment of cytokine responses was performed in human immunodeficiency virus (HIV)–seronegative patients with active tuberculosis and in healthy controls. Patients and controls showed no significant difference in expression of IFN-γ. However, patients showed a striking increase in production of IL-4 in CD4⁺ as well as CD8⁺ T cells. Most remarkably, the expression of IL-4 was especially elevated in patients with cavitary tuberculosis. The Th2-type response with increased production of IL-4 in patients with tuberculosis may antagonize host defense and lead to tissue necrosis.

In tuberculosis (TB), cell-mediated immunity (CMI) is responsible for the eradication of mycobacteria. The major effector mechanism of CMI is thought to be the activation of infected macrophages by Th1-type cytokines, particularly interferon (IFN)-γ. The protective effects of Th1-type cytokines may be antagonized by Th2-type cytokines, primarily interleukin (IL)-4 [1]. As is the case in leprosy, the balance between Th1-type and Th2-type cytokine responses in TB may influence mycobacterial growth as well as immunopathology.

In human TB, studies of the patterns of T cell cytokines have met with conflicting results [1–5]. Uncertainty also remains about the cellular source of the various cytokines in TB. CD4⁺ T cells have long been regarded as the major immune regulatory cells. CD8⁺ T cells were viewed as CD4-dependent, IFN-γ–secreting cytotoxic cells, whose main function was the major histocompatibility complex class I–restricted lysis of infected host cells. Therefore, most studies of T cell cytokine profiles have concentrated on CD4⁺ T cells, although expression of IL-4 has also been shown in CD8⁺ T cells in mycobacterial infections.

In atypical mycobacterial infections in humans, a Th1-type response is essential [6]. In murine TB, Th1-type cytokines are necessary for protective immunity [7], whereas increased production of Th2-type cytokines may be responsible for tissue damage [8, 9].

To further evaluate the Th cytokine response in human TB and to establish the cellular source, as well as the possible role in tissue damage, cytokine production by circulating T lymphocytes of patients with active TB both with and without cavitation was investigated by flow cytometry and compared with TB skin-test–positive controls.

Methods

**Subjects.** Eighteen patients who were seronegative for human immunodeficiency virus (HIV) and had active pulmonary TB were included at a University Hospital in Jakarta, Indonesia. Eleven (61%) were male, and the average age was 32.8 years (range 16–66 years). Most patients had extensive pulmonary involvement; 11 had pulmonary cavities, and 4 had miliary disease. Fourteen healthy individuals of similar age and sex whose skin test results were positive for TB were recruited as controls.

**Cell stimulation and immunofluorescent staining.** Intracellular cytokines by circulating T lymphocytes were detected as described elsewhere [10], with minor modifications. In brief, 1 × 10⁶ peripheral blood mononuclear cells (PBMC), isolated by density centrif-
Flow cytometric analysis. Cells were analyzed for immunofluorescence in a Coulter Epic XL (Coulter, Hialeah, FL). IL-4+ and IFN-γ+ cells were determined in the CD4+ CD3+ and CD8+ CD3+ cell populations in separate measurements (minimum, 50,000 cells). Spontaneous expression of cytokines was similarly low in patients and control subjects (mean: 0.75% for IFN-γ and 0.6% for IL-4). For final analysis, residual expression of cytokines was subtracted from expression after maximal stimulation.

Statistical analysis. Comparisons between groups were performed by use of the Mann-Whitney U test and χ² test. P values were 2-sided and the level of significance was set at P < .05.

Results

T cells from patients with TB do not produce more IFN-γ than those from controls. After stimulation of PBMC, IFN-γ was detected in CD8+ and CD4+ T cells from all patients and controls. Within the subset of CD4+ T lymphocytes, the percentage of cells staining positive for IFN-γ was almost identical in the 2 groups, with a range of 7.8%–29.9% (mean, 12.7%) in patients and 4.7%–21.3% (mean, 11.8%) in controls. Within CD8+ T cells, expression of IFN-γ was detected in 29.0% (range, 10%–65%) of stimulated cells from patients, compared with 21.3% (range, 5.7%–42%) in controls (P = .16).

Both CD4+ and CD8+ T cells from patients with TB produce more IL-4 than do similar cells from controls. After stimulation of PBMC, T lymphocytes also expressed IL-4. In both CD4+ and CD8+ T cells, production of IL-4 was significantly higher in patients with TB than in healthy controls. The percentage of CD4+ T cells positive for IL-4 ranged from 1.5%–7.3% in patients (mean, 3.9%) and from 0.4%–2.4% (mean, 1.4%) in control subjects (P < .001). In CD8+ T cells, expression of IL-4 exhibited a wide range in patients (0.4%–21.0%; mean, 4.7%) and was low or undetectable (range, 0%–2.0%; mean, 0.7%) in control subjects (P < .005). Five patients expressed IL-4 in >5% of CD8+ T cells. Analysis of a second sample of these 5 individuals yielded similarly high expression (range, 6.9%–18.7%).

The ratio of IFN-γ and IL-4 production is decreased in patients with TB. The ratio of production of IFN-γ to IL-4 in single individuals has been used as a marker for Th1-Th2 balance, with a lower ratio pointing toward a Th2 response [1]. For CD4+ cells, this ratio was much lower in patients than in controls (3.9 vs. 9.6; P < .001). The same was true for CD8+ T cells (21.6 vs. 36.2; P < .05). Figure 1 depicts the simultaneous detection of IFN-γ and IL-4 in circulating T cells from a representative patient after in vitro stimulation of PBMC. Unstimulated cells from this patient show spontaneous ex vivo production of IFN-γ in 0.9% of CD3+ cells (figure 1A). Stimulation of cells induces expression of IFN-γ in 10.1% of T cells.
Cavitary TB is associated with increased IL-4 expression. Next, expression of cytokines was related to clinical characteristics. Extent and nature of pulmonary involvement, as exemplified by the presence of cavities on chest radiograph, showed a correlation with production of IL-4, but not with IFN-γ (figure 2). The median number of CD8⁺ T cells expressing IL-4 was 3.7% in 11 patients with pulmonary cavities compared with 1.1% in 7 patients without cavities \((P < .05)\). In contrast, the number of CD8⁺ T cells expressing IFN-γ was almost similar in patients with or without pulmonary cavities \((27.0\% \text{ vs. } 22.4\%; \ P = .22)\). As a result, the ratio of IFN-γ to IL-4 production in CD8⁺ T cells was much lower in the presence of cavitary disease \((median, 7.4 \text{ vs. } 26.5; \ P < .05)\). Within the subset of CD4⁺ cells, expression of IL-4 also tended to be higher in patients with pulmonary cavities \(4.5\% \text{ vs. } 3.0\%; \ P = .19\). Expression of IFN-γ and ratio of IFN-γ to IL-4 in CD4⁺ were similar in both groups. IL-4 was more frequently expressed in CD8⁺ T cells than in CD4⁺ cells in the majority \(63\%\) of patients with pulmonary cavities, whereas this never occurred in patients without cavities \((P = .01)\).

Four patients with miliary disease, all severely ill, exhibited much lower IL-4 expression, especially in CD8⁺ T cells. The percentage of CD8⁺ T cells expressing IL-4 was 0.8% in miliary and 7% in cavitary TB \((P < .05)\), whereas expression of IFN-γ was not significantly different \(27.3\% \text{ vs. } 32\%, \ P = .6\).

Discussion

In this study, we showed that patients with pulmonary TB exhibit a markedly increased capacity for IL-4 production in circulating T lymphocytes. Production of IL-4 was established not only in CD4⁺ T cells, the primary cytokine-producing cell in the Th1-Th2 concept, but also in CD8⁺ T cells. Of interest, the highest expression of IL-4 in these 2 cellular subsets occurred in patients with pulmonary cavities, suggesting a role for IL-4 in the development of tissue damage.

It is unclear whether increased production of IL-4 causes, or merely reflects, severe disease. With regard to the former, IL-4 may reduce killing of mycobacteria by phagocytes through inhibition of IFN-γ production [1]. However, our study in vitro production of IFN-γ was not affected. With regard to the latter possibility, severely ill patients with miliary TB did not show increased expression of IL-4, suggesting that IL-4 is not just a marker of disease severity as such. Our findings suggest that increased production of IL-4 plays a role in tissue necrosis. The deleterious role of IL-4 in various infectious diseases, including leprosy and leishmaniasis, has been ascribed to its suppression of the protective inflammatory response of Th1-type cytokines [1]. However, IL-4 may also induce or intensify tissue damage; only when primed for production of IL-4 do mice infected with saprophytic mycobacteria develop peribronchial and interstitial necrosis [8]. Likewise, overexpression of IL-4 in PPD-sensitized mice increased the size and cellularity of PPD-induced granulomas [9]. Thus, these reports point to involvement of IL-4 in tissue damage in mycobacterial infections in mice; our observations extend this conclusion to human TB.

Another interesting finding is the expression of IL-4 in CD8⁺ T cells, which are usually viewed as CD4⁺-dependent, IFN-γ-secreting cytotoxic cells. Until now, the role of CD8⁺ T cells in human TB has been controversial. Specific CD8⁺ T cell clones, which recognize Mycobacterium tuberculosis-infected cells and react with high expression of IFN-γ, have been obtained from patients [12]. Other reports have challenged a protective role of CD8⁺ T cells. Killing of M. tuberculosis by human macrophages is dependent on CD4⁺ cells but independent of CD8⁺ cells [13]. High numbers of CD8⁺ cells in alveolar lavage of patients with TB correlated with delayed resolution of disease.
It is conceivable that CD8+ T cells exert their counterproductive role through secretion of IL-4. The Th2-type response found in this study of human TB is in agreement with that found in previous reports [2, 3]. In culture supernatants, IL-4 may not be detectable [4, 5] because production of IL-4 splice variants, or binding of secreted IL-4 to soluble or cellular receptors, interferes with this assay [15]. To circumvent this problem and to allow for cell-specific analysis of Th status in TB, we used flow cytometric detection of intracellular cytokine expression, which is unaffected by natural release of cytokine inhibitors or receptors [10].

Because protective immunity in TB depends on a cellular host response, with an important role for CD4+ T-helper cells, the Th1-Th2 concept has long been thought to be important in the outcome after infection with *M. tuberculosis*. As is the case with several other intracellular infections, a Th1-type response would be beneficial, whereas a Th2-type response would be ineffective or even harmful in TB. An essential role for Th1-type cytokines has been established in murine TB [7] and in nontuberculous mycobacterial infections in humans [6]. Assessment of Th1- and Th2-type cytokine patterns after experimental murine infection has shown high production of IFN-γ in early infection versus increased production of IL-4 during the chronic phase of infection that was characterized by progressive fibrosis and necrosis [8].

The finding of a correlation between a systemic Th2-type cytokine response and tissue necrosis in patients with pulmonary TB is instrumental in explaining the divergent manifestations of mycobacterial disease in humans. Increased understanding of the role of cytokines in determination of the delicate balance between protective immunity and immunopathology in TB may help the design of successful immunotherapy.

References


