

## Absence of a Prominent Th2 Cytokine Response in Human Tuberculosis

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**Depressed Th1 responses are a prominent feature of human tuberculosis, but an enhanced Th2 response has not been detected in peripheral blood T cells stimulated in vitro with *Mycobacterium tuberculosis*. In disease due to *Mycobacterium leprae*, Th2 cells predominate in tissue lesions of patients with extensive disease but are absent from peripheral blood. To determine if Th2 cells are present in tissue lesions of tuberculosis patients, we evaluated patterns of cytokine expression in lymph nodes from tuberculosis patients with or without human immunodeficiency virus infection and in controls without tuberculosis. Gamma interferon and interleukin-10 (IL-10) mRNA expression in tuberculosis patients with or without human immunodeficiency virus infection was high, whereas IL-4 expression in the same patients was low. Immunolabeling studies showed that macrophage production of IL-12 was increased in lymph nodes from tuberculosis patients, that gamma interferon was produced by T cells, and that IL-10 was produced by macrophages rather than Th2 cells. These results indicate that Th2 responses are not enhanced either systemically or at the site of disease in human tuberculosis.**

Immunologic resistance and susceptibility to intracellular pathogens are mediated by CD4<sup>+</sup> T cells with specific patterns of cytokine secretion. In murine models, Th1 cells that produce gamma interferon confer resistance to infection with mycobacteria and members of the genus *Leishmania* (13, 19, 22), whereas Th2 cells that produce interleukin-4 (IL-4) exacerbate disease due to *Leishmania* spp. (13, 22). In patients infected with *Mycobacterium leprae*, Th1 cells predominate in tissue lesions of tuberculoid leprosy patients with a resistant immune response, whereas Th2 cells are dominant in lesions from lepromatous leprosy patients with ineffective immunity (30).

Healthy tuberculin reactors are a useful model of protective immunity against *Mycobacterium tuberculosis*, as they are infected but remain well and are resistant to exogenous reinfection (26). Human immunodeficiency virus (HIV)-negative tuberculosis patients have serious local disease and ineffective immunity, and HIV-infected tuberculosis patients have very extensive disease, which frequently affects multiple organs (1a). Production of Th1 cytokines by *M. tuberculosis*-stimulated peripheral blood mononuclear cells (PBMC) is highest in healthy tuberculin reactors, intermediate in HIV-negative tuberculosis patients, and lowest in HIV-infected tuberculosis patients. We have not found enhanced production of Th2 cytokines in PBMC from HIV-negative or HIV-infected tuberculosis patients (32, 33), although others have noted an increased frequency of IL-4-producing cells in some tuberculosis patients (27). It is possible that our experimental system does not permit sensitive detection of Th2 cells. Alternatively, Th2 cells may be sequestered at the site of disease in patients with severe tuberculosis. To investigate these possibilities, we evaluated production of Th2 cytokine IL-4 by PBMC under experimental conditions that favored development of Th2 cells. In

addition, we investigated cytokine production and mRNA expression in lymph nodes of patients with tuberculous lymphadenitis, a moderately severe manifestation of tuberculosis.

### MATERIALS AND METHODS

**Patient population.** Blood was obtained from 15 patients upon their first episode of culture-proven pulmonary tuberculosis. All patients had positive acid-fast smears of sputum and had received less than 2 weeks of antituberculosis therapy. Fourteen patients had negative enzyme-linked immunosorbent assay (ELISA) tests for HIV antibody, and one was infected with HIV. Blood was also obtained from 11 healthy tuberculin reactors who had been immunized with *Mycobacterium bovis* BCG in childhood.

Frozen lymph node tissue samples were obtained from 10 HIV-negative patients and 10 HIV-infected patients with tuberculous lymphadenitis. *M. tuberculosis* was cultured from the lymph nodes of 8 of 10 HIV-negative patients and 10 of 10 HIV-infected patients. In the two HIV-negative patients with negative cultures, such characteristic changes as caseous necrosis and granulomatous inflammation were present in combination with a favorable response to antituberculosis chemotherapy. All lymph node specimens were obtained within 4 weeks of the beginning of the administration of antituberculosis medications. Tissue samples were also obtained from 10 healthy adults whose lymph nodes showed benign follicular hyperplasia and from 6 HIV-infected patients whose lymph nodes showed florid follicular hyperplasia. Follow-up of these six patients for 12 to 36 months showed no evidence of tumors or opportunistic infection in the lymph nodes.

**Cytokine production in response to live *M. tuberculosis*.** PBMC were isolated by differential centrifugation over Ficoll-Paque (Pharmacia Fine Chemicals, Piscataway, N.J.) and plated in 200- $\mu$ l wells at  $2 \times 10^5$  cells per well in RPMI (GIBCO, Grand Island, N.Y.) and 10% heat-inactivated human serum. Live *M. tuberculosis* H37Ra ( $2 \times 10^5$ ) was then added to each well, and the cells were cultured for 4 days at 37°C in an atmosphere containing 5% CO<sub>2</sub>. Supernatants were harvested and frozen at -70°C.

**Expansion of *M. tuberculosis*-reactive T cells in the presence of IL-4.** PBMC were plated in 2-ml wells at  $10^6$  cells per ml in RPMI containing penicillin-streptomycin (GIBCO), 10% heat-inactivated human serum, heat-killed whole washed *M. tuberculosis* Erdman (10  $\mu$ g/ml), and recombinant IL-4 (2,500 U/ml [a kind gift from Schering Plough Research Institute, Kenilworth, N.J.]). After 6 days of culture at 37°C in an atmosphere containing 5% CO<sub>2</sub>, the cells were centrifuged over Ficoll-Paque to enrich for lymphoblasts, and 10% IL-2 (Schiparelli Biosystems, Inc., Columbia, Md.) was added. After 6 days of culture, cells ( $10^6$ /ml) were cultured with 10 ng of phorbol myristate acetate (Sigma, St. Louis, Mo.) per ml and 1  $\mu$ M ionomycin (Sigma) for 24 h. Supernatants were harvested and frozen at -70°C.

**Measurement of cytokine concentrations by ELISA.** Cytokine concentrations were measured by ELISA. Antibodies used for the measurement of gamma

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interferon (detection limit, 25 pg/ml) were kindly provided by Genentech, South San Francisco, Calif. Antibodies to measure IL-4 and IL-10 (Pharmingen, San Diego, Calif.) were used according to the manufacturer's instructions. The detection limits for these assays were 10 and 20 pg of IL-4 and IL-10 per ml, respectively.

**Isolation of RNA from lymph nodes and cDNA synthesis.** Cryostat sections that were 6  $\mu$ m thick were lysed with 4 M guanidinium isothiocyanate and stored at  $-20^{\circ}\text{C}$  prior to the preparation of RNA, which was isolated by phenol-chloroform extraction as described previously (2). Complementary DNA was synthesized from RNA by standard methods (33).

**Quantification of cytokine cDNA content by competitive PCR.** To accurately compare different lymph node samples in terms of cytokine mRNA expression, it is critical to use equivalent amounts of substrate cDNA. In evaluating cytokines produced predominantly by T cells, we normalized all samples for CD3 cDNA content by competitive PCR as previously described (33). Briefly, the target CD3 sequence and a competitor DNA construct were coamplified with the same pair of primers, and the PCR product was subjected to electrophoresis and visualized by staining with ethidium bromide. For the quantification of the PCR product, gels were photographed with an imaging and analysis system that permits accurate comparisons of the integrated densities of the PCR product bands for target and competitor cDNA. When the ratio of the integrated density of the sample to that of the competitor PCR product is plotted against the known amount of competitor substrate cDNA, the amount of substrate CD3 cDNA in each sample can be calculated. This method allows for the accurate detection of twofold differences in substrate cDNA concentrations (32, 33).

For each sample, aliquots containing 0.6 amol of CD3 cDNA were used as substrate and were amplified by PCR with primers specific for the cDNA of gamma interferon, IL-2, IL-4, and IL-10. The primer sequences and the reaction conditions for the amplification of cytokine cDNA have been previously published (20, 33). Serial dilutions of competitor cDNA were added to each reaction, and substrate cytokine cDNA was quantitated as outlined above for CD3 cDNA.

**Immunohistochemistry of lymph node tissue.** Cryostat sections (5  $\mu$ m thick) of lymph nodes were mounted on poly-L-lysine-coated slides (Sigma). For staining with anti-IL-12, the sections were fixed in cold acetone for 5 min. For staining with anti-IL-10 and anti-gamma interferon, the sections were fixed in 4% paraformaldehyde (Sigma), pH 7.4, for 10 to 15 min, washed three times in 0.01 M phosphate-buffered saline (PBS; Sigma), pH 7.4, air dried, and stored at  $-20^{\circ}\text{C}$  prior to immunostaining.

The primary antibodies used were mouse anti-human CD3 (Leu 4 [immunoglobulin G1 (IgG1)]; Becton-Dickinson Monoclonal Center, San Jose, Calif.), mouse anti-human CD14 (LeuM3 [IgG2b]; Becton-Dickinson Monoclonal Center, and MO2 [IgM]; Coulter Immunology, Hialeah, Fla.), murine anti-human gamma interferon (IgG2a; Genzyme Diagnostics, Cambridge, Mass.), rat anti-human IL-10 (JES3-19F1 and JES3-12G8 [IgG2a]; Pharmingen), and rat anti-human IL-12 (20C2 [IgG1]; kindly provided by Maurice Gately, Hoffmann-La Roche, Nutley, N.J.). Irrelevant isotype-matched monoclonal antibodies (Zymed Laboratories Inc., South San Francisco, Calif.) or myeloma-purified rat IgG (ICN Immunologicals, Costa Mesa, Calif.) was used as controls for nonspecific staining.

For single staining with anti-IL-12, endogenous peroxidase in tissue sections was blocked with 0.3%  $\text{H}_2\text{O}_2$  for 5 min and nonspecific binding was blocked with 1.5% normal rabbit serum for 15 min. The sections were then incubated with rat anti-IL-12 (120  $\mu\text{g}/\text{ml}$ ) for 2 h and then with a 1:250 dilution of horseradish peroxidase-labeled rabbit anti-rat IgG (Sigma) for 1 h and developed with 3-amino-9-ethylcarbazole (AEC substrate kit; Vector Laboratories, Burlingame, Calif.) for 10 to 15 min. The slides were washed with PBS three times between each step. For double staining with anti-CD14, the slides were incubated with 40  $\mu\text{g}$  of biotinylated mouse anti-human CD14 (MO2) per ml overnight at  $4^{\circ}\text{C}$ . The next day, a 1:50 dilution of avidin-biotinylated alkaline phosphatase complex (Vectastain ABC-AP kit; Vector Laboratories) was added for 1 h at room temperature and then developed with Vector-blue (alkaline phosphatase substrate kit III; Vector Laboratories) for 20 to 30 min.

For single staining with anti-gamma interferon- and anti-IL-10, 0.1% saponin (Sigma) was added at all incubation and washing steps to increase the permeability of the cell membrane and facilitate the detection of intracellular cytokine. The sections were rehydrated for 10 min in a balanced salt solution that contained calcium and magnesium and that was supplemented with 0.01 M HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer (GIBCO), and then they were incubated with 0.1% saponin-balanced salt solution for 5 min. The sections were blocked with 1%  $\text{H}_2\text{O}_2$  for 15 min, incubated overnight at  $4^{\circ}\text{C}$  with mouse anti-gamma interferon (5  $\mu\text{g}/\text{ml}$ ) or rat anti-IL-10 (10  $\mu\text{g}$  of JES3-19F1 per ml and 10  $\mu\text{g}$  of JES3-12G8 per ml), incubated with 1% normal horse or goat serum for 15 min, incubated with biotinylated horse anti-mouse (for gamma interferon) or goat anti-rat (for IL-10) IgG (15  $\mu\text{g}/\text{ml}$ ; Vector Laboratories) for 30 min, and then developed with 3-amino-9-ethylcarbazole or diaminobenzidine tetrahydrochloride. The slides were rinsed three times in saponin-balanced salt solution between each of the steps described above. Finally, the slides were counterstained with hematoxylin for 1 min and mounted.

For double staining, the sections were stained with anti-cytokine antibodies as outlined above and then rehydrated with PBS, blocked with 1.5% normal horse

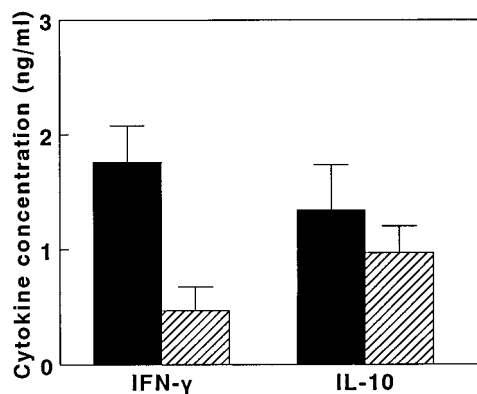


FIG. 1. Production of cytokines by PBMC from tuberculosis patients (hatched bars) and healthy tuberculin reactors (solid bars) after coculture with live *M. tuberculosis*. PBMC were cocultured with live *M. tuberculosis* H37Ra. Cytokine concentrations in cell culture supernatants were determined by ELISA. Error bars show standard errors. IFN- $\gamma$ , gamma interferon.

serum for 15 min, and incubated with either mouse anti-CD3 (1:40) or mouse anti-CD14 (LeuM3; 1:20) for 30 min. This step was followed by the addition of a 1:100 dilution of biotinylated horse anti-mouse IgG (15  $\mu\text{g}/\text{ml}$ ) for 30 min and a 1:50 dilution of avidin-biotinylated alkaline phosphatase complex for 30 min and development with Vector-blue for 20 to 30 min.

**Immunolabeling of *M. tuberculosis*-stimulated PBMC.** PBMC at  $10^6$  cells per ml were plated in 2-ml wells containing RPMI supplemented with penicillin-streptomycin, 10% heat-inactivated human serum, and heat-killed *M. tuberculosis* Erdman (10  $\mu\text{g}/\text{ml}$ ). The wells were incubated at  $37^{\circ}\text{C}$  in an atmosphere containing 5%  $\text{CO}_2$ . After 24, 48, or 96 h, cytocentrifuge preparations were prepared with a Shandon Cytospin III (Shandon, Inc., Pittsburgh, Pa.), and slides were air dried and fixed in acetone for 5 min. Single staining with anti-IL-10 and double staining with anti-IL-10 and either anti-CD3 or anti-CD14 were performed as outlined above for tissue sections, except that the concentration of anti-IL-10 antibodies used was 20  $\mu\text{g}/\text{ml}$  and blocking with  $\text{H}_2\text{O}_2$  was not done.

**Statistical analysis.** Continuous variables were compared with one another by Student's *t* test. Variables that were not normally distributed (IL-4 mRNA expression and the percentages of gamma interferon-producing cells in lymph nodes) were compared with each other by the nonparametric Wilcoxon rank-sum test.

## RESULTS

**Cytokine production in response to live *M. tuberculosis*.** We have previously shown that PBMC that were taken from tuberculosis patients and that were cocultured with heat-killed *M. tuberculosis* Erdman reduced the production of Th1 cytokines but did not enhance the production of Th2 cytokines IL-4 and IL-10 (33). We considered the possibility that live mycobacteria more effectively induced a Th2 response. We therefore measured cytokine production by PBMC that were taken from 12 HIV-negative tuberculosis patients and 11 healthy tuberculin reactors and that were cocultured with live *M. tuberculosis*. Concentrations of gamma interferon in supernatants from healthy tuberculin reactors were significantly higher than those from tuberculosis patients ( $1,760 \pm 333$  pg/ml versus  $474 \pm 218$  pg/ml [ $P = 0.004$ ]) (Fig. 1). Concentrations of IL-10 were similar in both groups ( $1,339 \pm 421$  pg/ml versus  $970 \pm 242$  pg/ml [ $P = 0.44$ ]) (Fig. 1). IL-4 was not detectable in supernatants from either group. These results suggest that live *M. tuberculosis* does not elicit enhanced production of Th2 cytokines by peripheral blood lymphocytes from tuberculosis patients.

**Cytokine production by *M. tuberculosis*-reactive cells expanded with IL-4.** To facilitate the expansion of *M. tuberculosis*-reactive Th2 cells, we established short-term T-cell lines in the presence of IL-4, which favors the development of Th0 and Th2 cells (16). PBMC from four healthy tuberculin reactors and eight HIV-negative tuberculosis patients were cocultured

with heat-killed *M. tuberculosis* and IL-4 for 6 days and then were expanded with IL-2, as outlined in Materials and Methods. When these cells were restimulated with *M. tuberculosis*, IL-4 was not detectable in any supernatants. When the cells were restimulated with phorbol myristate acetate and ionomycin, mean IL-4 concentrations in supernatants of T-cell lines derived from healthy tuberculin reactors were similar to those in supernatants derived from tuberculosis patients (means of  $183 \pm 105$  pg/ml versus  $157 \pm 120$  pg/ml, respectively [ $P = 0.89$ ]). These results suggest that Th2-like cells are not more readily expanded from peripheral blood of tuberculosis patients.

**Cytokine mRNA expression in lymph nodes.** In lepromatous leprosy patients, Th2 cytokines are prominent in tissue lesions (30), but *M. leprae*-stimulated PBMC do not produce increased amounts of IL-4 or IL-10 (1, 24). To investigate the possibility that Th2 cytokines predominate at the site of disease in tuberculosis, we compared cytokine mRNA expression in lymph nodes from HIV-negative tuberculosis patients with that in healthy controls whose lymph nodes showed histologic evidence of benign follicular hyperplasia. Because lymphadenitis in tuberculosis patients with HIV coinfection is particularly severe and often associated with widely disseminated disease, we hypothesized that these patients were most likely to demonstrate enhanced Th2 responses. We therefore determined cytokine mRNA expression in lymph nodes from HIV-infected tuberculosis patients. As additional controls, we also evaluated HIV-infected patients whose lymph nodes showed florid reactive hyperplasia without clinical or histologic evidence of opportunistic infections or tumors.

Cytokine mRNA expression was quantitated by competitive reverse transcriptase PCR. For each sample, 0.6 amol of CD3 cDNA was amplified by PCR with cytokine-specific primers. mRNA expression for gamma interferon in HIV-negative tuberculosis patients was increased threefold compared with that in HIV-negative controls ( $P = 0.0003$ ) (Fig. 2A), but IL-2 mRNA was similar in both groups ( $P = 0.45$ ). IL-4 mRNA was decreased ( $P = 0.005$ ) (Fig. 2B), but IL-10 mRNA in HIV-negative tuberculosis patients was increased two- to threefold compared with that in HIV-negative controls ( $P = 0.007$ ). Thus, mRNA expression of gamma interferon and IL-10 in HIV-negative tuberculosis patients was increased, but mRNA expression for IL-4 in these patients was decreased.

In HIV-infected patients, mRNA expression for gamma interferon in tuberculosis patients was also increased compared with that in HIV-infected patients without tuberculosis ( $P = 0.05$ ) (Fig. 2A), but mRNA expression for IL-2 was similar for both groups. IL-4 mRNA expression in HIV-infected tuberculosis patients was reduced ( $P = 0.04$ ) (Fig. 2B) and IL-10 mRNA expression for the same group was increased compared with that in HIV-negative controls ( $P = 0.008$ ). These results indicate that the changes in cytokine mRNA expression induced by tuberculosis are similar for patients with or without HIV coinfection.

**Cytokines in lymph nodes.** Because mRNA expression for gamma interferon and IL-10 was enhanced in tuberculosis patients, we wished to identify the cellular source of these cytokines. Single staining with anti-gamma interferon antibodies was performed with lymph nodes from five HIV-negative controls, five HIV-negative tuberculosis patients, three HIV-infected tuberculosis patients, and three HIV-infected controls. Most gamma interferon-positive cells were in the interfollicular areas where T cells usually predominate. The percentages of positively staining cells for five fields were calculated for each lymph node specimen. For these areas, the mean percentages of gamma interferon-positive cells were

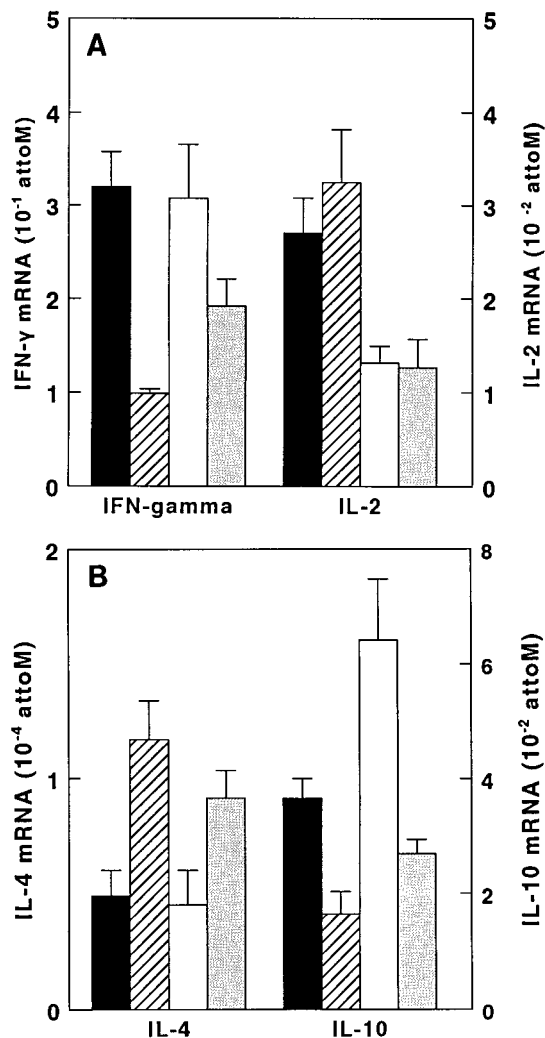


FIG. 2. Expression of cytokine mRNA in lymph nodes from HIV-negative and HIV-infected persons with or without tuberculosis. Lymph nodes were obtained from HIV-negative tuberculosis patients (solid bars), healthy HIV-negative persons without tuberculosis (hatched bars), HIV-infected tuberculosis patients (open bars), and HIV-infected patients without tuberculosis (shaded bars). Cytokine mRNA expression was determined by quantitative reverse transcriptase PCR after normalization for CD3 cDNA content. Expression of mRNA for gamma interferon (IFN- $\gamma$ ) and IL-2 is shown in panel A, and expression of mRNA for IL-4 and IL-10 is shown in panel B. Error bars show standard errors.

$20\% \pm 18\%$  for HIV-negative controls and  $81\% \pm 12\%$  for HIV-negative tuberculosis patients ( $P < 0.001$ ). Among HIV-infected patients, the mean percentages of gamma interferon-positive cells were  $24\% \pm 11\%$  for controls and  $76\% \pm 10\%$  for tuberculosis patients ( $P < 0.0001$ ). In HIV-negative and HIV-infected tuberculosis patients, most gamma interferon-positive cells were in granulomas, which were more discrete and well-formed in HIV-negative patients. In the HIV-negative and HIV-positive controls without tuberculosis, the gamma interferon-positive cells were not organized in granulomas and were scattered more diffusely in the lymph node.

To determine the phenotype of the cells producing gamma interferon, double immunolabeling with anti-gamma interferon and anti-CD3 was performed in lymph nodes from two HIV-negative tuberculosis patients and one HIV-infected tuberculosis patient. In these patients, 81 to 85% of the gamma interferon-positive cells were CD3+, indicating that T cells are



FIG. 3. Double immunolabeling with anti-cytokine antibodies and anti-CD3 or anti-CD14 in lymph nodes (A and B) and *M. tuberculosis*-stimulated PBMC (C and D) from an HIV-negative tuberculosis patient. (A) Staining with anti-gamma interferon (red) and anti-CD3 (blue). Double-stained cells are purple. Magnification,  $\times 260$ . (B) Staining with anti-IL-12 (red) and anti-CD14 (blue). Arrows show two purple double-stained cells. Magnification,  $\times 650$ . (C) Staining with anti-IL-10 (red) and anti-CD14 (blue). The single-stained CD14+ cell is blue (single arrow), and double-stained CD14+ IL-10-positive cells are purple (double arrows). Magnification,  $\times 260$ . (D) Staining with anti-IL-10 (red) and anti-CD3 (blue). Arrows show red single-staining IL-10-positive cells and blue CD3+ cells. Magnification,  $\times 260$ .

the major source of gamma interferon in tuberculous lymph nodes. Figure 3A shows examples of purple CD3+ gamma interferon-positive cells.

Because Th1-like gamma interferon-producing cells were prominent in the lymph nodes of tuberculosis patients, we wished to determine if this resulted from IL-12 production, as IL-12 is a potent stimulus for differentiation of Th1 cells (15, 17, 18). Immunolabeling with anti-IL-12 was performed in lymph nodes from three HIV-negative controls, five HIV-negative tuberculosis patients, three HIV-infected controls, and six HIV-infected tuberculosis patients. IL-12-producing cells were rare in nodes from HIV-negative and HIV-infected controls. In contrast, in nodes from HIV-negative and HIV-infected tuberculosis patients, many IL-12-positive cells were found in the subcapsular area, where the mean numbers of IL-12-positive cells per field were  $30 \pm 10$  and  $26 \pm 9$ , respectively ( $P = 0.24$ ). Double immunolabeling with anti-IL-12 and anti-CD14 was then performed in nodes from tuberculosis patients. For each node, cells from three randomly selected fields in the subcapsular area from four consecutive sections were counted and the number of double-stained cells was compared with the number of single-stained CD14+ cells. Examples of typical purple CD14+ IL-12-positive cells are shown in Fig. 3B. The mean percentages of CD14+ cells that were also IL-12-positive were  $39\% \pm 6\%$  for HIV-negative tuberculosis patients and  $37\% \pm 4\%$  for HIV-infected tuberculosis patients

( $P = 0.82$ ). Essentially all of the IL-12-positive cells were CD14+, indicating that macrophages are the dominant source of IL-12 in the local immune response to *M. tuberculosis*.

**Cellular source of IL-10.** IL-10 mRNA expression was increased in tuberculosis patients with or without HIV infection and could be produced either by T cells or macrophages. To distinguish these possibilities, we attempted to perform double immunolabeling with anti-IL-10 in combination with anti-CD3 or anti-CD14. Immunolabeling with anti-IL-10 yielded scattered diffuse staining that did not permit identification of the cellular source of this cytokine. As a surrogate means to identify the IL-10-producing cells, we stimulated PBMC from one HIV-negative and one HIV-infected tuberculosis patient with *M. tuberculosis* in vitro and stained cytocentrifuge preparations after 1, 2, and 4 days of culture. Maximal staining for IL-10 was seen after 4 days of culture. Approximately 90% of the IL-10-producing cells were CD14+. Examples of purple CD14+ IL-10-positive cells are shown in Fig. 3C. Staining with anti-IL-10 and anti-CD3 revealed no double-stained cells (Fig. 3D). These results indicate that macrophages were the dominant source of IL-10.

## DISCUSSION

The data presented in this report provide evidence that Th2 cytokine responses are not enhanced either in the peripheral blood or at the site of disease in tuberculosis patients, includ-



ing those who are coinfecting with HIV. Coculture of peripheral blood cells with IL-4 or with live mycobacteria did not markedly expand Th2 cells, and cytokine mRNA for IL-4 was depressed in lymph nodes from tuberculosis patients. The most prominent cytokines in tuberculous lymph nodes were gamma interferon produced by T cells as well as IL-10 and IL-12 produced by macrophages.

Human infection with *M. tuberculosis* displays a spectrum of manifestations that reflect the efficacy of the immune response. At one end of the spectrum, most healthy tuberculin reactors manifest protective immunity and remain well, the only evidence of infection being a positive tuberculin skin test. HIV-negative patients with tuberculous lymphadenitis have ineffective immunity and severe local disease that generally does not spread to other sites. HIV-infected patients with tuberculous lymphadenitis have the most severe manifestations of disease, with frequently disseminated disease and high mortality rates (1a). Production of mRNA expression for Th1 cytokines gamma interferon and IL-2 by *M. tuberculosis*-stimulated PBMC correlate well with the clinical manifestations of tuberculous infection. Production of Th1 cytokines is highest in healthy tuberculin reactors, intermediate in HIV-negative tuberculosis patients, and lowest in HIV-infected tuberculosis patients (32, 33). Our current findings indicate that in contrast to the depressed Th1 response of *M. tuberculosis*-stimulated PBMC from tuberculosis patients, the local immune response in tuberculosis is characterized by enhanced T-cell production of gamma interferon but not IL-2 or IL-4 and that this response is similar for HIV-infected and HIV-negative patients. These results confirm and extend previous observations demonstrating a prominent Th1 cytokine response at the site of disease in HIV-negative patients with pleural (4) and pulmonary (21) tuberculosis. Macrophage IL-12 production was prominent in tuberculous lymph nodes, and IL-12 favors development of Th1 responses in several experimental systems, including that with mice infected with *M. tuberculosis* (6, 12, 14, 15, 18, 23, 25, 28). These data strongly suggest that IL-12 is a central initiator of the local Th1 response in human tuberculosis. Direct infection of lymph node macrophages with *M. tuberculosis* may lead to IL-12 production, as human macrophages exposed to *M. tuberculosis* in vitro and murine macrophages infected with *M. bovis* BCG produce IL-12 (11, 28, 31). Alternatively, mycobacterial components and the cytokine milieu may stimulate IL-12 production by macrophages in the absence of direct infection. For example, lipopolysaccharide can induce IL-12 production by human PBMC (8), and gamma interferon and tumor necrosis factor alpha enhance IL-12 production (11). In tuberculous lymph nodes, such mycobacterial components as lipoarabinomannan, which is structurally similar to lipopolysaccharide, in concert with gamma interferon and tumor necrosis factor alpha, both of which are prominent in the local immune response to tuberculosis (3), may elicit IL-12 production by surrounding macrophages.

We found that local production of and mRNA expression for gamma interferon were similar in tuberculosis patients with or without HIV infection, implying that the extent of local production of this cytokine does not influence the likelihood of the hematogenous spread of *M. tuberculosis*. Gamma interferon produced in tissue may instead contribute more to local inflammation and immunopathology than to protective immunity. It is intriguing to speculate that *M. tuberculosis*-stimulated cytokine production by peripheral blood T cells provides a more accurate reflection of the capacity to contain mycobacterial infection than does cytokine production at the site of disease. These findings contrast with those for disease due to

*M. leprae*, for which cytokine profiles at the site of disease correlate best with the efficacy of the immune response (30).

The Th2 response was not increased in lymph nodes of tuberculosis patients with or without HIV infection. Thus, local expansion of cells producing Th2 cytokines does not appear to be responsible for the severe local disease in HIV-negative patients with tuberculous lymphadenitis or for the disseminated disease characteristic of tuberculosis in HIV-infected patients. PBMC of tuberculosis patients exposed to live *M. tuberculosis* or expanded with IL-4 did not produce increased amounts of IL-4, indicating no expansion of systemic Th2 cells. These results confirm and extend prior observations that depressed Th1 responses in the peripheral blood of HIV-negative and HIV-infected tuberculosis patients are not accompanied by an enhanced Th2 response (32, 33). Downregulation of the Th1 response in tuberculosis may occur through alternative mechanisms, such as increased production of transforming growth factor  $\beta$  by monocytes (29).

IL-10 mRNA expression was abundant in the lymph nodes of tuberculosis patients, particularly in those with HIV coinfection. Although IL-10 protein could not be unequivocally demonstrated in lymph nodes, macrophages stimulated in vitro with *M. tuberculosis* produced IL-10, and it is likely that macrophages in lymph nodes were also the cellular source of IL-10. The functional role of IL-10 at the site of infection in tuberculosis remains speculative. In the setting of a marked Th1 response, IL-10 could minimize local tissue damage by downregulating production of Th1 cytokines through the reduced production of IL-12 (7) and inhibition of macrophage activation (5, 9, 10). Additional studies are necessary to understand the interactions among IL-10, IL-12, and gamma interferon in the human immune response to mycobacterial infection.

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