INFECTION AND IMMUNITY, Sept. 1987, p. 2126–2131
0019-9567/87/092126-06$02.00/0

Vol. 55, No. 9

Recombinant Human Interleukin-2 Reverses In Vitro-Deficient Cell-Mediated Immune Responses to Tuberculin Purified Protein Derivative by Lymphocytes of Tuberculous Patients

HIROE SHIRATSUCHI,* YASUHISA OKUDA, AND IZUO TSUYUGUCHI

Osaka Prefectural Habikino Hospital, Habikino-shi, Osaka 583, Japan

Received 4 February 1987/Accepted 9 June 1987

In vitro lymphocyte proliferative response to purified protein derivative of tuberculin (PPD) was investigated in patients with tuberculosis. Peripheral blood lymphocytes (PBL) from patients with advanced, refractory tuberculosis showed a significantly depressed response compared with the response of PBL from patients with newly diagnosed tuberculosis ($P < 0.01$). A further characterization of this low responsiveness to PPD revealed that PBL from these advanced tuberculous patients failed to generate interleukin-2 (IL-2) in response to PPD stimulation. IL-2 receptor (Tac antigen) expression on the surface of T cells after PPD stimulation was also impaired, although to a lesser extent, in the patients with advanced, refractory tuberculosis. We attempted to overcome the depressed in vitro response observed in PBL from patients with advanced, refractory tuberculosis and found that the addition of exogenous, recombinant IL-2 returned the depressed PPD-induced PBL proliferation in these patients to the level of response observed in PBL from patients with newly diagnosed tuberculosis. The addition of recombinant IL-2 also had a restorative effect (up regulation) in vitro on the partly impaired PPD-induced IL-2 receptor expression by PBL from the patients with advanced, refractory tuberculosis. Our results suggest that recombinant IL-2 may offer a novel approach to the therapy of advanced, drug-resistant tuberculosis.

In tuberculosis, the T-cell-mediated immune response plays an important role in the pathogenesis of the disease and also in the protective immunity against the bacillus. This T-cell-mediated immune response can be detected in vitro by lymphocyte transformation in response to purified protein derivative of tuberculin (PPD). It is widely accepted that antigen-induced T-cell proliferation requires both the expression of receptors for and the secretion of interleukin-2 (IL-2). Subsequent interaction of IL-2 with its receptors leads to DNA synthesis and expansion of appropriate clones of antigen-reactive T cells (2, 7). The concentration of IL-2 and the expression of IL-2 receptors on T cells determine the extent of T-cell proliferation (3).

Tuberculosis is a chronic infectious disease and exhibits a broad clinical as well as immunological spectrum (1) as has been seen in leprosy. Leprosy is associated with alterations of immune responses which vary along the spectrum of the disease (28). Patients with lepromatous leprosy show an impaired cell-mediated immunity to *Mycobacterium leprae* antigen. A number of studies about this anergy in lepromatous leprosy have been described, including impaired IL-2 production by lymphocytes after lepromin stimulation (13–16, 20, 24). Haregewoin et al. (13) reported that peripheral blood lymphocytes (PBL) of patients with lepromatous leprosy fail to proliferate after in vitro stimulation with *M. leprae* antigen but that the cells are made fully reactive by the addition of exogenous IL-2.

Patients with far-advanced or miliary tuberculosis are often anergic to tuberculin PPD, as judged by in vivo skin testing and in vitro lymphocyte transformation (18, 23). However, little is known about the mechanisms underlying the anergy in tuberculosis. In both humans and mice (36), it has been shown that antigen-specific unresponsiveness is accompanied by a failure of T cells to release IL-2. Some possible reasons for tuberculin anergy include a relative loss of IL-2-producing cells, refractoriness of IL-2-producing cells to the putative stimulator (i.e., IL-1), and unresponsiveness of effector T cells to IL-2 stimulation. In experimental systems, T suppressor cells (12, 19, 21, 25) and T-cell-derived suppressor factors (17) have been described. Previously, we reported an increase in the percentage of OKT8-positive (suppressor/cytotoxic) T cells in advanced refractory tuberculosis after in vitro PPD stimulation (31).

In the present study, attempts were made to characterize further the low PPD-induced PBL transformation in advanced tuberculosis. The results obtained show that the low responsiveness to PPD was mostly due to the impaired IL-2 production. PPD-induced acquisition of IL-2 responsiveness was, however, not impaired. Therefore, the addition of exogenous IL-2 substantially restored the PPD-induced proliferative responses in PBL from patients with advanced, refractory tuberculosis, up to the high level of responsiveness seen in PBL from newly diagnosed tuberculosis patients.

MATERIALS AND METHODS

Patients. A total of 46 patients with active pulmonary tuberculosis, all of whom were inpatients in our hospital, were studied. These patients were divided into two groups: 23 patients (17 males and 6 females, 1 to 73 years old; mean age, 41 years) with newly diagnosed tuberculosis and 23 patients (18 males and 5 females, 26 to 70 years old; mean age, 51 years) with advanced, refractory tuberculosis. Patients classified as having newly diagnosed tuberculosis were those in whom pulmonary tuberculosis was diagnosed for the first time by chest X ray and by the demonstration of acid-fast bacilli in the sputum. Patients considered to have advanced, refractory tuberculosis were those who had been hospitalized for several years without improvement. Cultures of sputum specimens from patients with advanced,

* Corresponding author.

TABLE 1. In vitro proliferative response to PPD or lectin stimulation

Group	[^{3}H]thymidine incorporated (cpm, 10^3) ± SD with[a]:			
	PPD	ConA	PHA	PWM
Pulmonary tuberculosis				
Advanced (n = 23)	20.0 ± 10.8	14.4 ± 7.0	24.3 ± 8.5	34.4 ± 9.4
Newly diagnosed (n = 23)	30.5 ± 10.5[b]	16.7 ± 5.9	27.0 ± 8.8	35.3 ± 7.3
Healthy controls (n = 32)	22.6 ± 9.1	14.7 ± 4.7	28.8 ± 8.3	27.0 ± 9.9[c]

[a] PBL (10^6/ml) were cultured in vitro for 3 days with ConA (10 μg/ml) or PHA (1 μg/ml) or for 6 days with PPD (10 μg/ml) or PWM (2.5 μg/ml).
[b] Significantly different from results with advanced tuberculosis ($P < 0.01$) and from results with healthy controls ($P < 0.05$).
[c] Significantly different from results with advanced tuberculosis ($0.01 < P < 0.02$) and from results with newly diagnosed tuberculosis ($P < 0.01$).

refractory tuberculosis were continuously positive for acid-fast bacilli resistant to almost all antituberculosis drugs. Most patients with newly diagnosed tuberculosis showed a positive delayed-type skin reaction (erythema, induration, and in some cases, blister formation) to the standard dose (5 TU) of tuberculin injection, whereas most of the patients with advanced, refractory tuberculosis showed weak (erythema only) or no reaction at all to the standard dose of tuberculin. Healthy volunteers (16 males and 16 females, 20 to 69 years old; mean age, 37 years), all of whom were positive for the tuberculin skin test, served as control subjects.

PBL preparation. PBL were separated from heparinized venous blood by the Ficoll-Hypaque sedimentation method.

Antigen and lectins. PPD was kindly donated by the Institute for Microbial Diseases, Osaka University, Japan. Concanavalin A (ConA) was purchased from Miles-Yeda Ltd., Rehovot, Israel, phytohemagglutinin (PHA) was obtained from Difco Laboratories, Detroit, Mich., and pokeweed mitogen (PWM) was obtained from GIBCO Laboratories, Grand Island, N.Y.

Preparation of rIL-2. Highly purified human recombinant IL-2 (rIL-2), obtained from *Escherichia coli* carrying the cloned human IL-2 gene, was a gift from Takeda Chemical Industries, Ltd., Osaka, Japan, and was used as a source of exogenous IL-2 at a final concentration of 10 ng of protein per ml. This material yields only a single band at 15,000 daltons by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (22). A 1-mg amount of this rIL-2 contained 4×10^6 units in our assay system as described below.

Generation of IL-2-containing supernatants and assay for IL-2. For PPD-driven IL-2 production, PBL at 2×10^6/ml were activated with 10 μg of PPD or ConA per ml. After 24 h, the supernatants were collected, sterile filtered, and assayed for IL-2 activity. The IL-2 activity of culture supernatants was determined on the basis of incorporation of [^{3}H]thymidine by the IL-2-dependent murine cytotoxic T-cell line NRB (22). NRB was kindly provided by Eiichi Nakayama of the Center for Adult Disease, Osaka, Japan. NRB was maintained in the presence of rat spleen-conditioned medium. Units of activity were defined as the reciprocal of the dilution necessary to obtain half-maximal incorporation of [^{3}H]thymidine by the NRB cells. In the absence of added IL-2, the background incorporation was only 200 to 400 cpm; peak incorporation (at 8 U of IL-2 per ml) was approximately 4×10^4 cpm.

PBL activation with PPD and analysis for IL-2 receptors. PBL (10^6/ml) were cultured in a 24-well culture plate (no. 3047; Becton Dickinson Labware, Oxnard, Calif.) at 37°C and 7.5% CO_2 in air for 6 days with 10 μg of PPD per ml. The culture medium used was RPMI 1640 supplemented with 10% fetal calf serum. At the end of the culture period, the cells were harvested, washed twice, suspended in RPMI 1640 medium containing 10% fetal calf serum, and assayed for IL-2 receptors. IL-2 receptors were determined by using monoclonal anti-Tac antibody (35), which was kindly donated by T. Uchiyama and J. Yodoi, Kyoto University, Japan. Cell suspension (100 μl) was incubated with anti-Tac antibody (1/5,000 dilution in ascites fluid) for 30 min at 4°C and washed, and 100 μl of fluorescein-conjugated rabbit anti-mouse immunoglobulin G antibody (1/20) (Miles-Yeda) was then added. After further 30-min incubation on ice, the cells were washed and suspended in 1 drop of fetal calf serum, and the number of positive cells was assessed by using a fluorescence microscope. Tac-positive cells were blastogenic and belonged to E-rosetting T cells.

In vitro assay of proliferative responses. The in vitro proliferative responses were measured by a modification of the method reported previously (30, 33). In brief, 5×10^4 cells in 0.2 ml of RPMI 1640 medium containing 10% heat-inactivated pooled human sera were placed in a flat-bottom microplate (no. 3072; Becton Dickinson) with PPD or lectins and were cultured for 3 days with 10 μg of ConA or 1 μg of PHA per ml or for 6 days with 10 μg of PPD or 2.5 μg of PWM per ml in humidified 7.5% CO_2 in air at 37°C. [^{3}H]thymidine (0.2 μCi) was added to each well 18 h before the cells were harvested, and the incorporated radioactivity was counted in a scintillation counter (Tricarb; Packard Instrument Co., Inc., Rockville, Md.). Each determination was performed in triplicate, and the data were expressed as counts per minute ± the standard error of the mean.

To assess the effects of exogenous IL-2 on antigen-specific proliferation, 20 μl of rIL-2 (10 ng/ml) was added to the test culture at day 3 of the culture period. Appropriate control cultures were set up containing the same dose of rIL-2 but no antigen. Results of dose-response experiments with rIL-2 indicated that a dose of 10 ng of rIL-2 per ml gave the highest enhancing effect on PPD culture, while giving lowest values in control cultures.

Statistical analysis. The significance of the difference between groups was calculated by Student's t test. Computer-assisted evaluations of the results were used to calculate the P value in the data. A P value of 0.05 was used as the limit of statistical significance.

RESULTS

Proliferative responses to PPD and lectins. PBL from patients with pulmonary tuberculosis and from tuberculin skin test-positive healthy donors were cultured with PPD or lectins (ConA, PHA, and PWM), and the proliferative responses were evaluated (Table 1). There was a significant difference between the two groups of tuberculous patients in responsiveness of PBL to PPD. The extent of proliferation of

TABLE 2. IL-2 activity in the supernatant of PPD- or ConA-stimulated cultures

Group	IL-2 units (no. of subjects) with[a]:	
	PPD	ConA
Pulmonary tuberculosis		
Advanced	4.7 ± 1.6 (n = 11)[b]	20.7 ± 17.3 (n = 9)
Newly diagnosed	15.2 ± 13.1 (n = 12)	24.6 ± 3.1 (n = 5)
Healthy controls	9.0 ± 7.2 (n = 13)	18.5 ± 13.8 (n = 7)

[a] PBL (2×10^6/ml) were cultured for 24 h in the presence of PPD (10 μg/ml) or ConA (10 μg/ml). Culture supernatants were assayed for IL-2 activity. For a definition of units, see Materials and Methods.

[b] $0.01 < P < 0.05$ compared with results from newly diagnosed tuberculosis.

PBL from advanced tuberculous patients was considerably lower than that of PBL from patients with newly diagnosed tuberculosis ($P < 0.01$) but comparable with that of PBL from healthy controls. PBL from patients with newly diagnosed tuberculosis showed a higher proliferation in response to PPD stimulation than PBL from healthy controls ($0.01 < P < 0.05$). In the responses to ConA or PHA, no significant differences were seen among the groups examined. On the other hand, the responses to PWM were significantly higher in PBL from tuberculous patients than in PBL from healthy controls ($0.01 < P < 0.02$).

IL-2 production. PBL (2×10^6) were stimulated with PPD or ConA, and the culture supernatants were harvested after centrifugation for 10 min at 2,000 rpm, membrane filtered (Millipore Corp., Bedford, Mass.), and subjected to the IL-2 assay as described above. PPD-stimulated cultures of PBL from patients with newly diagnosed tuberculosis gave higher IL-2 activity than did PPD-stimulated PBL from patients with advanced tuberculosis ($0.01 < P < 0.05$), whereas no significant differences were observed in IL-2 activity in ConA-stimulated culture supernatants among the groups examined (Table 2).

PPD-induced IL-2 receptor (Tac antigen) expression. T-cell proliferation is dependent on the interaction of IL-2 with its cellular receptor. We examined the effect at PPD-induced IL-2 receptor expression on PBL after 6 days of in vitro culture. IL-2 receptor-bearing lymphocytes were assessed by using monoclonal anti-Tac antibody as described in Materials and Methods. The percentage, as well as the total number, of Tac^+ cells recovered in each lymphocyte group was significantly increased after in vitro stimulation with PPD ($P < 0.01$ compared with the control culture without PPD) (Table 3). It is noteworthy that after PPD stimulation, the Tac^+ cells were substantially increased in PBL from patients with advanced, refractory tuberculosis, although the extent of the response was not so large as that of PBL from patients with newly diagnosed tuberculosis or healthy controls.

These results suggest that the low proliferative response to PPD of PBL from the patients with advanced tuberculosis is due to the PPD-induced depressed IL-2 production and not to the depressed expression of IL-2 receptors. The addition of exogenous IL-2 was, therefore, expected to restore the proliferative response or to increase IL-2 receptor expression or both in PPD-treated cultures of PBL from such patients.

Effects of exogenous IL-2 (rIL-2) on the proliferative responses to PPD. Next, we examined whether the defective responses of PBL from advanced tuberculous patients were restored by the addition of exogenous IL-2 (Fig. 1 and 2). PBL were cultured in the presence of PPD, and rIL-2 was added at day 3 of the culture period. rIL-2 significantly increased the PPD-induced proliferation of PBL from advanced tuberculous patients ($P < 0.01$) (Fig. 1). The proliferative responses of their PBL were restored by the addition of rIL-2 to the level of PPD-induced proliferation of PBL from patients with newly diagnosed tuberculosis. The augmenting effect of the addition of rIL-2 was seen in PBL of patients with advanced, refractory tuberculosis, whereas no significant increase was seen in responses to PPD of PBL from patients with newly diagnosed tuberculosis or from healthy controls (Fig. 2).

Effect of rIL-2 on PPD-induced Tac expression. As described above, rIL-2 augmented the PPD-induced proliferation of PBL from patients with advanced, refractory tuberculosis. We further investigated whether rIL-2 also increased the PPD-induced IL-2 receptor (Tac) expression. Tests were performed on PBL from 7 advanced tuberculosis patients, 7 newly diagnosed tuberculosis patients, and 11 healthy donors (Table 4). The addition of rIL-2 to the PPD-stimulated PBL culture caused significant augmentation of the PPD-induced increase in Tac-positive cells in cultures of PBL from patients with advanced tuberculosis (36.1 ± 12.0% versus 22.7 ± 12.5%, $P < 0.01$) and in cultures from healthy controls (34.3 ± 11.4% versus 27.2 ± 10.3%, $P < 0.01$), whereas no significant augmentation by rIL-2 addition was observed in cultures from patients with newly diagnosed tuberculosis (41.0 ± 15.0% versus 35.3 ± 13.0%, $0.2 < P$).

DISCUSSION

The present study demonstrates that PBL from patients with advanced, refractory tuberculosis had defective PPD-induced in vitro proliferative responses. The responses of these PBL to the nonspecific mitogens ConA, PHA, and PWM, however, were comparable to those of patients with newly diagnosed tuberculosis or those of healthy donors. PPD-induced T-cell proliferation consists of two parts: IL-2 production and IL-2 receptor expression by sensitized T cells. In patients with advanced, refractory tuberculosis, PPD-induced IL-2 production, but not IL-2 responsiveness, was impaired as compared with production and responsiveness in PBL from newly diagnosed tuberculosis patients or healthy individuals, although the PPD-induced increase of Tac-positive cells was also not as high in advanced, refractory tuberculosis patients. The addition of rIL-2 to the culture, however, restored the impaired PPD-induced proliferative responses of PBL from advanced, refractory tuberculosis patients by the stimulation with PPD.

Vismara et al. (36) reported that the proliferative unresponsiveness observed in human PBL to antigenic extract from *Candida albicans* or to PPD of the spleen cells of mice infected with a high dose of *Mycobacterium bovis* BCG was due to a primary lack of IL-2 production. In the present study, Tac antigen-positive, IL-2 receptor-bearing T cells which increased after in vitro PPD stimulation were further increased in PBL of advanced tuberculosis patients by the addition of exogenous IL-2. In antigen-specific T-cell activation, the IL-2 that was produced up regulated its own receptors on activated T cells and triggered the proliferation of T cells (27, 37). Thus, in advanced, refractory tuberculosis an impaired IL-2 production gave an impaired IL-2 expression and led to an impaired proliferation of PPD-reactive T cells.

One plausible explanation for the impaired PPD-induced proliferation of PBL in advanced, refractory tuberculosis is

TABLE 3. Increase in Tac-positive cells upon PPD stimulation

Group	No. of Tac-positive cells ± SD with or without PPD[a]			
	% of total		No. of Tac-positive cells (10^{-4})/culture	
	No PPD	PPD	No PPD	PPD
Pulmonary tuberculosis				
Advanced (n = 23)	4.7 ± 2.4	21.3 ± 11.4[b]	1.7 ± 1.1	12.7 ± 9.9[b]
Newly diagnosed (n = 23)	5.3 ± 2.2	27.1 ± 14.7	2.6 ± 1.5	23.0 ± 20.8
Healthy controls (n = 32)	5.1 ± 2.6	30.3 ± 9.8	2.5 ± 1.5	25.1 ± 16.5

[a] PBL (10^6/ml) were cultured in vitro in the presence or absence of PPD for 6 days. Tac-positive lymphocytes were assessed by the indirect-fluorescence technique.
[b] $0.01 < P < 0.05$ compared with healthy controls.

that suppressor cells might be generated and inhibit PPD-induced IL-2 production. We reported previously (34) that in advanced, refractory tuberculosis, immunoglobulin G Fc receptor-bearing T cells or OKT8-positive T cells (31) were increased in vitro after stimulation of PBL with PPD. The Fc receptor-bearing T cells that were isolated suppressed the PPD-induced proliferative response of autologous PBL. In advanced tuberculosis, PBL depleted of OKT8-positive cells gave a higher proliferative response to stimulation with PPD than unfractionated PBL (31) did. In experimental systems, mice injected intravenously with a high dose of BCG failed to develop delayed-type hypersensitivity to BCG and were described as anergic (6). Colizzi et al. (5) demonstrated that spleen cells from these anergic mice produce inhibitory factors which block the IL-2 production. This state of unresponsiveness can be reversed both in vitro (14) and in vivo (4) by the administration of an IL-2-containing preparation. Together with these observations, our results suggest that in advanced, refractory tuberculosis, the proliferative T-cell defect in response to PPD stimulation is not due to the absence of or a generalized lack of triggering of immune T cells but may be related to a deficiency in events leading to IL-2 production. Addition of rIL-2 also augmented the PPD-induced Tac-antigen-positive (IL-2 receptor-bearing) T cells in PBL from advanced, refractory tuberculosis patients.

Recently, Toossi et al. (32) described a similar in vitro analysis of lymphocytes of patients with pulmonary tuberculosis. They reported that patients with newly diagnosed pulmonary tuberculosis have a tuberculin-specific defect in IL-2 production. However, purified IL-2 fails to correct

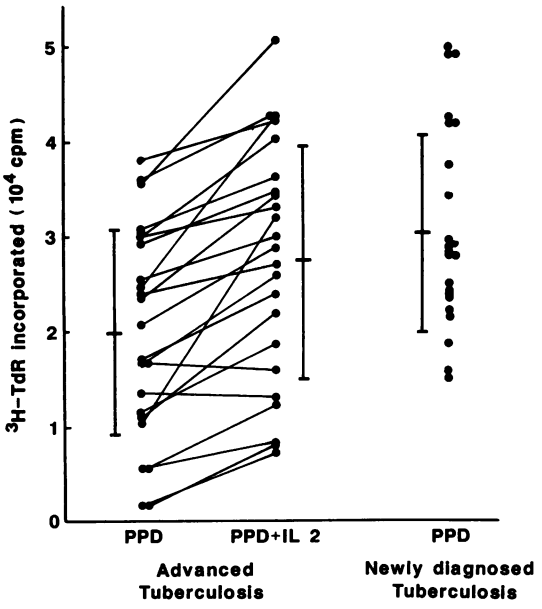

FIG. 1. Effect of exogenous IL-2 addition on PPD-induced in vitro proliferation of PBL from patients with advanced tuberculosis. PBL (5×10^4 per well) were cultured in vitro in the presence of PPD (10 μg/ml) for 6 days. rIL-2 (10 ng/ml) was added to the culture on day 3. The results are expressed as counts per minute of [^{3}H]thymidine incorporated during the last 18 h of the culture period. The points connected by a line represent data from the same donor.

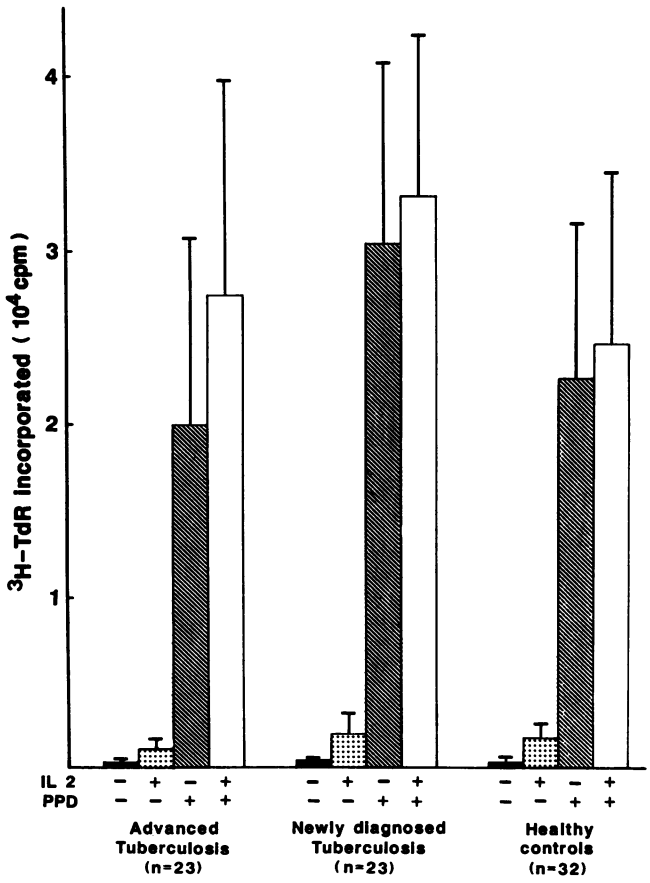

FIG. 2. Summarized data of the effects of exogenous rIL-2 addition on PPD-induced PBL proliferation. The results are expressed as mean counts per minute of [^{3}H]thymidine incorporation ± the standard deviation (bar).

TABLE 4. Effects of rIL-2 addition on PPD-induced increase of Tac-positive cells

Group and case no.	% Tac-positive cells with or without PPD and rIL-2[a]					
	No PPD			PPD		
	No rIL-2	rIL-2	% Increase	No rIL-2	rIL-2	% Increase
Pulmonary tuberculosis						
Advanced						
1	3.5	12.1	8.6	17.3	28.5	11.2
2	3.6	9.7	6.1	16.7	32.8	16.1
3	4.3	6.8	2.5	12.8	34.3	21.5
4	3.7	13.5	9.8	45.8	56.3	10.5
5	3.4	5.3	1.9	5.9	15.4	9.5
6	3.2	4.4	1.2	29.7	44.0	14.3
7	2.6	5.0	2.4	30.9	41.4	10.5
Mean	3.5 ± 0.5	8.1 ± 3.4	4.6 ± 3.2	22.7 ± 12.5	36.1 ± 11.9	13.4 ± 4.0
Newly diagnosed						
1	5.3	5.3	0.0	49.1	46.1	−3.0
2	10.8	10.8	0.0	45.1	43.6	−1.5
3	3.2	5.1	1.9	21.2	22.8	1.6
4	2.6	9.2	6.6	24.1	29.7	5.6
5	6.3	13.0	6.7	40.7	58.3	17.6
6	2.4	10.2	7.8	49.6	63.1	13.5
7	2.2	5.9	3.7	17.0	23.7	6.7
Mean	4.7 ± 2.9	8.5 ± 2.9	3.8 ± 3.0	35.3 ± 13.0	41.0 ± 15.0	5.8 ± 7.0
Healthy controls (n = 11)	4.1 ± 2.0	6.2 ± 2.0	2.1 ± 2.0	27.2 ± 10.3	34.3 ± 11.4	7.1 ± 6.5

[a] PBL (10^6/ml) were cultured in vitro for 6 days in the presence or absence of PPD and rIL-2 (10 ng/ml). Tac antigen-positive cells were assessed by the indirect-immunofluorescence method.

PPD-induced blastogenesis in these patients. In Japan, most patients with newly diagnosed tuberculosis reveal positive tuberculin skin reactions and their PBL are highly responsive in vitro to PPD stimulation, as has been shown, with a few exceptions, for the patients in the present study. In our study, PBL from patients with newly diagnosed tuberculosis showed the highest response to PPD stimulation (Table 1). In contrast, PBL from patients with advanced, refractory tuberculosis with a disease history of greater than 3 years showed significantly lower PPD responses than PBL from newly diagnosed tuberculous patients did, although sputum specimens from the former group were continuously positive for acid-fast bacilli resistant to antituberculosis drugs. In addition, in the present study, Tac antigen-positive, IL-2 receptor-bearing cells did appear in vitro after stimulation with PPD of PBL from advanced, refractory tuberculosis patients; the cells were further increased upon introduction of rIL-2 (Tables 3 and 4). Our results indicate that even in PBL from patients with advanced, refractory tuberculosis, PPD-sensitized T cells did exist and became responsive to exogenous IL-2 after PPD stimulation.

A lack of IL-2 production has also been reported to be responsible for T-cell unresponsiveness in several other diseases. T-cell unresponsiveness in Hodgkin's disease (10) and rheumatoid arthritis (8) is reversed in vitro by the addition of IL-2. Clinical trials using IL-2 administration in vivo have been conducted in children with Nezelof's syndrome (9) and in cancer patients (29). Preliminary results of these trials have suggested that IL-2 is able to produce a high degree of reconstitution of T-cell activity. In the present study, the restoration by rIL-2 of depressed PPD-induced T-cell proliferation in advanced tuberculosis was not complete (Fig. 2). Monocyte-mediated suppressor abnormalities have been reported in Hodgkin's disease (11) and diffuse cutaneous leishmaniasis (26). In these diseases, the addition of indomethacin, an inhibitor of prostaglandin synthesis, to cultures restores the lymphocyte responses. In addition to the deficiency of IL-2 production, prostaglandin-mediated suppression of IL-2 receptor expression might be partly responsible for the impaired proliferation of PBL from patients with advanced tuberculosis in the present study.

To date, we have no feasible means of controlling far-advanced, drug-resistant tuberculosis. From a number of studies, evidence has accumulated that immunity to intracellular parasites such as *Mycobacterium tuberculosis* or *M. leprae* is effected by T cells. This immunity is mediated by the release of lymphokines which may activate macrophages to kill parasites. Although we are not at all certain that the in vitro studies presented here parallel the in vivo regulation of cellular immunity in advanced, refractory tuberculosis, future studies of the effect of IL-2 administration in vivo on cellular immunity in these patients appear warranted. If the effects in vitro presented here can be reproduced in vivo, in vivo administration of IL-2 might be beneficial for patients with chronic refractory infections.

ACKNOWLEDGMENTS

We thank S. Kishimoto, Osaka University, Japan, for his valuable advice throughout this study and H. Takahashi, the president of our hospital, for his critical review of the manuscript. We also thank T. Uchiyama and J. Yodoi, Kyoto University, Japan, for supplying us with the monoclonal antibody anti-Tac; E. Nakayama, Center for Adult Diseases, Osaka, Japan, for supplying us with the murine cytotoxic T-cell line NRB; and Central Research Division, Takeda Chemical Industries, Ltd., Osaka, Japan, for supplying us with rIL-2.

LITERATURE CITED

1. **Bhatnagar, R., A. N. Malaviya, S. Narayanan, P. Rajgopalan, R. Kumar, and O. P. Bharadwaj.** 1977. Spectrum of immune response abnormalities in different clinical forms of tuberculosis. Am. Rev. Respir. Dis. **115**:207–212.
2. **Cantrell, D. A., and K. A. Smith.** 1983. Transient expression of interleukin 2 receptor consequence for T cell growth. J. Exp. Med. **158**:1895–1911.
3. **Cantrell, D. A., and K. A. Smith.** 1984. The interleukin 2 T cell system: a new cell growth model. Science **224**:1312–1316.
4. **Colizzi, V.** 1984. In vivo and in vitro administration of interleukin 2-containing preparation reverses T-cell unresponsiveness in *Mycobacterium bovis* BCG-infected mice. Infect. Immun. **45**:25–28.
5. **Colizzi, V., J. Ferluga, F. Garreau, M. Malkousky, and G. L. Asherson.** 1984. Suppressor cells induced by BCG release nonspecific factors *in vitro* which inhibit DNA synthesis and interleukin-2 production. Immunology **51**:65–71.
6. **Collins, F. M., and S. R. Watson.** 1979. Suppressor T-cells in BCG-infected mice. Infect. Immun. **25**:491–496.
7. **Cotner, T., J. M. Williams, L. Christenson, H. M. Shapiro, T. B. Strom, and J. S. Stomenger.** 1983. Simultaneous flow cytometric analysis of human T cell activation antigen expression and DNA content. J. Exp. Med. **157**:461–472.
8. **Emery, P., G. S. Panayi, and A. M. E. Nouri.** 1984. Interleukin-2 reverses deficient cell-mediated immune responses in rheumatoid arthritis. Clin. Exp. Immunol. **57**:123–129.
9. **Flomenberg, N., K. Welte, R. Mertelsmann, N. Kernan, N. Ciobanu, S. Venuta, S. Feldman, G. Kruger, D. Kirkpatrick, B. Dupont, and R. O'Reilly.** 1983. Immunologic effects of interleukin 2 in primary immunodeficiency. J. Immunol. **130**:2644–2650.
10. **Ford, R. J., J. Tsao, N. M. Kouttab, C. G. Sahasrabuddhe, and S. R. Mehta.** 1984. Association of an interleukin abnormality with the T cell defect in Hodgkin's disease. Blood **64**:386–392.
11. **Goodwin, J. S., R. P. Messner, A. D. Bankhurst, G. T. Peake, J. H. Saiki, and R. C. Williams.** 1977. Prostaglandin-producing suppressor cells in Hodgkin's disease. N. Engl. J. Med. **297**:963–968.
12. **Gullberg, M., and E. L. Larsson.** 1982. Studies on induction and effector functions of concanavalin A-induced suppressor cells that limit TCGF production. J. Immunol. **128**:746–750.
13. **Haregewoin, A., T. Godal, A. S. Mustafa, A. Belehu, and T. Yemaneberhan.** 1983. T-cell conditioned media reverse T-cell unresponsiveness in lepromatous leprosy. Nature (London) **303**:342–344.
14. **Hoffenbach, A., P. H. Lagrange, and M.-A. Bach.** 1983. Deficit of interleukin 2 production associated with impaired T-cell proliferative responses in *Mycobacterium lepraemurium* infection. Infect. Immun. **39**:109–116.
15. **Horwitz, M. A., W. R. Levis, and Z. A. Cohn.** 1984. Defective production of monocyte-activating cytokines in lepromatous leprosy. J. Exp. Med. **159**:666–678.
16. **Kaplan, G., D. E. Weinstein, R. M. Steinman, W. R. Levis, U. Elvers, M. E. Potarroyo, and Z. A. Cohn.** 1985. An analysis of *in vitro* T cell responsiveness in lepromatous leprosy. J. Exp. Med. **162**:917–929.
17. **Kramer, M., and U. Koszinowski.** 1982. T cell-specific suppressor factor(s) with regulatory influence on interleukin 2 production and function. J. Immunol. **128**:784–790.
18. **McMurray, D. N., and A. Echeverri.** 1978. Cell-mediated immunity in anergic patients with pulmonary tuberculosis. Am. Rev. Respir. Dis. **118**:827–834.
19. **Mehra, V., J. Convit, A. Rubinstein, and B. R. Bloom.** 1982. Activated suppressor T cells in leprosy. J. Immunol. **129**: 1946–1951.
20. **Mohagheghpour, N., R. H. Gelber, J. W. Larrick, D. T. Sasaki, P. J. Brennan, and E. G. Engleman.** 1985. Defective cell-mediated immunity in leprosy: failure of T cells from lepromatous leprosy patients to respond to *Microbacterium leprae* is not reconstituted by interleukin 2. J. Immunol. **135**:1443–1449.
21. **Mustafa, A. S., and T. Godal.** 1985. BCG-induced suppressor T cells optimal conditions for *in vitro* induction and mode of action. Clin. Exp. Immunol. **62**:474–481.
22. **Naruo, K., S. Hinuma, K. Kato, M. Koyama, H. Tada, O. Shiho, and K. Tsukamoto.** 1985. Comparison of the biological properties of purified natural and recombinant human interleukin-2. Biochem. Biophys. Res. Commun. **128**:257–264.
23. **Nash, D. R., and J. E. Douglass.** 1980. Anergy in active tuberculosis: a comparison between positive and negative reactors and an evaluation of 5 TU and 250 TU skin test doses. Chest **77**:32–37.
24. **Nath, I., M. Sathish, T. Jayaraman, L. K. Bhutani, and A. K. Sharma.** 1984. Evidence for the presence of *M. leprae* reactive T lymphocytes in patients with lepromatous leprosy. Clin. Exp. Immunol. **58**:522–530.
25. **Ohta, N., M. Minai, and T. Sasazuki.** 1983. Antigen specific suppressor T lymphocytes ($Leu2a^+$ $3a^-$) in human schistosomiasis japonica. J. Immunol. **131**:2524–2528.
26. **Petersen, E. A., F. A. Neva, C. N. Oster, and H. B. Diaz.** 1982. Specific inhibition of lymphocyte-proliferation response by adherent suppressor cells in diffuse cutaneous leishmaniasis. N. Engl. J. Med. **306**:387–392.
27. **Reen, G. H., and N. N. Yeh.** 1984. Interleukin 2 regulates expression of its receptor and synthesis of gamma interferon by human T lymphocytes. Science **224**:429–430.
28. **Ridley, D. S., and W. H. Jopling.** 1966. Classification of leprosy according to immunity: a five group system. Int. J. Lepr. **34**: 255–273.
29. **Rosenberg, S. A., M. T. Lotze, L. D. Muul, S. Leitman, A. E. Chang, S. E. Ettinghausen, Y. L. Matory, J. M. Skibber, E. Shiloni, J. T. Vetto, C. A. Seipp, C. Simpson, and C. M. Reichert.** 1985. Observations on the systemic administration of autologous lymphokine-activated killer cells and recombinant interleukin-2 to patients with metastatic cancer. N. Engl. J. Med. **313**:1485–1492.
30. **Shiratsuchi, H., and I. Tsuyuguchi.** 1981. Tuberculin purified protein derivative-reactive T cells in cord blood lymphocytes. Infect. Immun. **33**:651–657.
31. **Shiratsuchi, H., and I. Tsuyuguchi.** 1984. Analysis of T cell subsets by monoclonal antibodies in patients with tuberculosis after *in vitro* stimulation with purified protein derivative of tuberculin. Clin. Exp. Immunol. **57**:271–278.
32. **Toossi, Z., M. E. Kleinhenz, and J. J. Ellner.** 1986. Defective interleukin 2 production and responsiveness in human pulmonary tuberculosis. J. Exp. Med. **163**:1162–1172.
33. **Tsuyuguchi, I., H. Shiratsuchi, H. Fujiwara, and O. Teraoka.** 1982. Nonspecific recruitment of lymphocytes in purified protein derivative-induced lymphocyte proliferative response of patients with tuberculosis. Infect. Immun. **37**:702–709.
34. **Tsuyuguchi, I., H. Shiratsuchi, O. Teraoka, and T. Hirano.** 1980. Increase in T cells bearing IgG Fc receptors in peripheral blood of patients with tuberculosis by *in vitro* stimulation with purified protein derivative. Am. Rev. Respir. Dis. **121**:951–957.
35. **Uchiyama, T., S. Broder, and T. A. Waldmann.** 1981. A monoclonal antibody (anti-Tac) reactive with activated and functionally mature human T cells. 1. Production of anti-Tac monoclonal antibody and distribution of Tac(+) cells. J. Immunol. **126**: 1393–1397.
36. **Vismara, D., G. Lombardi, E. Piccolella, and V. Colizzi.** 1985. Dissociation between interleukin-1 and interleukin-2 production in proliferative response to microbial antigens: restorative effect of exogenous interleukin-2. Infect. Immun. **49**:298–304.
37. **Welte, K., M. Andreeff, E. Platzer, K. Holloway, B. Y. Rubin, M. A. S. Moore, and R. Mertelmann.** 1984. Interleukin 2 regulates the expression of Tac antigen on peripheral blood T lymphocytes. J. Exp. Med. **160**:1390–1403.