Cytokine Production in Children with Tuberculous Infection and Disease

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To determine if the manifestations of initial infection with Mycobacterium tuberculosis reflect changes in the balance of T cell cytokines, we evaluated cytokine production by M. tuberculosis–stimulated peripheral blood mononuclear cells (PBMCs) from 24 children with tuberculosis and 22 children who were healthy tuberculin reactors. PBMCs from patients with tuberculosis had lower production and mRNA expression of interferon γ (IFN-γ) than did PBMCs from healthy tuberculin reactors. IFN-γ production was most severely depressed in patients with moderately advanced and far-advanced pulmonary disease and in malnourished patients. Production of IL-12, IL-4, and IL-10 was similar in tuberculosis patients and healthy tuberculin reactors. These results indicate that, during the initial immune response to M. tuberculosis, development of tuberculosis is associated with diminished IFN-γ production, which is not due to reduced production of IL-12 or enhanced production of IL-4 or IL-10.

The manifestations of infection with Mycobacterium tuberculosis reflect the adequacy of the immune response. Healthy tuberculin reactors generally mount a protective immune response, as 85%–95% of them never develop tuberculosis [1]. In contrast, patients with tuberculosis have ineffective immunity [2]. IFN-γ plays a critical role in immunologic resistance to tuberculosis, as patients who lack the IFN-γ receptor are highly susceptible to mycobacterial infection [3, 4]. Furthermore, in adult patients with tuberculosis, M. tuberculosis–stimulated peripheral blood mononuclear cells (PBMCs) produce reduced amounts of IFN-γ, compared with amounts produced by PBMCs from healthy tuberculin reactors [5–7].

Most studies of the immune response in patients with tuberculosis have evaluated adults. Because most adult patients with tuberculosis were infected in the distant past, the initial response to infection cannot be assessed. Furthermore, many adult patients with tuberculosis have coexistent conditions such as alcoholism and diabetes that affect cellular immunity. To characterize the cytokine response during initial infection and to minimize the confounding effect of other medical conditions, we evaluated cytokine production by PBMCs from children with M. tuberculosis infection and disease.

Methods

Patient population. We studied 24 children with tuberculosis. The diagnosis was culture-proven for 16 patients and was based on clinical findings for the other eight patients. In these eight cases, routine bacterial cultures were negative and there was no clinical response to broad-spectrum antibiotics, but the chest radiographic and clinical abnormalities resolved with antituberculosis therapy. One patient had caseating granulomatous lymph node biopsy, 3 had household exposure to tuberculosis and chest radiographic abnormalities, 2 had mediastinal lymphadenopathy, and 1 had a left-upper-lobe infiltrate. One child had meningitis with CSF findings that were typical of tuberculosis, and the child’s grandfather had recently died of tuberculosis.

The mean (± SD, here and elsewhere in text) age of the patients was 6.0 ± 3.4 years, the mean weight was 16.0 ± 6.9 kg, and 12 patients were male. The mean tuberculin skin test size (induration) was 15.2 ± 5.9 mm, including 0 mm of induration in two patients. Twenty-four patients had pulmonary tuberculosis, one had meningitis, and one had miliary disease. All patients were seronegative for HIV by ELISA, and all had received <7 days of antituberculosis therapy, prior to providing blood for the study. Seven patients had minimal disease evident on a chest radiograph, and 17 patients had moderately advanced or far-advanced disease, on the basis of standard criteria [8]. Ten patients were malnourished, defined as being <75% of their predicted body weight for age.

We studied 22 healthy HIV-seronegative tuberculin reactors, who were children without tuberculosis presenting to the hospital with minor complaints. The mean age was 6.6 ± 3.8 years, the mean weight was 17.0 ± 6.6 kg, and 10 children were male. The mean tuberculin skin test induration size was 16.2 ± 3.3 mm. Thirteen healthy tuberculin reactors were malnourished.

Stimulation of mononuclear cells with M. tuberculosis. PBMCs were isolated by standard techniques [6] and cultured...
at 2 × 10^6 cells/mL in triplicate in medium alone or with heat-killed M. tuberculosis Erdman (10 μg/mL), PPD (10 μg/mL; Fisheries Department, Weybridge, United Kingdom), or phytohemagglutinin (10 μg/mL; Wellcome Diagnostics, Dartford, United Kingdom).

**Measurement of cytokine concentrations.** Supernatants from cultured cells were harvested after 24, 48, 72, and 96 hours and stored at −70°C. Cytokine concentrations were measured by ELISA (IL-12: Hoffmann-LaRoche, Nutley, NJ; IFN-γ: Genentech, South San Francisco, CA; IL-4 and IL-10: Pharmingen, San Diego, CA). Preliminary experiments revealed that IFN-γ concentrations in supernatants of M. tuberculosis–stimulated PBMCs were maximal after 96 hours of culture and are reported for that time point. Concentrations of IL-10 and IL-12 were maximal after 48 hours. IL-4 was not detected at any time points. Cytokine concentrations are shown as those in supernatants of M. tuberculosis–stimulated cells, minus those in supernatants of cells cultured in medium alone.

**Measurement of IFN-γ cDNA by competitive PCR.** Cells stimulated with M. tuberculosis were harvested after 24 hours, which is the optimal time point for expression of IFN-γ mRNA [6]. Cells were lysed and stored at −20°C, cellular RNA was extracted, and cDNA was synthesized as previously described [6]. To evaluate production of IFN-γ, which is produced predominantly by T cells, we normalized samples for CD3 cDNA content by competitive reverse transcription–PCR [9]. Aliquots containing equal amounts of CD3 cDNA were amplified by PCR with primers specific for IFN-γ, and the PCR product was quantitated by densitometry, as previously described [9].

**Statistical analysis.** Values are expressed as the mean and standard deviation. Student’s t test was used to compare cytokine concentrations in two groups.

**Results**

**IFN-γ production.** IFN-γ concentrations in M. tuberculosis–stimulated PBMCs were higher for healthy tuberculin reactors than for patients with tuberculosis (1,363 ± 1,549 pg/mL vs 465 ± 632 pg/mL; P = .02) (figure 1). Similar results were obtained when PBMCs were exposed to PPD (1,057 ± 1,410 pg/mL vs 302 ± 289 pg/mL; P = .02). This reduction in IFN-γ production was antigen-specific, as stimulation of PBMCs with the mitogen phytohemagglutinin yielded similar IFN-γ concentrations in eight patients with tuberculosis and eight healthy tuberculin reactors (1,634 ± 913 pg/mL and 1,552 ± 780 pg/mL, respectively; P = NS).

**Clinical findings and IFN-γ production.** We wished to determine if IFN-γ production in patients with tuberculosis was related to radiographic extent of disease or to nutritional status. In PPD-stimulated PBMCs, the mean IFN-γ concentration in 17 patients with moderately or far-advanced disease was lower than that in seven children with minimal disease (215 ± 56 pg/mL vs 534 ± 145 pg/mL; P = .02). IFN-γ production was also reduced in 10 malnourished patients, compared with that in 14 patients who were not malnourished (175 ± 69 pg/mL vs 407 ± 94 pg/mL; P = .03). There was no significant correlation between IFN-γ production and nutritional status in healthy tuberculin reactors, nor was there one between IFN-γ concentrations and age or sex of the patients or healthy tuberculin reactors (data not shown).

**IFN-γ mRNA expression.** To determine if reduced IFN-γ production in patients with tuberculosis was mediated by changes in mRNA expression, we evaluated mRNA expression for IFN-γ in M. tuberculosis–stimulated PBMCs. mRNA was successfully isolated from the PBMCs of five patients with tuberculosis and five healthy tuberculin reactors. Among these individuals, IFN-γ mRNA expression was substantially lower in patients with tuberculosis than in healthy tuberculin reactors (figure 2), suggesting that production of IFN-γ in patients with tuberculosis was diminished through decreased transcription of the IFN-γ gene or through reduced stability of IFN-γ mRNA.

**Production of IL-4, IL-10, and IL-12.** IL-4 was not detected in any supernatants of M. tuberculosis–stimulated PBMCs. PBMCs from 10 patients with tuberculosis and 12 healthy tuberculin reactors produced similar concentrations of IL-10 upon exposure to M. tuberculosis Erdman (1,476 ± 1,758 pg/mL and 881 ± 879 pg/mL, respectively; P = NS) or to PPD (746 ± 630 pg/mL and 768 ± 524 pg/mL, respectively; P = NS). PBMCs from 10 patients with tuberculosis and 10 healthy tuberculin reactors produced similar concentrations of IL-12 p40 upon stimulation with heat-killed M. tuberculosis Erdman (316 ± 261 pg/mL and 260 ± 354 pg/mL, respectively; P = NS) or to PPD (179 ± 165 pg/mL and 293 ± 414 pg/mL, respectively; P = NS).

**Discussion**

The data presented in this report demonstrate that the clinical manifestations of infection with M. tuberculosis during the
initial immune response in children reflect alterations in the balance of T cell cytokines. Specifically, *M. tuberculosis*–stimulated PBMCs from patients with tuberculosis with ineffective immunity have lower production and mRNA expression of IFN-γ than do those from healthy tuberculin reactors with protective immunity. IFN-γ production is most severely depressed in patients with extensive disease and malnutrition. The depressed production of IFN-γ in tuberculosis is not due to reduced production of IL-12, nor to enhanced production of IL-4 and IL-10.

In animals, IFN-γ plays a central role in mediating protection against tuberculosis, as mice with a disrupted IFN-γ gene cannot control infection with *M. tuberculosis* or *Mycobacterium bovis* [10, 11]. In humans, IFN-γ is also critical for immunity against tuberculosis. Children who lack the IFN-γ receptor are susceptible to severe mycobacterial infections [3, 4]. Furthermore, aerosolized IFN-γ reduced the bacterial burden in patients with multidrug-resistant tuberculosis who were not responding to chemotherapy [12]. Finally, the clinical manifestations of *M. tuberculosis* infection and disease in adults correlate with *M. tuberculosis*–induced IFN-γ production by PBMCs, which is highest in healthy tuberculin reactors, intermediate in patients with moderately advanced tuberculosis, and lowest in patients with far-advanced disease [5, 6, 13].

Evaluation of the immune response in children with *M. tuberculosis* infection and disease is logistically problematic, because tuberculosis in children is usually not confirmed by culture and because limited numbers of PBMCs can be obtained from children. In our study, 67% of the patients had culture-confirmed tuberculosis, compared with 22%–39% in other series [14, 15]. In addition, there was convincing evidence of tuberculosis in culture-negative patients. Prior studies suggest that fewer than 10% of children with tuberculosis have reactivation disease [14, 16], and most of these children are adolescents. As only three of 24 patients in our study were of age 12 years or older, it is likely that virtually all of our patients had primary tuberculosis.

We found that *M. tuberculosis*–induced IFN-γ production by PBMCs during the initial immune response to *M. tuberculosis* correlates strongly with clinical outcome. IFN-γ production was lower in patients with tuberculosis than in healthy tuberculin reactors and was lowest in patients with more severe disease. Mitogen-stimulated production of IFN-γ was normal in patients with tuberculosis, suggesting no intrinsic defect in the capacity of PBMCs to produce IFN-γ. Because of limitations in the number of PBMCs and in the quality of mRNA, production of some cytokines and IFN-γ mRNA expression could be evaluated in only a subset of study subjects, so our results should be interpreted with some caution. However, we believe it unlikely that systematic bias was introduced.

In patients with extensive disease from *Mycobacterium leprae*, depressed IFN-γ production probably results from enhanced production of the Th2 cytokines IL-4 and IL-10, which inhibit development of T cells that produce IFN-γ [17]. In tuberculosis, reduced IFN-γ production is not associated with an enhanced Th2 response [18]. Our findings confirm and extend these observations, demonstrating that depressed IFN-γ production during the initial immune response to *M. tuberculosis* is not associated with enhanced production of IL-4 or IL-10, nor with reduced production of IL-12, which is a central initiator of Th1 responses [19]. Additional studies are needed to delineate the mechanisms underlying the depressed Th1 response in children and adults with tuberculosis.

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References


