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Interleukin-10 Downregulates *Mycobacterium tuberculosis*-Induced Th1 Responses and CTLA-4 Expression

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To characterize the mechanism by which interleukin 10 (IL-10) inhibits Th1 responses to intracellular pathogens, we evaluated the interaction between IL-10 and *Mycobacterium tuberculosis*-induced gamma interferon (IFN-γ) production by peripheral blood mononuclear cells from persons across the spectrum of tuberculous infection. *M. tuberculosis*-induced IFN-γ production was highest in healthy tuberculin reactors, intermediate in human immunodeficiency virus (HIV)-negative tuberculosis patients, and lowest in HIV-infected tuberculosis patients. Neutralizing antibodies to IL-10 increased IFN-γ production in HIV-infected and HIV-negative tuberculosis patients by enhancing monocyte IL-12 production. Expression of the T-cell-costimulatory molecule CTLA-4 was depressed in *M. tuberculosis*-stimulated peripheral blood mononuclear cells from tuberculosis patients, and anti-IL-10 and IL-12 upregulated expression of CTLA-4. These findings provide evidence that intracellular pathogens can inhibit Th1 responses and downregulate expression of specific costimulatory molecules.

An ineffective cell-mediated immune response is a characteristic feature of human disease due to a wide variety of intracellular pathogens, including *Mycobacterium tuberculosis*, *M. leprae*, and *Leishmania* species. Ineffective immunity in these infections is associated with a depressed Th1 cytokine response and reduced production of gamma interferon (IFN-γ) (12, 13, 26, 29, 30). Administration of IFN-γ reduces the extent of disease in patients with nontuberculous mycobacterial disease, leprosy, and leishmaniasis (14, 19, 20, 24) but requires administration of supraphysiologic doses that can cause significant toxicity (20). An alternative approach to immunotherapy for these diseases is to administer agents that enhance production of physiologic amounts of IFN-γ through immunoregulatory mechanisms.

Human tuberculosis provides an excellent model with which to study production of IFN-γ in response to infectious agents. Tuberculous infection causes a spectrum of clinical manifestations that correlate with production of IFN-γ (3). Healthy tuberculin reactors have protective immunity against disease, and *M. tuberculosis*-stimulated peripheral blood mononuclear cells (PBMC) produce high concentrations of IFN-γ (21, 30). Human immunodeficiency virus (HIV)-negative patients with pulmonary tuberculosis have moderately severe disease, and *M. tuberculosis*-stimulated PBMC produce significantly lower amounts of IFN-γ (30). HIV-infected tuberculosis patients have the most severe disease, with frequent extrapulmonary dissemination, and IFN-γ production is markedly depressed (29).

Interleukin-10 (IL-10) is a central mediator of the depressed IFN-γ response in disease from intracellular pathogens, as the pathogens themselves elicit IL-10 production by mononuclear phagocytes (1, 6, 12, 13, 22), and antibodies to IL-10 enhance IFN-γ production by PBMC in response to microbial antigens (6, 11, 29). Because IL-10 has a wide range of effects on antigen-presenting cells, including downregulation of major histocompatibility complex molecules and inhibition of monokine synthesis (5, 7–10), the mechanism by which IL-10 inhibits IFN-γ production remains uncertain. To investigate this question, we studied the interaction between IL-10 and *M. tuberculosis*-induced IFN-γ production by PBMC from persons across the spectrum of tuberculous infection.

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MATERIALS AND METHODS

Patient population. Blood was obtained from 27 patients with their first episode of culture-proven pulmonary tuberculosis. Acid-fast stains of sputum were positive in all patients. Twelve patients had antibodies to HIV confirmed by Western blot (immunoblot) analysis, and 15 patients had negative enzyme-linked immunosorbent assay (ELISA) tests for HIV antibody. All patients had received less than 2 weeks of antituberculosis therapy at the time that blood samples were obtained. Blood was also obtained from 11 healthy tuberculin reactors.

Cell culture conditions. PBMC were isolated by differential centrifugation over Ficoll-Paque (Pharmacia Fine Chemicals, Piscataway, N.J.) and plated in 200-μl flat-bottom wells at 2×10^5 cells per well in RPMI (GIBCO, Grand Island, N.Y.) containing penicillin-streptomycin (GIBCO) and 10% heat-inactivated pooled human serum. PBMC were cultured in triplicate in the presence of medium alone or with heat-killed *M. tuberculosis* Erdman (10 μg/ml). In some experiments, neutralizing antibodies to IL-10 (10 μg/ml; Biosource International, Camarillo, Calif.) or IL-12 (5 μg/ml of a 50:50 mix of the antibodies 4A1 and 20C2 [28]; kind gifts of Maurice Gately, Hoffmann-La Roche, Nutley, N.J.), recombinant IL-12 (10 ng/ml), CTLA-4–immunoglobulin (10 μg/ml), or Fab fragments of the anti-CTLA-4 antibody 11D4 (10 μg/ml) were added to the cultures. Cells were maintained at 37°C and 7.5% CO_2, and supernatants were collected at various time points for measurement of cytokine concentrations. In some experiments, cells were collected after different time points for cytofluorometric analysis or lysed for measurement of intracellular CTLA-4 concentrations.

For experiments involving separation of macrophages and T cells, adherent cells were prepared from PBMC by standard methods (28), and T cells were obtained by passage of nonadherent cells through columns that bind B cells and macrophages (T-cell enrichment columns; R & D Systems, Minneapolis, Minn.). Adherent cells were 90 to 95% monocytes by Giemsa staining and by cytofluorometric analysis with anti-CD14 (LeuM3; Becton Dickinson Immunocytometry Systems, San Jose, Calif.). T cells were 95 to 99% $CD3^+$ by cytofluorometric analysis with fluorescein isothiocyanate-conjugated anti-CD3 (Pharmingen, San Diego, Calif.). During the initial 24 h, T cells or macrophages were cocultured with heat-killed *M. tuberculosis* Erdman, with or without combinations of optimal concentrations of anti-IL-10, anti-IL-12, or recombinant IL-12. After 24 h, cells

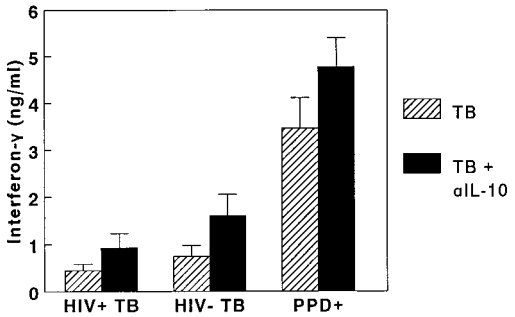

FIG. 1. Effects of anti-IL-10 antibodies on *M. tuberculosis*-induced production of IFN-γ. PBMC were obtained from tuberculosis patients with (HIV+ TB) or without (HIV− TB) HIV infection or from healthy tuberculin reactors (PPD+). PBMC were cocultured with heat-killed *M. tuberculosis* Erdman, with or without anti-IL-10 antibodies. IFN-γ concentrations were measured in culture supernatants by ELISA.

were washed three times to remove antibodies and recombinant cytokines, and then the reciprocal subpopulation was added together with *M. tuberculosis* Erdman. Supernatants were collected after an additional 72 h of culture for measurement of IFN-γ concentrations.

Measurement of cytokine concentrations by ELISA. PBMC supernatants were harvested after 24 h for measurement of IL-12 and after 72 to 96 h for measurement of IFN-γ by ELISA. These time points were optimal for detection of these cytokines, as determined in preliminary experiments. Materials for two ELISAs for IL-12 (28) were generously provided by Maurice Gately. One measures both heterodimeric IL-12 and free p40, and the other measures only heterodimeric IL-12. Materials for measurement of IFN-γ were kindly supplied by Genentech Inc., South San Francisco, Calif. Concentrations of IL-12 and IFN-γ were not detectable in supernatants of cells cultured in medium alone.

Cytofluorometric analysis of CD28 expression. Single and double immunolabeling were performed by standard techniques (2), using fluorescein isothiocyanate-conjugated anti-CD3 and phycoerythrin-conjugated anti-CD28 (Chromaprobe, Inc., Mountain View, Calif.).

Measurement of CTLA-4 expression. CTLA-4 concentrations were measured in cell extracts by ELISA as previously described (18). Briefly, microtiter plates were coated with the anti-CTLA-4 monoclonal antibody 11D4. Nonionic detergent extracts of cells were prepared and incubated on the plates. After washing, biotinylated anti-CTLA-4 monoclonal antibody 7F8 was added for 1 h. Plates were washed, incubated with streptavidin-horseradish peroxidase, and developed with *o*-phenylenediamine substrate (Sigma Chemical Co., St. Louis, Mo.). The A_{490} was recorded, and the amount of CTLA-4 in each sample was determined from a standard curve, using known amounts of purified CTLA-4–immunoglobulin as a standard. The intracellular concentration of CTLA-4 was then expressed as the number of molecules per cell.

Statistical analysis. Continuous variables that were normally distributed were compared by Student's *t* test or the paired *t* test. Variables that were not normally distributed were compared by the nonparametric Wilcoxon rank-sum test.

RESULTS

Effects of anti-IL-10 on IFN-γ production. We have previously demonstrated that antibodies to IL-10 enhance IFN-γ production by PBMC from HIV-infected tuberculosis patients (29). To confirm and extend these results, we evaluated the effects of IL-10 neutralization on IFN-γ production by PBMC from patients across the spectrum of tuberculosis infection. *M. tuberculosis*-induced IFN-γ production was highest in 8 healthy tuberculin reactors, intermediate in 15 HIV-negative tuberculosis patients, and lowest in 10 HIV-infected tuberculosis patients (Fig. 1). Addition of neutralizing antibodies to IL-10 increased IFN-γ concentrations twofold in tuberculosis patients, with or without HIV infection ($P < 0.006$ for HIV-negative patients; $P = 0.04$ for HIV-infected patients). Addition of isotype control antibodies had no effect on IFN-γ production (data not shown). Anti-IL-10 also increased IFN-γ production slightly in healthy tuberculin reactors, but this increase was not statistically significant ($P = 0.16$). These findings indicate that depressed *M. tuberculosis*-induced IFN-γ production in tuberculosis patients with or without HIV infection can be partially reversed by neutralization of IL-10.

TABLE 1. Effect of anti-IL-10 on *M. tuberculosis*-induced IFN-γ production[a]

Expt	Day 0	Day 1	IFN-γ (pg/ml)
1	Adherent cells + TB	T cells + TB	45
	T cells + TB	Adherent cells + TB	34
	Adherent cells + TB + α-IL-10	T cells + TB	373
	T cells + TB + α-IL-10	Adherent cells + TB	48
2	Adherent cells + TB	T cells + TB	502
	Adherent cells + TB + α-IL-10	T cells + TB	1,027
	T cells + TB + α-IL-10	Adherent cells + TB	250

[a] Adherent cells or negatively selected T cells from PBMC of tuberculosis patients were cultured as indicated on day 0 for 24 h and then washed, and the reciprocal subpopulation was added. IFN-γ concentrations were measured after an additional 48 h of culture. TB, heat-killed *M. tuberculosis*; α-IL-10, anti-IL-10.

Anti-IL-10 enhances IFN-γ production through its effects on monocytes. IL-10 is made predominantly by monocytes when PBMC are cocultured with *M. leprae* (22). To confirm that anti-IL-10 increases IFN-γ production through neutralization of monocyte-derived IL-10 in our experimental system, we separated PBMC from HIV-negative tuberculosis patients into adherent cells (90 to 95% monocytes) and T cells as outlined in Materials and Methods. We incubated these cell subpopulations with or without *M. tuberculosis*, and with or without anti-IL-10, for 24 h, washed them to remove anti-IL-10, and then added the reciprocal subpopulation with *M. tuberculosis*. IFN-γ was undetectable if cells were cultured in medium alone or if anti-IL-10 was added in the absence of *M. tuberculosis* (data not shown). When T cells and adherent cells were cocultured with *M. tuberculosis*, IFN-γ concentrations were low (Table 1). IFN-γ production increased substantially when anti-IL-10 was added to the adherent cells but not when it was added to the T cells, indicating that anti-IL-10 elicits IFN-γ production through its effects on monocytes that are exposed to *M. tuberculosis.*

Anti-IL-10 enhances IFN-γ production by eliciting IL-12 production. IL-10 suppresses production of a broad array of monokines, including IL-1, IL-6, IL-8, tumor necrosis factor alpha, granulocyte-macrophage colony-stimulating factor, and

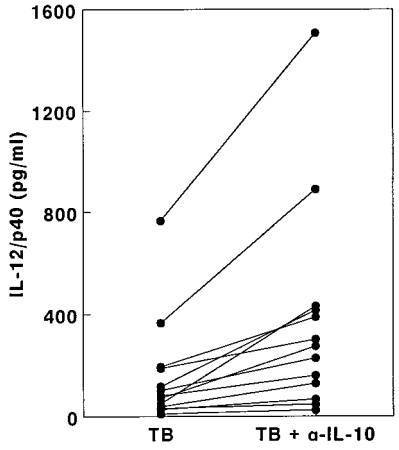

FIG. 2. Effects of anti-IL-10 antibodies on *M. tuberculosis*-induced production of IL-12/p40. PBMC from HIV-negative tuberculosis patients were cocultured with *M. tuberculosis* (TB), with or without anti-IL-10 (a-IL-10). Concentrations of IL-12 and p40 were measured in culture supernatants by ELISA.

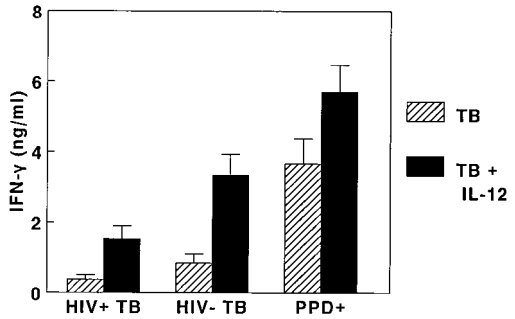

FIG. 3. Effects of recombinant IL-12 on *M. tuberculosis*-induced IFN-γ production. PBMC were obtained from tuberculosis patients with (HIV+ TB) or without (HIV− TB) HIV infection or from healthy tuberculin reactors (PPD+). PBMC were cocultured with heat-killed *M. tuberculosis* Erdman, with or without recombinant IL-12. IFN-γ concentrations were measured in culture supernatants by ELISA.

TABLE 2. Effects of anti-IL-10 and anti-IL-12 on *M. tuberculosis*-induced IFN-γ production[a]

Expt	Day 0	IFN-γ (pg/ml)
1	Adherent cells + TB	626
	Adherent cells + TB + α-IL-10	1,027
	Adherent cells + TB + α-IL-10 + α-IL-12	565
2	Adherent cells + TB	130
	Adherent cells + TB + α-IL-10	764
	Adherent cells + TB + α-IL-10 + α-IL-12	207

[a] Adherent cells or negatively selected T cells from PBMC of tuberculosis patients were cultured as indicated on day 0 for 24 h and then washed, and the reciprocal subpopulation was added. IFN-γ concentrations were measured after an additional 48 h of culture. In all assays, T cells plus heat-killed *M. tuberculosis* (TB) were added on day 1. α-IL-10, anti-IL-10.

IL-12 (5, 7, 8, 10). IL-12 is a potent stimulus for antigen-induced IFN-γ production (4), is present at the site of mycobacterial disease, and is induced by monocytes exposed to mycobacterial antigens (23, 28). We therefore hypothesized that anti-IL-10 increases IFN-γ production by eliciting production of IL-12 by monocytes. PBMC from 13 HIV-negative tuberculosis patients were stimulated with *M. tuberculosis* or with *M. tuberculosis* and anti-IL-10. Concentrations of heterodimeric IL-12 and p40 were more than twofold higher in supernatants of cells to which anti-IL-10 had been added (mean, 374 ± 114 versus 157 ± 57 pg/ml, $P = 0.004$; Fig. 2). Heterodimeric IL-12 was not detectable in the supernatants, perhaps because it was taken up by activated T cells expressing IL-12 receptors.

To obtain additional evidence that anti-IL-10 enhanced IFN-γ production through stimulating IL-12 production by monocytes, we separated PBMC from HIV-negative tuberculosis patients into adherent cells and T cells and then cocultured the adherent cells with *M. tuberculosis* and either anti-IL-10 alone or anti-IL-10 and anti-IL-12 (Table 2). Addition of anti-IL-12 abrogated the capacity of anti-IL-10 to stimulate IFN-γ production, indicating that anti-IL-10 acts through a pathway that requires the presence of IL-12.

Effect of IL-12 on IFN-γ production. Because our results suggested that anti-IL-10 increased IFN-γ production in an IL-12-dependent manner, we next evaluated the direct effect of recombinant IL-12 on *M. tuberculosis*-induced IFN-γ production in patients across the spectrum of tuberculosis infection (Fig. 3). IL-12 increased IFN-γ production three- to fourfold in 12 HIV-infected and 14 HIV-negative tuberculosis patients ($P < 0.001$ for both groups). IL-12 also enhanced IFN-γ production by PBMC from eight healthy tuberculin reactors, but the increase was not statistically significant. Addition of IL-12 to PBMC in the absence of *M. tuberculosis* did not elicit IFN-γ production (data not shown).

If anti-IL-10 upregulates IFN-γ production predominantly through enhancing IL-12 production, addition of both anti-IL-10 and IL-12 should not yield additive or synergistic effects. In eight HIV-negative tuberculosis patients, IL-12 increased *M. tuberculosis*-induced IFN-γ production to a greater extent than did anti-IL-10. The combination of anti-IL-10 and IL-12 did not yield any additional increment in IFN-γ production (Fig. 4). Identical results were obtained for *M. tuberculosis*-stimulated PBMC from nine HIV-infected tuberculosis patients (data not shown).

To identify the cell subpopulation through which IL-12 increases IFN-γ production, we cocultured adherent cells or T cells with *M. tuberculosis*, with or without IL-12, for 24 h, washed the cells to remove IL-12, and then added the reciprocal subpopulation with *M. tuberculosis* (Table 3). Addition of IL-12 to T cells increased IFN-γ production 6- to 18-fold, whereas addition of IL-12 to the adherent cells yielded 2- to 5-fold increases, suggesting that the predominant effect of IL-12 was directly on the T cells' capacity to produce IFN-γ. The smaller increase in IFN-γ induced by addition of IL-12 to adherent cells may have been due to the effect of small amounts of IL-12 being carried over and affecting the T cells. Alternatively, IL-12 may act directly on the antigen-presenting cell to enhance IFN-γ production through mechanisms that are as yet undefined.

Expression of CD28 and CTLA-4 in tuberculosis patients and healthy tuberculin reactors. Costimulatory molecules are thought to be critical in eliciting cellular proliferation and cytokine production upon antigen stimulation. We therefore wished to determine if depressed IFN-γ production in tuberculosis patients was related to inadequate expression of the T-cell-costimulatory molecules CD28 and CTLA-4.

Single and double immunolabeling with anti-CD28 and anti-CD3 monoclonal antibodies revealed that the percentages of T cells expressing CD28 in freshly isolated PBMC from 14 tuberculosis patients and 10 healthy tuberculin reactors were similar (65% ± 19% and 66% ± 12%, respectively). The percentages of *M. tuberculosis*-stimulated T cells expressing CD28 in tuberculosis patients and healthy tuberculin reactors were also similar (70% ± 8% and 85% ± 6%).

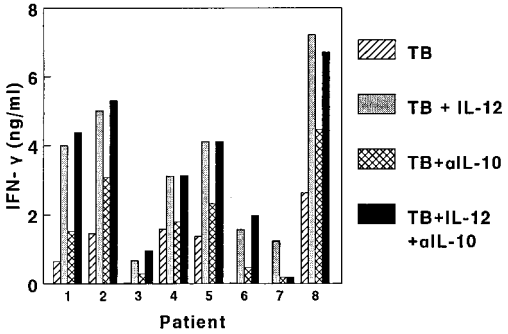

FIG. 4. Effects of recombinant IL-12 and anti-IL-10 on *M. tuberculosis*-induced IFN-γ production. PBMC were obtained from eight HIV-negative tuberculosis patients and cocultured with heat-killed *M. tuberculosis* Erdman (TB) alone or with addition of IL-12, anti-IL-10 antibodies (a-IL-10), or both. IFN-γ concentrations were measured in culture supernatants by ELISA.

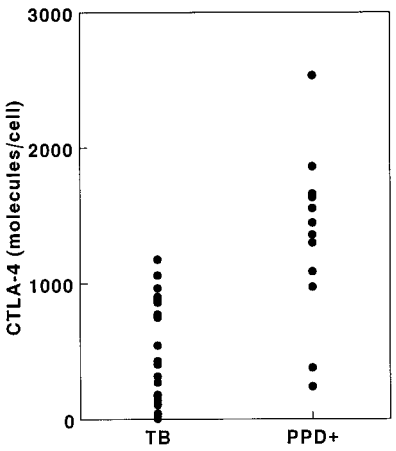

FIG. 5. CTLA-4 expression in *M. tuberculosis*-stimulated cells from HIV-negative tuberculosis patients (TB) and healthy tuberculin reactors (PPD+). PBMC were cocultured with *M. tuberculosis* Erdman and lysed after 4 to 5 days of culture, and CTLA-4 concentrations were measured in cell extracts by ELISA.

By cytofluorometric analysis with anti-CTLA-4, we found no detectable surface expression of CTLA-4 on freshly isolated or *M. tuberculosis*-stimulated PBMC, consistent with the predominant intracellular expression of this molecule (16). We therefore measured intracellular CTLA-4 concentrations by ELISA. CTLA-4 was not detectable in lysates of freshly isolated PBMC but was present in *M. tuberculosis*-stimulated cells, consistent with the marked upregulation of CTLA-4 during T-cell activation (18). CTLA-4 expression was significantly lower in 21 HIV-negative tuberculosis patients than in 12 healthy tuberculin reactors (486 ± 90 versus 1,332 ± 198 molecules per cell, $P = 0.00005$; Fig. 5), and CTLA-4 expression showed a linear correlation with IFN-γ concentrations in *M. tuberculosis*-stimulated supernatants from 11 HIV-negative tuberculosis patients (correlation coefficient = 0.96). Reduced CTLA-4 expression in tuberculosis patients could not be explained by lower numbers of T cells, as the percentages of $CD3^+$ cells after coculture with *M. tuberculosis* were similar in tuberculosis patients and healthy tuberculin reactors (means, 70 and 72%).

Anti-IL-10 and IL-12 upregulate CTLA-4 expression. Because anti-IL-10 and IL-12 enhanced *M. tuberculosis*-induced IFN-γ production, we wished to determine if this effect was mediated through upregulation of CD28 or CTLA-4. Addition of anti-IL-10 or IL-12 did not change the percentage of CD28-expressing cells (data not shown). However, IL-12 increased CTLA-4 expression in 12 of 13 tuberculosis patients (mean, 396 ± 90 versus 216 ± 47 molecules per cell, $P = 0.005$; Fig. 6A), and anti-IL-10 had similar effects (mean, 450 ± 72 versus 252 ± 72 molecules per cell, $P = 0.04$; Fig. 6A). IL-12 and anti-IL-10 did not enhance CTLA-4 expression in healthy tuberculin reactors (Fig. 6B). These data indicate that CTLA-4 expression correlates with *M. tuberculosis*-induced IFN-γ production and that IL-12 and anti-IL-10 upregulate both CTLA-4 expression and IFN-γ production.

To determine if anti-IL-10 and IL-12 enhance *M. tuberculosis*-induced IFN-γ production through an essential intermediary step that involves upregulation of CTLA-4, we used anti-CTLA-4 Fab antibodies to block CTLA-4 upregulation. The Fab antibodies did not abrogate the capacity of IL-12 to enhance *M. tuberculosis*-induced IFN-γ production in three tuberculosis patients (Table 4), indicating that CTLA-4 upregulation is not required for IL-12 to increase IFN-γ production.

TABLE 3. Effect of IL-12 on *M. tuberculosis*-induced IFN-γ production[a]

Expt	Day 0	Day 1	IFN-γ (pg/ml)
1	Adherent cells + TB	T cells + TB	36
	T cells + TB	Adherent cells + TB	17
	Adherent cells + TB + IL-12	T cells + TB	170
	T cells + TB + IL-12	Adherent cells + TB	675
2	Adherent cells + TB	T cells + TB	155
	T cells + TB	Adherent cells + TB	132
	Adherent cells + TB + IL-12	T cells + TB	342
	T cells + TB + IL-12	Adherent cells + TB	978

[a] Adherent cells or negatively selected T cells from PBMC of tuberculosis patients were cultured as indicated on day 0 for 24 h and then washed, and the reciprocal subpopulation was added. IFN-γ concentrations were measured after an additional 48 h of culture. TB, heat-killed *M. tuberculosis.*

DISCUSSION

Mycobacteria and other intracellular pathogens are potent inducers of IL-10, and disease due to these organisms is frequently associated with immunologic unresponsiveness and failure to produce IFN-γ (12, 13, 26, 30). The findings in this report provide insight into the mechanism by which IL-10 inhibits immune responses through the network of cytokines and costimulatory molecules. Antigen-induced IFN-γ production and CTLA-4 expression are decreased in tuberculosis patients compared with healthy tuberculin reactors. Neutralization of IL-10 enhances IFN-γ production in response to mycobacteria through increasing antigen-induced IL-12 production by monocytes. IL-12 in turn acts on the T cells to increase IFN-γ production and upregulate CTLA-4 expression.

A critical component of the immune response to infectious pathogens is innate immunity mediated by antigen-presenting cells and natural killer (NK) cells, which produce specific cytokines that favor development of Th1 or Th2 responses. IL-12 produced by macrophages and IFN-γ produced by NK cells both favor development of a Th1 response. In contrast, IL-10 produced by macrophages and IL-4 produced by CD4+ $NK1.1^+$ cells bias toward a Th2 response (15, 27). Our findings indicate that the depressed *M. tuberculosis*-induced IFN-γ production in tuberculosis patients is mediated in part through IL-10's capacity to inhibit IL-12 production, confirming the importance of the balance between IL-10 and IL-12 in eliciting

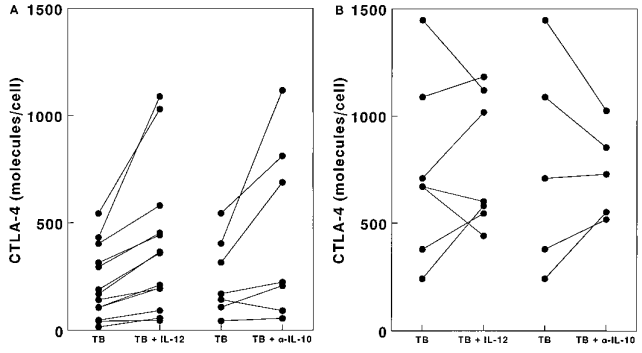

FIG. 6. Effects of recombinant IL-12 and anti-IL-10 on *M. tuberculosis*-induced CTLA-4 expression. PBMC were obtained from HIV-negative tuberculosis patients (A) or healthy tuberculin reactors (B) and cocultured with heat-killed *M. tuberculosis* (TB), with or without recombinant IL-12 or anti-IL-10 antibodies (a-IL-10). Cells were lysed after 4 to 5 days of culture, and CTLA-4 concentrations were measured in cell extracts by ELISA.

TABLE 4. Effects of anti-CTLA-4 on *M. tuberculosis* and IL-12-induced IFN-γ production

Sample	IFN-γ (pg/ml) in patient:		
	1	2	3
PBMC	3	0	1
PBMC + TB[a]	310	46	170
PBMC + TB + IL-12	1,904	1,599	1,562
PBMC + TB + IL-12 + anti-CTLA-4	1,884	1,850	1,543
PBMC + TB + anti-CTLA-4	383	62	114

[a] TB, heat-killed *M. tuberculosis.*

a strong Th1 response to the intracellular pathogen *M. tuberculosis*. Neutralization of IL-10 or addition of IL-12 enhanced IFN-γ production by PBMC from tuberculosis patients. Although IFN-γ production in tuberculosis patients is significantly less than that in healthy tuberculin reactors, *M. tuberculosis*-induced production of IL-12 in tuberculosis patients is normal (28) and production of the Th2 cytokines IL-4 and IL-10 is not enhanced (30). One may speculate that the reduced Th1 response in tuberculosis patients is mediated through differences in IL-10 or IL-12 receptor expression or signalling pathways, rather than through differences in cytokine concentrations. Alternatively, it is possible that the reduced Th1 response in tuberculosis patients is mediated by overproduction of immunosuppressive cytokines such as transforming growth factor β (25).

The T-cell-costimulatory molecules CD28 and CTLA-4 and their counterreceptors B7-1 and B7-2 on antigen-presenting cells play a critical role in T-cell activation and cytokine production. CD28 is present on resting T cells and is therefore likely to predominate in initial costimulatory activity. CTLA-4 probably plays a more important role after antigen activation, as it is expressed only on activated T cells, and its affinity for B7 is 20 times that of CD28 (17). Our results demonstrate that CTLA-4 but not CD28 expression is depressed in tuberculosis patients. Furthermore, addition of anti-IL-10 and IL-12 enhances CTLA-4 expression, suggesting that IL-10 in tuberculosis patients specifically reduces CTLA-4 expression. IL-10 has been shown to downregulate expression of B7-1 and B7-2 on antigen-presenting cells (11), but its effects on T-cell-costimulatory molecules have not been previously reported. Further studies are needed to clarify the interactions between the network of cytokines and costimulatory molecules.

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