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T-Cell Cytokine Responses in Human Infection with *Mycobacterium tuberculosis*

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Compared with healthy tuberculin reactors, *Mycobacterium tuberculosis*-stimulated peripheral blood mononuclear cells from tuberculosis patients had diminished production and mRNA expression of the Th1 cytokines gamma interferon and interleukin 2 (IL-2), with no change in production and mRNA expression for the Th2 cytokines IL-4, IL-10, and IL-13. These results were confirmed by evaluation of T cells and $CD4^+$ cells. At the level of systemic T cells, development of tuberculosis is associated with diminished Th1 but not enhanced Th2 responses.

The increasing frequency of drug-resistant tuberculosis has fueled deadly outbreaks of disease that are poorly responsive to chemotherapy (5, 9). Development of alternative immunomodulatory approaches to treatment is attractive but depends on an improved understanding of the human immune response to *Mycobacterium tuberculosis*. In patients infected with *Mycobacterium leprae*, Th1-like cells that produce gamma interferon (IFN-γ) and interleukin 2 (IL-2) predominate in tuberculoid leprosy patients with a resistant immune response, whereas Th2-like cells producing IL-4 and IL-10 are dominant in lepromatous leprosy patients with ineffective immunity (21). Most persons infected with *M. tuberculosis* are healthy tuberculin reactors with protective immunity, but a minority with ineffective immunity develop extensive pulmonary tuberculosis. To determine if the clinical manifestations of *M. tuberculosis* infection reflect alterations in the balance of T-cell cytokines, we evaluated cytokine production and mRNA expression by peripheral blood mononuclear cells (PBMCs) from healthy tuberculin reactors and pulmonary tuberculosis patients.

Patient population. Blood was obtained from 49 human immunodeficiency virus-negative patients (35 male, 14 female) with newly diagnosed sputum smear-positive pulmonary tuberculosis and from 30 healthy tuberculin reactors (21 male, 9 female). All subjects were 21 to 55 years old.

Cell preparation. PBMCs were cultured at 10^6 cells per well, with or without 10 μg of heat-killed *M. tuberculosis* Erdman per ml as described previously (3). In some cases, purified T cells (>90% $CD3^+$ by cytofluorometric analysis) were obtained by passage of PBMCs through T-cell enrichment columns (R & D Systems, Minneapolis, Minn.), and purified NK cells (>90% $CD56^+$) were obtained by immunomagnetic depletion of $CD3^+$, $CD4^+$, and $CD19^+$ cells from PBMCs.

Quantitation of cytokine mRNA. Cells stimulated with *M. tuberculosis* were harvested, and RNA was isolated by standard methods (23). Twenty-four hours was the optimal time point for expression of mRNA for IFN-γ, IL-2, IL-4, IL-10, and IL-13. In some cases, RNA was prepared from unstimulated PBMCs or from purified $CD4^+$ cells (95 to 99% $CD4^+$) isolated from *M. tuberculosis*-stimulated cells by positive immunomagnetic selection, as described previously (18).

cDNA was synthesized from RNA, and samples were normalized for CD3 cDNA by competitive PCR (23). PCR product was quantified with a gel documentation system. Aliquots containing 0.6 aM CD3 cDNA were amplified by competitive PCR with primers for cDNA of IFN-γ, IL-2, IL-4, IL-10, and IL-13. The 5′ and 3′ primers for IL-13 were AGGGAGCTC ATTGAGGAGCTGGTC and GAGCAGGTCCTTTACAAA CTGGGC, respectively. The primer sequences for the other cytokines and the PCR conditions have been published (15, 23).

Measurement of cytokine concentrations. Supernatants were harvested after 24 to 96 h. Cytokine concentrations are reported for *M. tuberculosis*-stimulated cells at the time points at which they were maximal (24 h for IL-2, IL-10, and IL-12; 48 to 72 h for IL-4; 96 h for IFN-γ). After culture in medium alone, IL-2, IL-4, and IL-12 were not detectable, and <100 pg of IFN-γ and IL-10 per ml was found in some cases. An enzyme-linked immunosorbent assay (ELISA) was used to measure concentrations of IL-2 (1), IL-4 (Genzyme, Cambridge, Mass.), IL-10 (Biosource International, Camarillo, Calif.), IL-12 (22), and IFN-γ.

Cytokine production by *M. tuberculosis*-stimulated PBMCs. We measured cytokine concentrations in supernatants of *M. tuberculosis*-stimulated PBMCs from 38 tuberculosis patients and 30 healthy tuberculin reactors. IFN-γ concentrations were reduced in tuberculosis patients ($P = 0.01$ [Fig. 1]), whereas concentrations of IL-2, IL-4, and IL-10 were similar in both groups. Reduced IFN-γ production in tuberculosis patients was not associated with reduced IL-12 production, because the concentrations of heterodimeric IL-12 were comparable in tuberculosis patients and healthy tuberculin reactors (mean of 6 versus 9 pg/ml, respectively).

Cytokine production by T cells and NK cells. IFN-γ can be produced by T cells and NK cells. When purified T cells and NK cells from nine persons were cultured with *M. tuberculosis*, mean IFN-γ concentrations were 1,089 pg/ml in T-cell supernatants but were not detectable in NK cell supernatants. In *M. tuberculosis*-stimulated T cells from six healthy tuberculin re-

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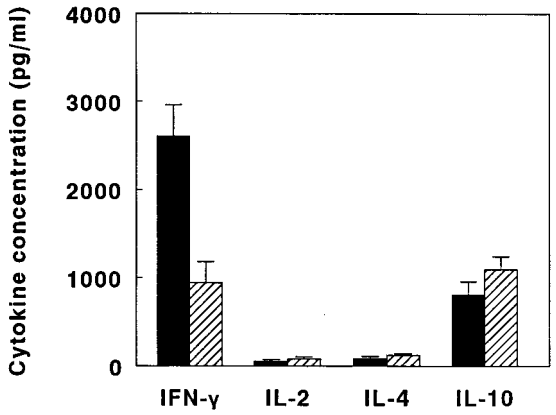

FIG. 1. Production of cytokines by PBMCs from 38 tuberculosis patients (hatched bars) and 30 healthy tuberculin reactors (solid bars). PBMCs were cocultured with heat-killed *M. tuberculosis*. Cytokine concentrations were determined in cell culture supernatants by ELISA.

actors and seven tuberculosis patients, IFN-γ concentrations were higher in healthy tuberculin reactors (mean of 2,338 ± 516 versus 492 ± 394 pg/ml; $P = 0.01$), whereas IL-4 and IL-10 concentrations were low (mean of <15 pg/ml) in both groups. These results confirm that T-cell production of IFN-γ is depressed, whereas production of Th2 cytokines is unchanged in tuberculosis patients.

Cytokine mRNA expression. IL-2 and IL-4 are difficult to detect in supernatants because they are utilized by cells during culture. We therefore used reverse transcriptase PCR to evaluate cytokine mRNA expression in *M. tuberculosis*-stimulated PBMCs from 19 tuberculosis patients and 19 healthy tuberculin reactors. mRNA expression for IFN-γ and IL-2 was reduced in tuberculosis patients ($P = 0.0005$ for IFN-γ and $P = 0.02$ for IL-2), whereas mRNA expression for IL-4, IL-10, and IL-13 was comparable in both groups (Fig. 2). These results provide additional evidence that PBMCs from tuberculosis patients have a diminished Th1 response without an enhanced Th2 response. Cytokine mRNA expression was similar in unstimulated PBMCs from 8 tuberculosis patients and 10 healthy tuberculin reactors, indicating no changes in baseline cytokine mRNA levels (data not shown).

To determine if the differences in cytokine production and mRNA expression in PBMCs and T cells reflected changes in $CD4^+$ cells, we isolated purified $CD4^+$ cells from *M. tuberculosis*-stimulated PBMCs of seven tuberculosis patients and seven healthy tuberculin reactors. mRNA expression for IFN-γ and IL-2 was reduced in tuberculosis patients ($P = 0.04$ for IFN-γ and $P = 0.02$ for IL-2), but mRNA expression for IL-4 and IL-10 was similar in both groups (Fig. 3). Thus, tuberculosis patients demonstrate reduced production of Th1 but not Th2 cytokines per $CD4^+$ cell.

Changes in cytokine production during therapy. To determine if the depressed *M. tuberculosis*-induced IFN-γ production by PBMCs of tuberculosis patients was permanent or reversible, we retested 11 patients after 9 months of antituberculosis therapy. Mean IFN-γ concentrations increased markedly after therapy, from 496 pg/ml to 2,085 pg/ml ($P = 0.001$ [Fig. 4]). In contrast, IL-10 concentrations did not change significantly (mean of 527 pg/ml initially versus 660 pg/ml after therapy).

Our data demonstrate that the clinical manifestations of human infection with *M. tuberculosis* reflect alterations in the balance of T-cell cytokines. PBMCs from tuberculosis patients

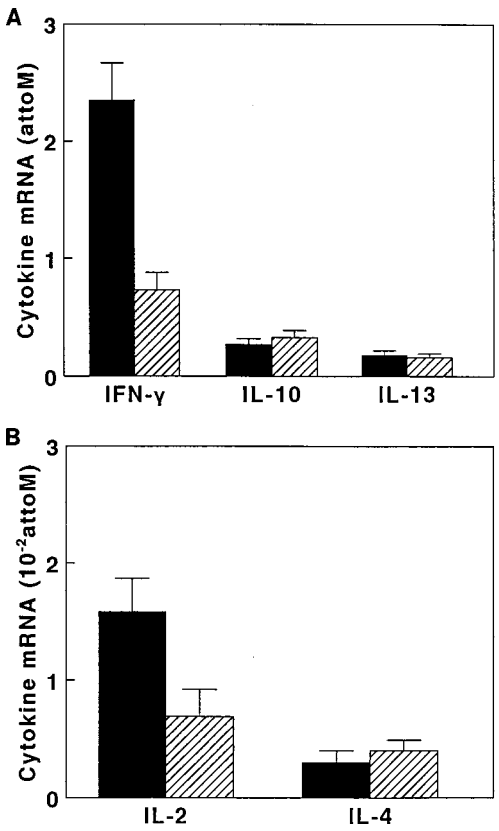

FIG. 2. Expression of cytokine mRNA by PBMCs from tuberculosis patients (hatched bars) and healthy tuberculin reactors (solid bars). PBMCs were cocultured with *M. tuberculosis*. Cytokine mRNA expression was determined by quantitative reverse transcriptase PCR, amplifying aliquots containing 0.6 aM CD3 cDNA. Expression of mRNA for IFN-γ, IL-10, and IL-13 is shown in panel A, and expression of mRNA for IL-2 and IL-4 is shown in panel B.

have reduced Th1 responses to *M. tuberculosis* compared with healthy tuberculin reactors with protective immunity, and this is not mediated by an enhanced Th2 response. Successful antituberculosis therapy is accompanied by recovery of the Th1 response, indicating that this defect is reversible.

In murine models of tuberculosis, T cells producing the Th1 cytokine IFN-γ mediate protective immunity (6, 7, 10, 14), whereas lymphocyte production of the Th2 cytokine IL-4 is associated with susceptibility to tuberculosis (12). In human tuberculosis, the role of Th1 and Th2 cells is uncertain. Some *M. tuberculosis*-reactive T-cell clones isolated from healthy tuberculin reactors are Th1-like, producing large quantities of IFN-γ but not IL-4 or IL-5 (8, 11), whereas other clones produce Th1 and Th2 cytokines, including IFN-γ, IL-4, IL-5, and IL-10 (1, 4). At the site of disease in tuberculosis, there is selective concentration of Th1 but not Th2 cells (2, 16), but it is unclear if Th1 cells contribute to protective immunity or to local immunopathology.

One approach to evaluating protective immunity to *M. tuberculosis* is to compare the immune response in healthy tuberculin reactors with protective immunity with that in pulmonary tuberculosis patients with ineffective immunity. Our results and one recent report (17) indicate that *M. tuberculosis*-induced production of the Th1 cytokines IFN-γ and IL-2 is depressed in tuberculosis patients. On the other hand, others have found that IFN-γ mRNA expression and the number of IFN-γ-producing cells are similar in PBMCs from tuberculosis

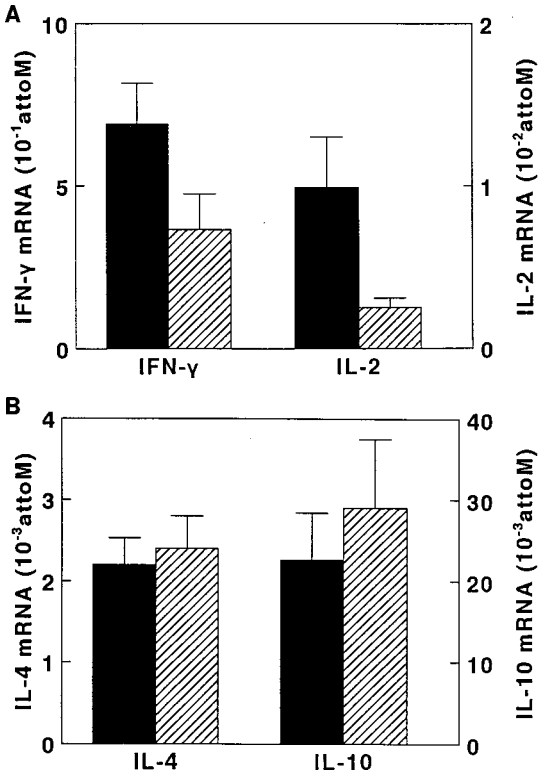

FIG. 3. Expression of mRNA for IFN-γ and IL-2 (A) and IL-4 and IL-10 (B) by $CD4^+$ cells from tuberculosis patients (hatched bars) and healthy tuberculin reactors (solid bars). PBMCs were cocultured with *M. tuberculosis*, and $CD4^+$ cells were isolated by immunomagnetic selection. Cytokine mRNA expression was determined in $CD4^+$ cells by quantitative reverse transcriptase PCR.

patients and healthy tuberculin reactors (13, 19). This discrepancy may result from the uncertain contribution of NK cells to IFN-γ production in these studies, because purified T cells were not obtained. Alternatively, the evaluation of patients after 2 to 3 months of therapy in one study (13, 13a) may have minimized the differences between groups, because IFN-γ production in tuberculosis patients would have recovered to some extent.

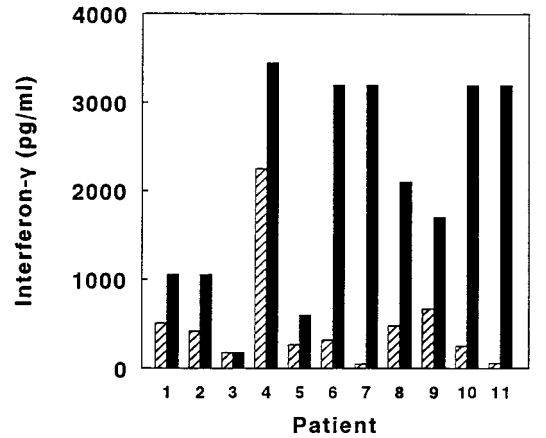

FIG. 4. Production of IFN-γ by PBMCs before (hatched bars) and after 9 months of therapy (solid bars) for tuberculosis. PBMCs were obtained from tuberculosis patients before and after antituberculosis therapy. PBMCs were cocultured with heat-killed *M. tuberculosis* and cytokine concentrations were determined by ELISA. Each bar represents results for a single patient.

We found that reduced Th1 responses in tuberculosis patients were not associated with enhanced Th2 responses. In contrast, one recent report suggested that IL-4 production by purified protein derivative (PPD)-stimulated PBMCs was greater in tuberculosis patients than in healthy tuberculin reactors (17). However, unstimulated and PPD-stimulated cells produced comparable amounts of IL-4, so that the antigen specificity of IL-4 production is uncertain in this system. Other investigators found that the frequency of IL-4-producing cells was increased in tuberculosis patients (19), but total IL-4 production was not evaluated. We believe that our findings accurately reflect the pattern of cytokine production in tuberculosis patients, because we evaluated several Th1 and Th2 cytokines at the level of mRNA expression and protein, studied purified T cells and $CD4^+$ cells, and included serial measurements of cytokine production before and after antituberculosis therapy. All of these approaches demonstrated that at the level of systemic T cells, the Th1 response is reduced, but the Th2 response is unchanged in tuberculosis patients. It is possible that Th2 responses may occur at early stages of disease prior to diagnosis. Alternatively, the Th1 response may be depressed through mechanisms independent of Th2 cells, such as dysregulation of costimulatory molecules, anergized Th1 cells, and enhanced production of immunosuppressive monokines such as transforming growth factor β (20). Further studies are needed to delineate the mechanism underlying the depressed Th1 response in human tuberculosis.

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