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Immunology

Cross-modulation by transforming growth factor β in human tuberculosis: Suppression of antigen-driven blastogenesis and interferon γ production

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ABSTRACT **In tuberculosis, *Mycobacterium tuberculosis* (MTB)-stimulated T-cell responses are depressed transiently, whereas antibody levels are increased. Lymphoproliferative responses of peripheral blood mononuclear cells (PBMCs) from Pakistani tuberculosis (TB) patients to both mycobacterial and candidal antigens were suppressed by ≈50% when compared to healthy purified protein derivative (PPD)-positive household contacts. Production of interferon γ (IFN-γ) in response to PPD also was depressed by 78%. Stimulation with PPD and the 30-kDa α antigen of MTB (30-kDa antigen) induced greater secretion of transforming growth factor β (TGF-β), but not interleukin 10 (IL-10) or tumor necrosis factor α (TNF-α), by PBMCs from TB patients compared to healthy contacts. The degree of suppression correlated with the duration of treatment; patients treated for <1 month had significantly lower T-cell blastogenesis and IFN-γ production and higher levels of TGF-β than did patients treated for >1 month. Neutralizing antibody to TGF-β normalized lymphocyte proliferation in response to PPD, partially restored blastogenesis to candidal antigen, and significantly increased PPD-stimulated production of IFN-γ in TB patients but not in contacts. Neutralizing antibody to IL-10 augmented, but did not normalize, T-cell responses to both PPD and candida in TB patients and candidal antigen in contacts. TGF-β, produced in response to MTB antigens, therefore plays a prominent role in down-regulating potentially protective host effector mechanisms and looms as an important mediator of immunosuppression in TB.**

In the past decade, the incidence of tuberculosis (TB) has been increasing in the United States and worldwide with nearly 12 million cases projected to occur in 2005. Management of multidrug-resistant (MDR) TB poses a difficult therapeutic problem. Understanding regulation of the human immune response in TB assumes particular importance, therefore, because of its potential implication for immune-based interventions. Therapies that boost the cellular immune response to *Mycobacterium tuberculosis* (MTB) may shorten the course of antituberculous chemotherapy and improve the outcome of MDR TB.

Depressed delayed-type hypersensitivity (DTH) skin test and *in vitro* T-cell responses in patients with TB result from an offset balance between stimulatory and immunosuppressive influences. Peripheral blood mononuclear cells (PBMCs from patients with pulmonary TB show depressed purified protein derivative (PPD)-stimulated blastogenesis (1), which is associated with negative DTH skin test reactivity to PPD. Both PPD-triggered production of interleukin 2 (IL-2) and responsiveness of PBMCs to IL-2 are depressed (2); furthermore, defective IL-2 production is associated with the extent of pulmonary TB and is most pronounced in far-advanced disease. Blood monocytes (MNs) suppress lymphocyte responses (1, 2) and are activated *in vivo* as manifest by constitutive expression and secretion of the IL-2 receptor (3). Furthermore, MTB antigen-induced production of interferon γ (IFN-γ), a cytokine that is crucial in the immune response to MTB (4, 5), is depressed in PBMCs of patients with active pulmonary TB compared to healthy tuberculin reactors (6–8). Reciprocal increases in antibody production in response to MTB antigens in TB patients (7–9) suggest a role for cross-modulatory cytokines. In support of this hypothesis, the frequency of cells producing IL-4, a cytokine that down-regulates potentially protective Th1-type responses, was increased in a recent study (10), as were serum antibody levels. Studies in TB pleuritis similarly indicate that, whereas mRNA for the Th2 cytokine IL-4 was increased in PBMCs, secretion of IFN-γ upon stimulation with MTB antigen was more pronounced in pleural mononuclear cells (11). As TB pleuritis usually resolves without therapy (12), compartmentalization of the protective cytokine IFN-γ rather than IL-4 in the pleural cavity, indicates a possible role for Th1 cytokines in modulating protective immune responses in human TB.

PPD and the 30-kDa antigen of MTB are direct stimuli for production of cytokines by both MNs of patients with active pulmonary TB and healthy tuberculin reactors (6–8, 13). Transforming growth factor β (TGF-β) is spontaneously expressed in tuberculosis granulomas in the lung as well as by MNs of patients with active pulmonary TB (14). Recently, we demonstrated that TGF-β also is secreted by MNs of health individuals in response to PPD (15) and is induced upon infection with MTB *in vitro* (16). As TGF-β down-regulates production of proinflammatory cytokines, deactivates MN effector function against MTB, decreases responsiveness of T cells to IL-2 (17–20), and inhibits production of IFN-γ (21), it is a good candidate for mediator of immunosuppression. The current studies, in fact, demonstrate a prominent role for TGF-β in modulating antigen-stimulated blastogenesis and production of IFN-γ in patients with pulmonary tuberculosis.

MATERIALS AND METHODS

Human Subjects. After obtaining informed consent 20 patients with active pulmonary TB and matched household

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Abbreviations: TB, tuberculosis; MTB, *Mycobacterium tuberculosis*; PBMC, peripheral blood mononuclear cell; PPD, purified protein derivative; IL, interleukin; MN, blood monocyte; IFN-γ, interferon γ; TGF-β, transforming growth factor β; HIV, human immunodeficiency virus; TNF-α, tumor necrosis factor α; HPRT, hypoxanthine phosphoribosyltransferase.

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contacts were studied. Patient/contact pairs were evaluated on the same day. A diagnosis of TB was established in the patients by routine radiographic, clinical, and bacteriological methods at Massoomeen Hospital Trust in Karachi, Pakistan. At the time of enrollment, 10 patients had undergone chemotherapy with isohiazid, rifampin, ethambutol, pyrazinamide for <1 month, the remaining 10 for 2–4 months. Nineteen of 20 patients were sputum-smear positive, the remaining patient had miliary TB without sputum confirmation. A diagnosis of TB was confirmed by positive culture in 15 of 20 patients and response to therapy in the others. DTH skin testing with PPD (5 TU; Connaught Laboratories) was performed on all patients and household contacts and was assessed 48 h later. None of the patients or contacts had either suspicion or clinical evidence of infection with the human immunodeficiency virus (HIV) or other concomitant illnesses leading to immunosuppression. Routine HIV testing was not done as HIV disease is essentially nonexistent in Pakistan (evaluation of >100 TB patients recruited from the same hospital between 1990 and 1993 revealed the absence of HIV disease in all; R.H., unpublished data).

Antigens and Antibodies. PPD, a gift from Lederle Laboratories (American Cyanamid), was used at a final concentration of 10 μg/ml. Candidal antigen (Greer Laboratories, Lenore, NC) was purchased and used at a concentration of 10 μg/ml. Purified 30-kDa antigen of MTB (H37Ra), prepared as described (22), was a gift from Thomas Daniel (Case Western Reserve University, Cleveland) and was used at 5 μg/ml. Rat anti-human IL-10 monoclonal antibody (clone JES3-9D7, without azide) and isotype control antibody ($IgG1_{-\kappa}$) (PharMingen) as well as chicken anti-TGF-β antibody and chicken immunoglobulin (R & D Diagnostics, Minneapolis) were purchased. All antibodies were used at a concentration of 5 μg/ml. The concentrations of antigens and antibodies used in these experiments were optimal as established in prior dose–response experiments. Lipopolysaccharide contamination as assessed by limulus lysate assay (BioWhittaker) was <0.01 ng per μg of protein for cytokine neutralizing and control antibodies as well as PPD and <0.04 ng per μg of protein for the 30-kDa antigen.

Preparation of Cells and Generation of Cytokine-Containing Supernatants. PBMCs of patients and contacts were obtained by sedimentation of heparinized blood over Ficoll Paque (Pharmacia Fine Chemicals, Piscataway, NJ) (23). To induce cytokines, PBMCs suspended in IMDM (BioWhittaker) containing 2% pooled human serum PHS (2×10^6 cells per ml) were incubated in round-bottomed tissue culture tubes (Falcon Plastics, Oxnard, CA) without stimulus or with MTB antigens. All stimuli were preincubated with polymyxin B (10 μg/ml; Sigma) for 30 min to inactivate any residual contaminating lipopolysaccharide. Supernatants were collected after 24 [tumor necrosis factor α (TNF-α)] and 72 (TGF-β, IL-10, and IFN-γ) of culture and stored frozen at −70°C.

Blastogenesis. PBMCs were suspended in RPMI 1640 culture medium (BioWhittaker) containing penicillin, (50 units/ml) streptomycin (50 μg/ml), and 2 mM L-glutamine (Sigma) supplemented with 10% fetal calf serum. Cells were incubated in triplicate (10^5 cells per well) with the appropriate antigens in the presence or absence of neutralizing or control antibodies to TGF-β or IL-10 in 96-well round-bottomed microtiter plates (Falcon) and cultured for 5 days at 37°C in 5% CO_2/95% air. Wells were pulsed with [^{3}H]thymidine (1 μCi per well; 1 Ci = 37 GBq; specific activity, 6.7 Ci/mMol) (ICN) and harvested 18–24 h later (PHD cell harvester; Cambridge Technology, Watertown, MA). [^{3}H]thymidine incorporation was measured in a liquid scintillation counter. Results are expressed as mean cpm of triplicate cultures minus background.

Immunoassays for Cytokines. Sandwich ELISAs were performed to assess cytokine immunoreactivity in culture supernatants. TNF-α immunoreactivity was measured with a mouse monoclonal antibody to TNF-α (Endogen, Boston) as coating antibody and a polyclonal rabbit anti-human antibody (Genzyme) as detecting antibody. This assay is sensitive to 62.5 pg of TNF-α activity per ml. IL-10 immunoreactivity was assessed with a pair of monoclonal antibodies to IL-10 (clones JES3-9D7 and JES3-12G8; PharMingen). This ELISA detects 32 pg of IL-10 activity per ml. The ELISA for TGF-β utilizes a mouse monoclonal antibody to TGF-β1,2,3 (Genzyme) as capture antibody and a polyclonal chicken anti-human antibody to TGF-β1 (R & D Diagnostics) as capping antibody. All samples were acid activated with 1 M HCl and then neutralized with 1 M NaOH·Hepes. The assay is sensitive to 0.15 ng of TGF-β activity per ml. IL-4 and IFN-γ immunoreactivity were assessed with commercially available ELISA kits (R & D Diagnostics and Endogen), which have lower limits of sensitivity of 15 pg/ml (IL-4) and 32 pg/ml (IFN-γ).

Detection of Cytokine-Specific mRNA by Reverse Transcription PCR and Southern Hybridization. PBMC pellets were lysed with 0.4 ml of RNA/zol B (Tel-Test, Friendswood, TX). Total cellular RNA was extracted as described (24). cDNA was synthesized from total RNA with Moloney murine leukemia virus reverse transcriptase (GIBCO/BRL). Aliquots of 4-fold diluted cDNA were used as template for amplification by PCR in the presence of 2 units of *Taq* DNA polymerase (Promega). Nucleotide sequences for the oligonucleotide primers for IFN-γ were 5′-CAGCTCTGCATCGTTTTGGGTTCT-3′ and 3′-TGCTCTTCGACCTTGAAACAGCAT-5′. The sequences of the oligonucleotide primers for hypoxanthine phosphoribosyltransferase (HPRT) have been described (25). Primers for IL-4 were purchased from Clontech and are based on the cDNA sequence described by Yokota *et al.* (26). PCR product was subjected to electrophoresis on 1.5% agarose gel and transferred to a Hybond N^+ nitrocellulose membrane. Southern blot transfers were probed with internal cytokine-specific oligonucleotides and visualized using the chemiluminescence detection system (Amersham). To control for the relative amount of products reverse transcribed and to assess the amount of mRNA in each sample, concurrent measurement of the housekeeping gene HPRT was made. To allow comparison between samples, signal intensity was assessed by densitometry.

Statistical analysis. Data were analyzed by Student's *t* test, paired *t* test, and linear correlation and regression analysis; $P \leq 0.05$ was considered significant.

RESULTS

Tuberculin Skin Test Reactivity in TB Patients and Contacts. Tuberculin skin test reactivity was assessed as induration at 48 h. The median reaction size was 16 mm in TB patients and 13 mm in contacts. There was a significant difference between TB patients studied within 1 month of initiation of treatment (median, 10 mm) and those treated for >1 month (median, 18 mm; $P = 0.02$).

Overproduction of TGF-β in Patients with TB Is Associated with a Depressed IFN-γ Response. First cytokine production and blastogenesis in response to MTB antigens in PBMCs were examined. Both PPD and the 30-kDa antigen, a secreted protein of growing mycobacteria (22) that has previously been linked to protective immunity (7–9), stimulated PBMCs to produce cytokines (Table 1). Significantly higher concentrations of immunoreactive TGF-β were present in supernatants of PBMCs from TB patients stimulated with either MTB antigen when compared to those of contacts (PPD, 1.6-fold; 30-kDa antigen, 1.9-fold; Table 1). TGF-β immunoreactivity was highest in supernatants of patients treated for <1 month (Table 2). Production of IL-10, another immunosuppressive cytokine previously described to play a prominent role in suppression of T-cell function in patients with leprosy (27), however, was comparable in TB patients and contacts (Table

Table 1. Cytokine production by PBMCs of TB patients and healthy contacts

Cytokine	Stimulus	TB	Contacts
TGF-β	Nil	2.68 ± 0.61	1.83 ± 0.30
	30-kDa Ag	7.17 ± 0.82*	3.76 ± 0.53
	PPD	6.68 ± 0.70*	4.22 ± 0.40
IL-10	Nil	<0.03	<0.03
	30-kDa Ag	1.39 ± 0.15	1.81 ± 0.26
	PPD	0.70 ± 0.07	0.89 ± 0.04
TNF-α	Nil	0.27 ± 0.07	0.25 ± 0.09
	30-kDa Ag	4.78 ± 0.69	5.94 ± 1.32
	PPD	4.05 ± 0.68	4.02 ± 1.10
IFN-γ	Nil	<0.03	<0.03
	30-kDa Ag	0.19 ± 0.05†	0.98 ± 0.28
	PPD	0.48 ± 0.13‡	2.23 ± 0.41

PBMCs of patients with TB and healthy contacts were stimulated as described. TNF-α, IL-10, TGF-β, and IFN-γ in supernatants were assessed by ELISA. Values represent means ± SE (ng/ml) of cytokine. Ag, antigen.
*$P \leq 0.001$ ($n = 18$) when compared to cytokine concentration in supernatants of healthy contacts.
†$P \leq 0.01$ ($n = 12$) when compared to concentration in supernatants of healthy contacts.
‡$P \leq 0.0001$ ($n = 12$) when compared to concentration in supernatants of healthy contacts.

1) and did not differ with duration of therapy (data not shown). As PPD and the 30-kDa antigen are known to induce TNF-α in mononuclear cells of healthy individuals (13) and TB patients (28, 29) and TNF-α also plays a central role in granuloma formation (30), we included it in the panel of cytokines studied. The amounts of TNF-α produced in response to both PPD and the 30-kDa antigen did not differ significantly in supernatants of patients and contacts (Table 1). PBMCs of patients with TB, on the other hand, produced 4.5-fold less IFN-γ in response to PPD and 4.0-fold less in response to 30-kDa antigen when compared to cells of healthy contacts (Table 1). Levels of IFN-γ immunoreactivity were significantly lower in patients treated for <1 month compared to patients treated for longer periods of time (Table 2).

Culture supernatants of PBMCs stimulated with both MTB antigens also were tested for IL-4, a cytokine implicated in mediating depressed immunity to infectious agents (27, 31–33). IL-4 was undetectable (<15 pg/ml) in supernatants of both patients and contacts.

Effects of Neutralizing Antibodies to TGF-β and IL-10 on Antigen-Driven Blastogenesis in Patients with TB and Household Contacts. To compare patterns of cytokine production with proliferative responses, blastogenesis was examined next. T-cell responses to both PPD and candidal antigen were significantly suppressed in patients when compared to tuberculin-positive contacts [10,550 ± 1389 vs. 22,209 ± 3664 cpm (mean ± SE); $P < 0.005$ for PPD; and 6332 ± 1802 vs. 13,228 ± 2671 cpm; $P < 0.04$ for candidal antigen]. A positive correlation also was noted between PPD-stimulated IFN-γ production and blastogenesis in TB patients ($r = 0.834$; $P \leq 0.0001$; $n = 12$). Depression of blastogenesis was inversely related to duration of treatment ($r = 0.786$; $P \leq 0.0001$; $n = 20$). T-cell blastogenic responses to PPD were reduced 3.0-fold in patients undergoing therapy for <1 month when compared to controls but only by 1.7-fold in patients treated for >1 month (Table 2).

Table 2. Effect of duration of antituberculous therapy on blastogenesis and cytokine production

Test	Stimulus	Duration < 1 month	Duration > 1 month
[³H]Thymidine	PPD	6,643 ± 1386	144,457 ± 1683*
(Δ cpm)	PPD + TGF-α	16,016 ± 1914	21,643 ± 1888
TGF-β (ng/ml)	30 kDa	8.76 ± 1.38	5.87 ± 0.84
	PPD	7.85 ± 1.13	4.86 ± 0.55†
IFN-γ (pg/ml)	30 kDa	166 ± 60	301 ± 67
	PPD	316 ± 74	947 ± 182‡
	PPD + TGF-α	957 ± 83	1755 ± 221

PBMCs of TB patients and contacts were isolated as described. T-cell responses to PPD were assessed by [³H]thymidine incorporation. TGF-β and IFN-γ were assessed in supernatants collected at 72 h. Values represent means ± SE for blastogenesis and for TGF-β and IFN-γ immunoreactivity.
*$P \leq 0.002$ ($n = 10$) compared to blastogenesis in PBMCs of patients treated for <1 month.
†$P \leq 0.02$ ($n = 9$) compared to levels of TGF-β in supernatants of TB patients treated for <1 month.
‡$P \leq 0.005$ ($n = 6$) compared to levels of IFN-γ in supernatants of TB patients treated <1 month.

To further evaluate the role of TGF-β and IL-10 in depressed lymphoproliferative responses of patients with TB, neutralizing antibodies in these cytokines or matched isotype control antibodies were added to the *in vitro* lymphocyte proliferation assays. Coculture with antibody to TGF-β, but not isotype control antibody, increased lymphocyte proliferation in response to PPD and candidal antigen in the TB patient group only (Fig. 1). Blastogenesis in response to PPD was restored to levels comparable to those of healthy contacts in the presence of neutralizing antibody to TGF-β. The increases were greater in the more suppressed patients ($r = 0.638$; $P \leq 0.002$). Candida-induced T-cell proliferation increased but failed to reach levels similar to those in healthy individuals (Fig. 1). Anti-TGF-β antibody had no effect on blastogenesis in healthy contacts irrespective of the antigen used (Fig. 1). Coculture with antibody to IL-10, but not isotype control antibody, similarly increased [³H]thymidine incorporation in response to both antigens in T cells of patients with active TB (Fig. 2). In contrast to findings with neutralizing antibody to TGF-β, however, coculture with antibody to IL-10 failed to restore blastogenesis to PPD in TB patients to levels encountered in healthy contacts. T-cell responses to PPD in contacts were not affected significantly by addition of antibody to IL-10 (Fig. 2*A*); blastogenic responses to candidal antigen increased significantly, however, after coculture with anti-IL-10 antibody (Fig. 2*B*).

Neutralization of TGF-β Restores IFN-γ Response to Mycobacterial Antigens in PBMCs of Patients with TB. Since depressed blastogenesis correlated with production of decreased levels of IFN-γ by PBMCs of patients with TB and was associated with production of increased concentrations of TGF-β, the effect of neutralization of TGF-β on IFN-γ expression was examined next. Coculture with neutralizing antibody to TGF-β, but not isotype control antibody, increased IFN-γ production in PBMC culture supernatants of TB patients (2-fold; $P \leq 0.002$; $n = 12$; Fig. 3). The increase in IFN-γ immunoreactivity in the presence of neutralizing antibody to TGF-β was most pronounced in supernatants of PBMCs of patients treated <1 month (Table 2). Production of IFN-γ in culture supernatants of healthy contacts also increased by 20% in the presence of antibody to TGF-β but did not reach statistical significance ($P = 0.07$) (Fig. 3).

Expression of cytokine-Specific mRNA in PBMCs of TB Patients and Healthy Contacts. To determine the relationship between patterns of secreted protein and induction of gene expression, we next extracted total RNA from PBMCs of patients and contacts. Total RNA was reverse transcribed into cDNA, amplified by PCR with cytokine-specific primers or primers for the housekeeping gene HPRT, and then visualized by Southern blot hybridization. The intensity of the signals generated was assessed by densitometry. Results are given as ratios of optical densities of cytokine to HPRT. At 24 h, the ratios of IFN-γ/HPRT signals generated in response to the 30-kDa antigen were 0.07 and 0.39, respectively, in patients and contacts and 0.21 and 6.62 in response to PPD (data not

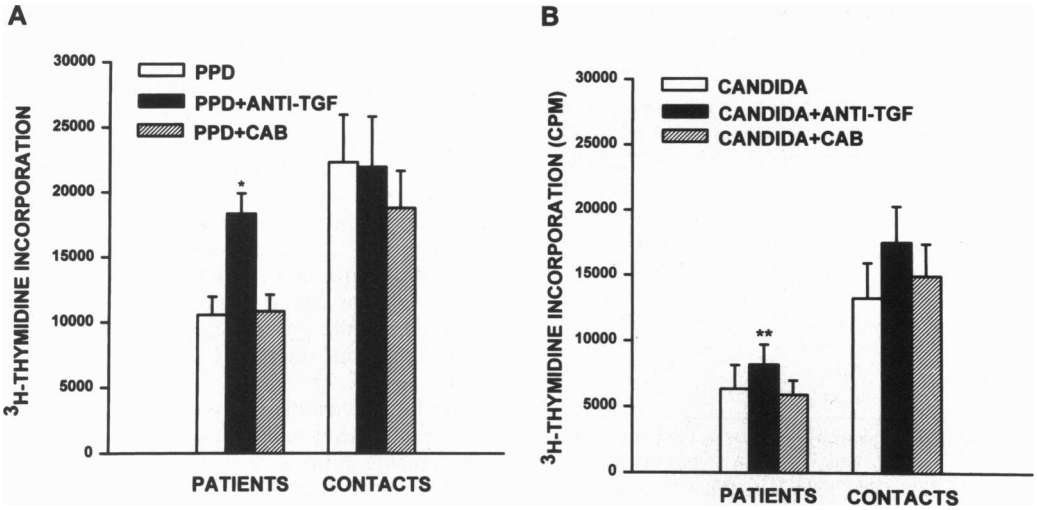

FIG. 1. Effect of neutralization of TGF-β on blastogenesis in TB patients and healthy household contacts. PBMCs from patients and contacts were incubated with appropriate antigens in the presence or absence of neutralizing or control antibody (CAB) to TGF-β for 6 days. [^{3}H]Thymidine was added for the final 18 h of culture and incorporation of radioactivity was assayed by liquid scintillation counting. Effect of neutralizing or control antibody to TGF-β on PPD- (*A*) or candida- (*B*) induced lymphocyte proliferation. Bars represent means ± SE (cpm) of 20 consecutive experiments. $P \leq 0.001$ (*) and $P \leq 0.04$ (**) when compared to cultures in the absence of antibody.

shown). Levels of IFN-γ mRNA (corrected for HPRT) by reverse transcription PCR assay at 72 h correlated with results for secreted protein (Fig. 4); when applying densitometry, PBMCs of TB patients expressed less IFN-γ than did those of contacts; coculture with neutralizing antibody to TGF-β increased expression of cytokine-specific mRNA to levels comparable to those found in contacts. The ratio of signals generated from PBMCs of patients cocultured with neutralizing antibody to TGF-β increased 3-fold in patients but only 1.3-fold in controls. mRNA for IL-4 was present in comparable amounts in samples obtained from both patients and contacts (Fig. 4).

DISCUSSION

The profile of cytokines produced in response to infectious agents is a major determinant of resistance or susceptibility to disease. Thus, modulation of cytokine expression by MTB and its major protein and polysaccharide antigens is an essential feature of the pathogenesis of TB (16, 34–36). Here we add TGF-β and IL-10 to the list of cytokines produced by PBMCs of TB patients and healthy contacts in response to both PPD and the 30-kDa antigen. Furthermore, the results of this study indicate a prominent role for TGF-β in depressing T-cell blastogenesis and IFN-γ responses in TB patients.

TGF-β, a product of activated MNs and other inflammatory cells, exhibits a panoply of immunomodulatory functions including inhibition of T-cell and B-cell mitogenesis, attenuation of generation and cytotoxicity of natural killer cells and T cells, reduction of monocyte HLA-DR expression, and down-regulation of IFN-γ, TNF-α, IL-1, and IL-6 protein release (17–20, 37–42). TGF-β is expressed in increased quantities in Langhans giant cells and epithelioid cells of tuberculous pulmonary granulomas (14). Additionally, the spontaneous release of immunoreactive and bioactive TGF-β is higher in MNs of patients with TB than in MNs of healthy tuberculin reactors (14). TGF-β is produced by MNs infected with MTB

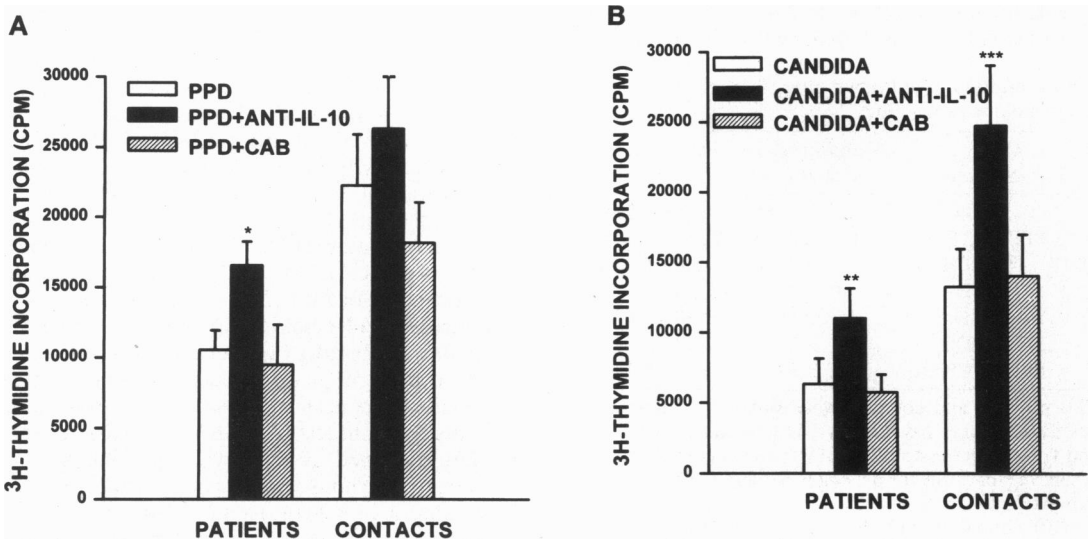

FIG. 2. Effect of neutralization of IL-10 on lymphocyte proliferation in TB patients and PPD-positive household contacts. PBMCs were cultured in the presence or absence of neutralizing or control antibody (CAB) to IL-10. Blastogenesis to PPD (*A*) or candidal antigen (*B*) in the presence or absence of neutralizing or control antibody to IL-10. Bars represent means ± SE (cpm) of 20 consecutive experiments. $P \leq 0.001$ (*), $P \leq 0.006$ (**), and $P \leq 0.001$ (***) when compared to cultures in the absence of antibody.

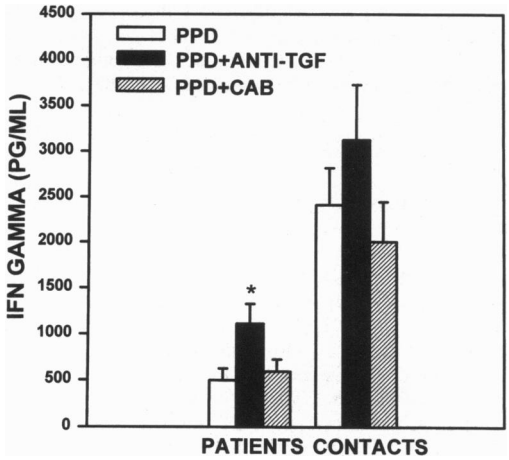

FIG. 3. Production of IFN-γ by cells of patients and contacts cocultured with neutralizing antibody to TGF-β. PBMCs were cultured with or without neutralizing or control antibodies (CAB) to TGF-β for 72 h. IFN-γ concentration in supernatants was assessed by ELISA. Bars represent means ± SE of cytokine concentration. $P \leq 0.002$; $n = 12$ (*).

(H37Ra) *in vitro* and acts to promote intracellular myocabacterial replication and block macrophage activation by IFN-γ and TNF-α (16).

In the current study, levels of TGF-β were increased in PBMC supernatants of patients with TB, but not significant differences were apparent in the amount of IL-10 produced by PBMCs of patients or healthy contacts. Both IL-10 and TGF-β appeared to play a role, however, in depressed lymphoproliferative responses in TB patients as evidenced by enhanced blastogenesis in cells cultured in the presence of neutralizing antibody to either cytokine. Antibody to TGF-β had its most

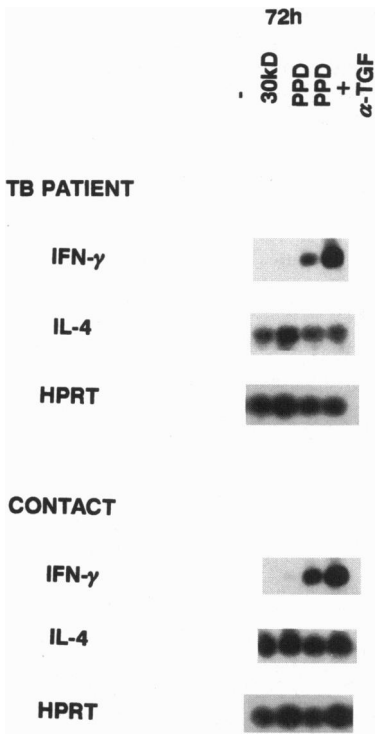

FIG. 4. IFN-γ, IL-4, and HPRT gene expression as assessed by reverse transcription PCR in PBMCs of patients and contacts cultured in the presence or absence of neutralizing antibody to TGF-β. Cytokine gene incubation after 72 h of culture. Lane 1, IFN-γ; lane 2, IL-4; lane 3, HPRT. One representative experiment of six performed is shown.

pronounced action on MTB antigen-driven blastogenesis in TB patients, which, in fact, normalized. Administration of neutralizing antibody to TGF-β did not affect IL-10 levels in culture supernatants of patients or contacts (data not shown), thus suggesting independent effects of TGF-β and IL-10. As TGF-β can modulate expression of cell surface receptors (43, 44), it is possible, however, that increased amounts of TGF-β available in cell cultures of TB patients enhanced sensitivity to IL-10 by up-regulating its receptor. It should also be noted that antibody to TGF-β had a greater effect on blastogenesis than on IFN-γ production. The limitation in cell number did not allow study of the regulatory role of IL-10 on IFN-γ production. It may well be the case, however, that IL-10 or other cytokines have an important adjunctive role in modulating IFN-γ production. A recent study in leprosy also linked IL-10 to depressed lymphocyte proliferation (31). In contrast to our findings, however, this effect was limited to responses to mycobacterial antigens. This discrepancy may relate to a differential capacity of the control antigens tetanus toxoid, used in the study by Sieling *et al.* (27), and candida, used in this study, to induce production of IL-10. In fact, we found candida to be a stimulus for induction of IL-10, TGF-β, and IFN-γ protein release from PBMCs of healthy individuals (data not shown).

Interestingly, both T-cell blastogenesis and cytokine levels differed between groups of patients undergoing therapy for <1 month and those on treatment for >1 month. Our earlier studies also indicated that blastogenesis normalized and that adherent suppressor cell activity declined with treatment (1). The current studies suggest that the mediator of this suppressive activity is TGF-β overproduced when MNs primed *in vivo* are restimulated by PPD or the 30-kDa antigen *in vitro*. Although the data are presented for TGF-β production by PBMCs, the cell population ultimately responsible for production of the cytokine was the MN (data not shown). It is presumably the case that treatment, with the associated declining bacterial load and inflammation, diminishes the priming of MN *in situ*, resulting in production of less amounts of TGF-β and restoration of T-cell responses.

An issue of considerable importance is the role of TGF-β in the local immunopathogenesis of TB. Circulating PBMCs clearly are the source of cells that constitute granulomas and the alveolitis in TB. Recent data indicate influx of a substantial number of peroxidase-positive MNs into the affected lung segments of TB patients (X. Schwander, personal communication). TGF-β expression has been demonstrated in Langhans multinucleated giant cells and epithelioid cells in TB granulomas (14) and more recently in MNs found in bronchoalveolar lavage cells and fluid from TB patients (Z.T., unpublished observation). It therefore seems likely that the deactivating and immunosuppressive properties of TGF-β are expressed locally as well as systemically and contribute to immunopathogenesis at the sites of disease. It should be noted that this scenario differs markedly from findings in TB pleuritis. The latter is a self-curing process in which Th1 cytokines predominate (11, 12). These Th1 cytokines are produced by antigen-specific T cells that have undergone *in situ* expansion in an area devoid of adherent suppressor cell activity (45). Pulmonary TB, on the other hand, is a progressive process requiring treatment with chemotherapy in which the immune response contributes to disease pathogenesis.

Depressed cellular immune responses in parasitic disease and leprosy also have been ascribed to IL-4 (27, 32, 33, 46). Its effect is linked to down-regulation of secretion of IFN-γ and enhanced production of antibodies, thus effecting a switch between cellular and humoral immunity. In the current study, IL-4 immunoactivity was not demonstrable in supernatants of PBMCs from either TB patients or contacts but cellular mRNA was constitutively expressed in both. Another study comparing HIV-seronegative and HIV-seropositive TB patients similarly found no IL-4 protein in supernatants of MTB

antigen-stimulated PBMCs of either group; mRNA was present in low levels in PBMCs of both (47). Consonant with the study described above but in contrast to work by Surcel *et al.* (10), our findings at both the protein and the gene level suggest that IL-4 is not directly involved in depressed cellular immunity in TB.

In conclusion, these data indicate that TGF-β, produced in response to MTB antigens, plays an important role in muting potentially protective host effector functions such as production of IFN-γ. TGF-β therefore looms as an important mediator of immunosuppression in TB and is a logical target for immune-based therapies in the future.

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