The Journal of Clinical Investigation

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Research Article

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Expression of Functional Interleukin 2 Receptors by Peripheral Blood Monocytes from Patients with Active Pulmonary Tuberculosis

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Abstract

Peripheral blood monocytes from patients with active tuberculosis are "activated" by a number of criteria, including selective depression of T-lymphocyte responses to the mycobacterial antigen, tuberculin-purified protein derivative (PPD). We studied monocytes from patients with tuberculosis and healthy skin test reactive controls for expression and function of IL 2 receptors (IL 2R). Depletion of adherent monocytes increased the IL 2 activity of supernatants of PPD-stimulated T cell cultures from patients by 30-fold. When cultured with purified IL 2, adherent cells from the patients depleted IL 2 activity by 66%. Monocytes from patients displayed IL 2R on their surface as evidenced by anti–Tac reactivity, and released soluble IL 2R into the medium during culture. The release of soluble IL 2R was augmented when monocytes were cultured with PPD. Finally, freshly isolated adherent monocytes from patients but not healthy individuals expressed the gene encoding Tac protein. Thus, blood monocytes from patients with tuberculosis express functional IL 2R constitutively. This property may be important in the immunoregulatory and effector function of mononuclear phagocytes during tuberculosis. (*J. Clin. Invest.* 1990. 85:1777–1784.) monocyte • IL 2R • tuberculosis

Introduction

Blood monocytes from patients with pulmonary tuberculosis (TB)[1] show a number of stigmata of activation. Such cells display increased stimulated release of IL 1 (1), augmented adherence to plastic (2), enhanced hexose monophosphate shunt activity (3), altered surface expression of HLA DR antigens (4), and increased killing of some but not all targets (5). Circulating monocytes from patients with TB also selectively depress lymphocyte responses to the mycobacterial antigen, tuberculin purified protein derivative (PPD) (6, 7); this activity may contribute to depression of delayed-type hypersensitivity (DTH), as assessed by tuberculin skin tests in such patients. The expression of cell-mediated immune response requires the interaction of IL 2 with its receptor (IL 2R) on the surface of antigen-responsive T cells (8). IL 2R also have been demon-

strated on the surface of B cells, and their expression can be induced on human monocytes (9–12). In sarcoidosis, a chronic granulomatous disease characterized by infiltration of the lungs with activated mononuclear cells, bronchoalveolar macrophages express IL 2R (13). We previously have shown depressed T cell expression of IL 2 in patients with active pulmonary TB, in part related to modulatory effects of monocytes (7). Monocytes may down regulate T cell synthesis of IL 2 and/or increase its consumption. The latter is a possibility since enhanced IL 2 absorption by lymphokine activated human myelomonocytic cells, U937 has been demonstrated (9). The biological consequences of IL 2R expression by mononuclear phagocytes, particularly as regards immunoregulation are unclear.

The high affinity ($K_d = 10^{-11}$) IL 2R on the surface of activated human mononuclear cells consist of two polypeptide chains (α and β) which confer the efficient binding of IL 2 to the receptor with subsequent activation of immunologic function (14). The more recently described α chain ($M_r = 75,000$), binds IL 2 sluggishly, however, with an intermediate affinity ($K_d = 10^{-9}$); and while its occupation by the ligand leads to signal transduction, this only occurs at nonphysiologically high levels of IL 2 (15). The β chain, also known as Tac protein ($M_r = 55,000$), reacts with IL 2 more rapidly but with a low affinity ($K_d = 10^{-8}$) (16). A soluble released form of the β chain ($M_r = 45,000$) has been described in several pathologic states (17–19) and in supernatants of lectin activated mononuclear cells (20). The mechanism of release of soluble IL 2R is presently under study; however since soluble IL 2R retain IL 2 binding capacity (21), they may have an immunomodulatory role in disease states.

In the present study we evaluated the expression and function of IL 2R on blood monocytes obtained from patients with TB. Freshly isolated adherent monocytes from patients with newly diagnosed pulmonary TB displayed IL 2R constitutively and such receptors were functional. These monocytes also expressed the gene for the β chain of IL 2R. Additionally, mycobacterial products induced IL 2R gene expression of monocytes from a healthy tuberculin nonreactor. Monocytes cultured from patients with TB released soluble IL 2R into their supernatants, a process augmented by tuberculin PPD. Both the enhanced expression of IL 2R on monocytes and increased release of soluble IL 2R may modulate T cell responsiveness in human TB.

Methods

Study population. Patients with newly diagnosed active pulmonary tuberculosis hospitalized at Cleveland Veterans Administration Hospital, University Hospitals of Cleveland, and Cleveland Metropolitan General Hospital were studied. Patients with factors potentially affecting the immune response were excluded: (*a*) age > 65 yr, (*b*) anti–tuberculosis therapy for > 21 d, (*c*) concomitant debilitating diseases,

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1. *Abbreviations used in this paper:* APAAP, alkaline phosphatase anti–alkaline phosphatase; IFN, interferon gamma; IL 1, interleukin-1; IL 2, interleukin-2, IL 2R, interleukin-2 receptor; PBMC, peripheral blood mononuclear cells; PPD, purified protein derivative; TB, tuberculosis.

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and (d) positive serology for HIV. Diagnosis of TB was based on the demonstration of acid fast bacilli in sputum and was eventually confirmed by positive culture for *Mycobacterium tuberculosis*. Chest roentgenograms of each patient obtained close to the time of study were graded for extent of tuberculosis according to National Tuberculosis and Respiratory Disease Association criteria (22). Of a total of 25 patients studied, one patient had minimal disease, 16 patients had moderately advanced, and eight patients had far advanced disease. Miliary TB was not seen. A group of healthy tuberculin reactors, age and sex-matched to the patients, was studied concurrently. Additionally, three healthy tuberculin nonreactors and three patients with pulmonary infection by mycobacteria other than *M. tuberculosis* were studied. Informed consent was obtained from all patients and control subjects.

Preparation of cells. 50 ml of heparinized blood (20 U/ml) was obtained from each subject by venipuncture. Peripheral blood mononuclear cells (PBMC) were separated by sedimentation on Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ) gradients (23). The monocyte content of PBMC was determined by peroxidase cytochemistry (24). PBMC (5×10^6/ml) were resuspended in RPMI 1640 (MA Bioproducts, Walkersville, MA) supplemented with 10% (vol/vol) heat inactivated human AB serum (Gibco Laboratories, Grand Island, NY) and incubated at 37° for 1 h on plastic petri dishes (100 $\times$ 20 mm; Falcon Labware, Oxnard, CA), which had been precoated with AB serum. After removing nonadherent cells, the adherent monolayers were dislodged by gentle scraping with a rubber policeman. The resultant adherent cell population consisted of 85–95% monocytes (peroxidase staining) and was 99% viable by trypan-blue dye exclusion. The adherent cells were > 85% LeuM3 (monocyte/macrophage; Becton Dickinson, Mountainview, CA) reactive and 5–8% OKT3 (Pan T cell; Orthodiagnostics Inc., Raritan, NJ) positive. In some experiments adherent cells were further depleted of contaminating CD3 T cells by complement lysis. 5 million adherent cells were incubated with 5 μl of OKT3 for 30 min at room temperature. Baby rabbit complement (Cedarlane, Hornby, Ontario, Canada) at a dilution of 1:4 then was added and the cells incubated for another 30 min. Washed cells were < 1% OKT3 reactive.

Nonadherent cells were washed onto 600 mg acid-treated nylon wool columns and incubated at 37°C for 30 min. The lymphocytes then eluted were T cell enriched (> 85% sheep erythrocyte rosetting) and monocyte depleted (< 1% peroxidase positive in both patients and healthy donors), and will be referred to as T cells.

Generation of IL 2-containing supernatants. PBMC or T cells were incubated in 24-well plates (Falcon Labware) at 3×10^6/ml in RPMI 1640 supplemented with L-glutamine (2 mM), penicillin (50 U/ml), gentamicin (50 μg/ml), and 2% (vol/vol) AB serum. PPD, a gift of Lederle Laboratories (American Cyanamid Co., Wayne, NJ) was added to cell cultures at a final concentration of 100 μg/ml. This concentration was found to be optimal for IL 2 generation in a group of healthy donors. After 48 h, supernatants were separated from cells by centrifugation at 500 g for 10 min and stored at −20°C until assayed.

Generation of adherent cell supernatants. Adherent cells were washed and resuspended in RPMI 1640 and 2% (vol/vol) AB serum at 1×10^6/ml in 24-well plates. Cultures were carried out in the absence or presence of purified IL 2 (Electronucleonics, Columbia, MD). This preparation of IL 2 is delectinized with no detectable interferon gamma or endotoxin activity and contains 640 U (BRMP)/ml of IL 2 activity.

IL 2 assay. The IL 2 dependent cytotoxic murine T cell line CTLL-20 was used for assay purposes (25). CTLL-20 cells were washed extensively, and resuspended in DME (MA Bioproducts) at 10^5/ml. 100 μl of the cell suspension was placed in flat bottom microtiter wells (Falcon Labware) along with 100 μl of supernatants in several dilutions. IL 2 determination was performed in triplicate. The total duration of culture was 24 h; 1 μCi of [^{3}H]thymidine specific activity 6.7 Ci/mmol (ICN Radiochemicals, Irvine, CA) was added per well for the last 5 h of culture. Cells then were harvested and ^{3}H activity was

assayed by liquid scintillation spectrometry. Results were analyzed by the probit method (26) using a standard IL 2 supernatant. This preparation of IL 2 is obtained from a gibbon cell line (MLA 144), a constitutive producer of IL 2. The standard contained 30 U/ml of activity in our assay.

Quantitation of IL 2 receptors. The monoclonal antibody anti-Tac (27) reacts with the β chain of human IL 2R and was a generous gift of Dr. T. Waldmann (National Cancer Institute), and it was used at 1:1,000 dilution. Reactivity to anti-Tac antibody was assessed by two methods: FACS analysis or alkaline phosphatase anti-alkaline phosphatase (APAAP) cytostaining (28). In the first, 0.5×10^6 adherent cells were incubated with 50 μl of the antibody at 4°C for 30 min. Washed cells were further incubated with fluorescein isothiocyanate conjugated anti-mouse IgG (Cappel Laboratories, Malvern, PA) at 1:20 dilution and analyzed on an EPICS V fluorescence activated cell sorter (FACS). Alternatively, adherent cells (0.3×10^6/ml) were cytocentrifuged onto glass slides. Cells were fixed in 100% acetone for 3 min and then incubated overnight at 4°C with the appropriate dilution of the monoclonal antibody or control antibody, mouse IgG in ascites (Pico Immunochemical Inc., Richmond Heights, OH). Cells were then incubated with alkaline phosphatase linked rabbit anti-mouse IgG antiserum (Z-259; Dako Corp., Santa Barbara, CA) (1:10) for 30 min followed by incubation with alkaline phosphatase anti-alkaline phosphatase (D651, Dako Corp.) at 1:25 for 30 min. Slides were washed extensively in Tris-saline buffer (0.1M, pH 7.6) between incubations. Substrate solution (0.1 M Tris buffer, pH 8.2, 2% formamide, 0.2% Naphtol AS-MX, 0.1% Fast Red and 1 nM Levamisole) was then added for 15 min. Levamisole inhibits endogenous alkaline phosphatase enzyme activity. Slides were then counterstained with Gill's hemotoxylin and mounted. Duplicate slides from each experiment were evaluated microscopically and 200 cells were scored on each slide. In some experiments anti-IL 2R antibody (2A3; Becton Dickinson) was used instead of anti-Tac at a 1:10 dilution. This monoclonal antibody recognizes a different epitope of the Tac protein than anti-Tac antibody.

IL 2 binding assay. The binding of radioiodinated IL 2 to adherent cells was measured according to the method described by Robb et al. (29). ^{125}I-labeled human recombinant (r) IL 2 was purchased (NEX 229, specific activity 36.6 μCi/μg; New England Nuclear, Boston, MA; DuPont Co., Wilmington, DE). Nonspecific binding was determined by addition of a 200-fold molar excess of unlabeled rIL 2 (generous gift of Hoffmann La Roche, Nutley, NJ). Briefly, 0.5×10^6 cells were suspended in 100 μl of RPMI 1640 10% (vol/vol) FCS containing ^{125}I-IL 2 in 1.5 ml Eppendorf micro test tubes (Brinkmann Instruments Co., Westbury, NY) and incubated for 60 min at 37°C. Cells were centrifuged for 15 s at 20,000 g and resuspended in 100 μl of media. The cell suspension was centrifuged through a 150-μl layer of a mixture of 84% silicone oil (550 fluids; Contour Chemical Co, North Reading, MA) and 16% Paraffin oil (Fisher Scientific Co., Pittsburgh, PA) for 2 min at 10,000 g. The tips of the tubes containing the cell pellets were then cut and radioactivity was measured in a gamma counter (Beckman Instruments Inc., Irvine, CA). All binding data were evaluated by the LIGAND program (30).

Measurement of soluble IL 2R. Soluble IL 2R levels in supernatants was measured with a sandwich enzyme immunoassay (Cell Free, interleukin-2 receptor test kit; T-cell Sciences, Cambridge, MA) (19) according to the manufacturers. Supernatants of adherent cell or standards were added to anti-IL 2R monoclonal antibody coated microtiter plates in duplicates. An enzyme-conjugated anti-IL 2R monoclonal antibody directed against a second epitope of the IL 2R molecule was added followed by addition of substrate. Absorption was read at 490 nm with a microelisa plate reader. A standard curve was prepared using the IL 2R standards. Unknown values were determined from the standard curve.

Preparation of RNA. Total RNA was extracted from T cells (20–30 $\times 10^6$) or adherent monocytes (10 $\times 10^6$) by the guanidinium-cesium method as previously described (31). Briefly, cells were washed and pellets were homogenized in 4 M guanidine isothiocyanate (Interna-

tional Biotechnologies, Inc., New Haven, CT) containing 25 mM Na citrate, 0.5% N-lauryl-sarcosine, 10 mM EDTA, 0.1 M 2-mercaptoethanol, and 0.1% antifoam A. Homogenates were layered onto a 5.7 M cesium chloride (International Biotechnologies, Inc.) cushion and centrifuged for 18 h at 175,000 g at 20°C. The RNA pellet was suspended in a solution of 25 mM Tris-HCl, 100 mM sodium chloride, 5 mM EDTA, and 0.1% SDS and precipitated twice in ethanol and then quantified.

Northern blot and dot blot hybridizations. For Northern blot analysis, 10 μg of RNA was electrophoretically separated through a 1% agarose-2.2 M formaldehyde gel, and was transferred to nitrocellulose paper (Millipore Corp., Bedford, MA). After washing in 6× standard saline citrate (SSC) (0.9 M sodium chloride–0.09 M sodium citrate), the filters were dried at 80°C for 90 min and were prehybridized at 42°C for 18 h in 10% dextran sulfate, 1 M NaCl, 50% formamide, 2 mM EDTA, 5× Denhardts' (0.1% Ficoll, 0.1% polyvinylpyrrolidone, 0.1% BSA) solution, and 1% SDS. An Eco RI insert of pIL 2R2 (a generous gift of Dr. W. C. Greene, National Cancer Institute) (32) was purified by preparative electrophoresis and labeled with ($α$-^{32}P) deoxycytidine 5′-triphosphate (dCTP; sp act 3,000 Ci/mmol; New England Nuclear) by nick translation. The filters were hybridized overnight to the heat-denatured probe at 42°C. Hybridization buffer was the same as prehybridization buffer except it contained 100 μg/ml of salmon sperm DNA. Filters were washed (2× SCC and 1% SDS at 60°C for 1 h with constant agitation) and exposed to XAR-5 film (Kodak Laboratories, Rochester, NY) at −70°C with an intensifying screen.

For dot blot analysis, dilutions of total RNA in 50% formamide–6.6% formaldehyde were applied to a Gene Screen Plus nylon membrane (New England Nuclear) using a 96-well manifold apparatus (Schleicher & Schuell, Keene, NH). Blots were then prehybridized (50% formamide, 10% dextran sulfate, 1% SDS, 1 M NaCl, and 100 μg/ml salmon sperm DNA) and then hybridized to ^{32}P-labeled IL 2R probe as above. Autoradiography then was performed.

Statistical analysis. Group means were compared using the t test.

Results

Characteristics of PPD-induced IL 2 production by blood mononuclear cells of TB patients.
PBMC or adherent cell-depleted, nylon wool–purified T cells were cultured with PPD for 48 h. IL 2 activity of cell free supernatants then was assessed. Supernatants of PPD-stimulated PBMC from tuberculin nonreactors were devoid of IL 2 activity. PPD-induced IL 2 activity was lower in supernatants of PBMC from patients with TB (mean 2.8±SE 1.4 U/ml) as compared with healthy skin test–reactive controls (103±30 U/ml) (Fig. 1). The relative abundance of monocytes in PBMC of patients in comparison to healthy donors (30±8% vs. 16±2%, peroxidase reactive) was
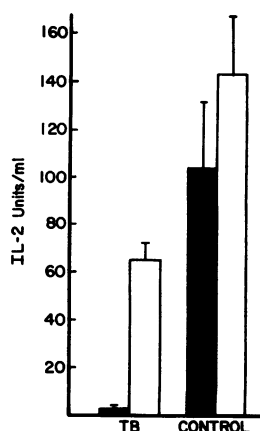

Figure 1. PPD-induced IL 2 activity of mononuclear cell supernatants of patients with TB, (n = 17; left) and healthy skin test–reactive controls (n = 17; right). 48-h culture supernatants of PBMC (solid bar) or T cells (open bar) were assessed for IL 2 activity as mitogenesis for the IL 2-dependent cell line, CTLL-20. Units per milliliter were calculated using the probit method.

insufficient to explain the discrepancy in IL 2 activity of supernatants of PBMC between the two groups (30-fold). Adherent cell depletion significantly enhanced IL 2 activity of T cell supernatants in patients only ($P < 0.05$). T cell enrichment alone does not explain improved IL 2 activity of PPD-induced T cell supernatants, since the observed value for IL 2 activity of PBMC (2.8 U/ml) was 10-fold lower than the value predicted if the 30% monocyte content of PBMC merely had been replaced by IL 2-producing T cells. Furthermore, in support of the active role for monocytes in depression of IL 2 activity, small numbers (2%) of adherent monocytes from patients with tuberculosis when cocultured with autologous T cells significantly reduced PPD-stimulated IL 2 activity (7). It should be noted, however, that mean IL 2 activity of supernatants of PPD-stimulated T cells of patients remained 50% lower than that of healthy controls ($P < 0.05$). These data indicate that adherent cells from TB subjects modulate PPD-induced expression of IL 2 activity of T cells and that this is superimposed on an existing defect in IL 2 metabolism of the T cells. Modulation of IL 2 could be due to inhibition of T cell production of the growth factor by the monocytes or to binding and active metabolism of IL 2 by monocytes. These mechanisms need not, however, be mutually exclusive.

Consumption of IL 2 by adherent monocytes from TB patients. Adherent cell preparations from patients or healthy controls were incubated with purified IL 2 (640 U/ml) at a concentration of 1% (vol/vol). IL 2 activity of cell-free supernatants was then assessed after 24, 48, and 72 h of culture. IL 2 activity decreased as a function of time when adherent cells from patients but not controls were cultured with IL 2. Maximal absorption of IL 2 by monocytes occurred after 72 h of culture (Fig. 2). IL 2 activity of cultures of adherent cells from TB patients was one-third that of cultures containing IL 2 with no cells ($P < 0.01$), whereas IL 2 activity was not decreased significantly in the presence of monocytes from healthy donors. Moreover, supernatants of adherent cells from TB patients did not interfere with the response of the indicator cells (CTLL-20) to exogenous IL 2 (data not shown). Additionally, in three experiments, nylon wool–prepared T cells obtained from patients with TB were unable to absorb IL 2 significantly when cultured with IL 2 for 72 h (data not shown). Adherent cell preparations were highly enriched for monocytes, but had a small number of CD3$^+$ cells (5–8%). In some experiments, therefore, OKT3 reactive cells were removed from adherent cell populations prepared from patients by complement lysis (< 1% CD3$^+$). IL 2 absorbing potential of adherent cells was unaffected by depletion of CD3$^+$ lymphocytes (data not shown). These data indicate that adherent monocytes from
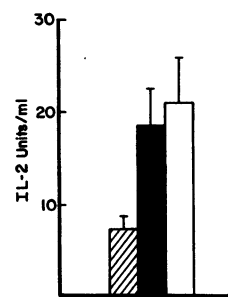

Figure 2. Depletion of IL 2 activity by adherent monocytes from TB patients. Purified IL 2 at 1% (vol/vol) was cultured with adherent cells (1 × 10^6/ml) from patients with TB (n = 22) (striped bar), healthy controls (n = 18) (solid bar), or cell-free (n = 18) (open bar); IL 2 activity of 72-h supernatants was then assessed. IL 2 activity was 7.1±1.3 U/ml (mean±SE) in cell cultures of TB patients, 18.8±4 U/ml in cell cultures of controls, and 21±5.3 U/ml in cell-free cultures.

Table I. Expression of IL 2R on Adherent Cells Isolated from Tuberculosis Patients and Healthy Donors

Experiment	TB patients	Healthy donors
1	10	4
2	17	0
3	18	0
4	11	0
5	57	ND
6	60	ND
7	33	7
8	44	0
Mean±SE	30±6.4	1.8±1.2*

Adherent cells were assessed for expression of IL 2R by indirect immunofluorescence using anti-Tac monoclonal antibody and FACS analysis. Data indicate percentage of Tac reactive cells.
ND, not done.
* $P < 0.01$.

patients with tuberculosis removed IL 2 from supernatants and imply the presence of a functional receptor for the molecule on the surface of monocytes.

Expression of IL 2R by adherent monocytes from TB patients. Next, freshly isolated adherent cells from TB patients and healthy PPD skin test reactive donors were examined for expression of IL 2R. Table I shows the results of FACS analysis, gaited for monocytes by size and light scatter, using anti–Tac antibody in eight experiments. Anti–Tac reactivity of cells from healthy donors was nil to minimal. This is not unexpected since normal peripheral blood monocytes express

IL 2R only when stimulated with cytokines (e.g., gamma-interferon) (9–11) or when exposed to bacterial products (11). In contrast, adherent cells freshly isolated from each of the TB patients were Tac reactive. Whereas the mean intensity of fluorescence of positive cells was rather uniform (140±30, mean±SD), the percentage of Tac reactivity varied considerably (10–60%). Maintenance of IL 2R expression on cultured adherent cells from patients with TB was examined at 24, 48, and 72 h in three experiments. In two of three experiments adherent cells remained Tac reactive at the same level as fresh cells, whereas in the third experiment, Tac reactivity declined by 30% (data not shown).

Monocyte IL 2R expression was also examined by APAAP staining using anti-Tac or anti–IL 2R (2A3) antibody. This technique is more sensitive than FACS analysis and allows for the direct visualization of reactive cells. Whereas TB monocytes were consistently and strongly reactive with anti–Tac, reactivity was weak with the anti–IL 2R antibody, 2A3. Other studies have shown that commercial preparations of 2A3 antibody do not react with monocyte IL 2R, although they react strongly with T cell IL 2R (13). In four experiments, adherent cell preparations from TB patients were 65±5.5% (range: 48–70%) anti–Tac positive and morphologically the reactive cells were 98% monocytes. Fig. 3 shows an example of such an experiment. Adherent cell preparations from healthy controls lacked staining when reacted with anti–Tac. Similarly patient cells were APAAP negative when reacted with control ascites containing IgG (Fig. 3 A).

IL 2 binding characteristics of adherent cells of TB patients. ^{125}I–IL 2 binding was performed on adherent cells freshly isolated from TB patients or healthy PPD reactive controls. In these studies we used IL 2 concentrations that ranged from 2 pM to 20 nM, a range that would allow assessment of
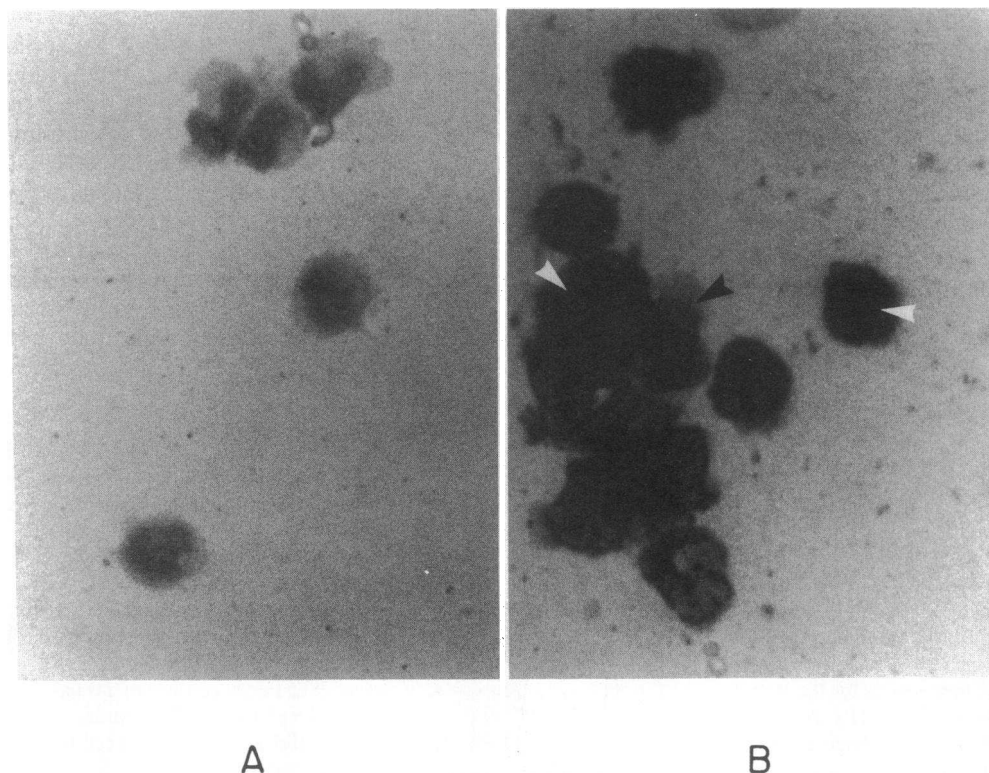

A

B

Figure 3. Cytostain of adherent cells from a TB patient with IgG in ascites (*A*) or anti–Tac (*B*) by APAAP technique. In *B* black arrow shows the only nonstaining cell, white arrows point to two of the reactive (red) cells (×400). In *A* all cells are nonreactive.
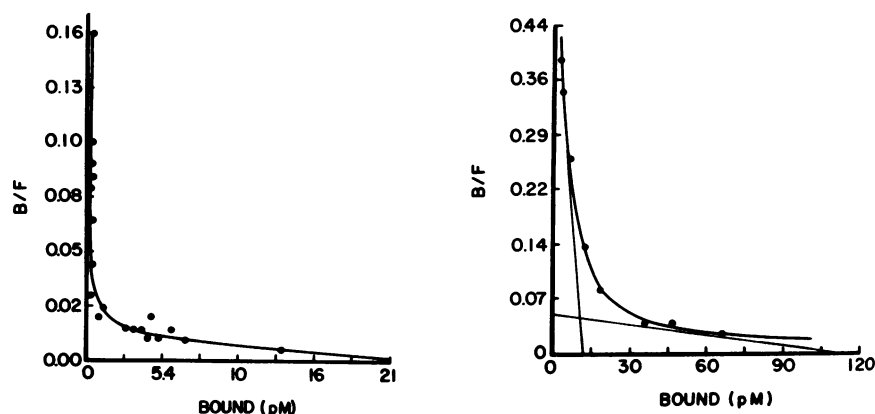
Figure 4. Scatchard analysis of ^{125}I–IL 2 binding to fresh adherent cells from patients with TB (*left*) and 72-h PHA-stimulated T cells from a healthy donor (*right*). The points representing actual data points and the computer-generated curves are shown. The Scatchard plot on left represents data obtained from ^{125}I–IL 2 binding of adherent cells from three patients.

both high- and low-affinity IL 2R (33). Adherent monocytes from controls did not bind the radiolabeled ligand in a specific manner. Unstimulated monocytes from TB patients demonstrated 84 high-affinity and 5,252 low-affinity binding sites per cell with respective dissociation constants (K_d) of 3.3 pM and 1.2 nM (Fig. 4). By comparison, T cells obtained from healthy donors and stimulated with phytohemagglutin (PHA) for 72 h expressed 1,800 high affinity (K_d = 20 pM) and 14,455 low affinity (K_d = 3 nM) binding sites per cell.

Expression of IL 2R RNA by adherent cells from TB patients. The expression of IL 2R by freshly isolated adherent cells obtained from patients with active TB could potentially be secondary to adsorption of IL 2R on the surface of, rather

Figure 5. Analysis of total RNA hybridized with an IL 2 receptor cDNA probe encoding for the Tac protein. In *A* total RNA extracted from fresh adherent cells of one healthy individual (lane *1*) and two TB patients (lanes *2* and *3*) was applied to nitrocellulose paper in dilutions (3 μg starting dilution) by a dot-blot apparatus. *B* is a Northern blot analysis of total RNA of unstimulated (lane *1*) and PHA-stimulated (lane *2*) T cells. Blots were hybridized to a ^{32}P-labeled Eco RI insert of pIL 2R2. RNA sizes are given in kilobases at the right of *B*.

than the synthesis of, the molecule by monocytes. To assess the latter, we used a cDNA probe encoding for the Tac protein (pIL 2R2). Fig. 5 *B* shows a Northern blot analysis of total RNA extracted from resting and PHA-stimulated T cells of a healthy individual. Both the 3.5- and 1.5-kb transcripts of IL 2R can be seen as described previously (32). We then isolated total RNA from adherent cells of two patients with TB, one with far advanced and one with moderately advanced disease as determined by chest roentgenogram, and one skin test–reactive control individual. In this experiment RNA was processed immediately after obtaining adherent cells. The amount of RNA (6 μg) obtained from each individual was inadequate to perform Northern blot analysis. Dot blot analysis of RNA was performed using the IL 2R probe. Whereas RNA prepared from adherent cells of the two TB subjects showed strong hybridization with the probe at all dilutions, RNA from monocytes of the healthy control was inactive (Fig. 5 *A*). These data indicate that circulating monocytes from patients are already activated to express IL 2R. Such activation could potentially be secondary to exposure of monocytes to mycobacterial products in vivo.

Previous studies have shown that both expression of IL 1 and TNF by monocytes from healthy skin test reactors are inducible by PPD and several other mycobacterial constituents. The ability of the mycobacterial products, PPD, and a recombinant heat shock protein of *Mycobacterium bovis* (r65kd; a generous gift of Dr. van Embden) (34) to induce adherent cell expression of IL 2R was examined next. Adher-
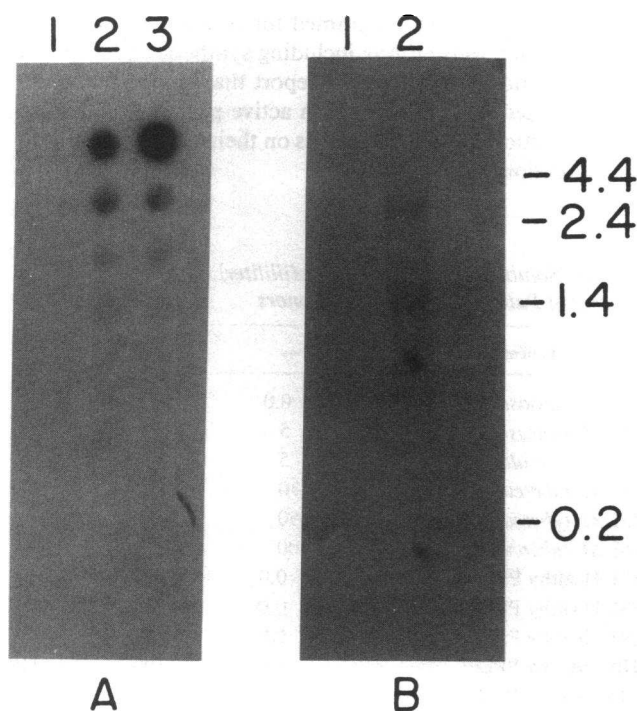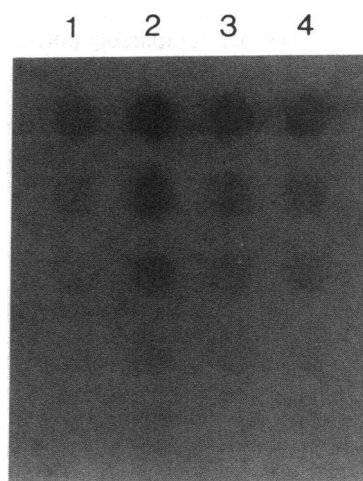

Figure 6. IL 2 receptor dot blot analysis of total RNA (in dilutions) extracted from monocytes of a PPD nonreactor in culture for 3 h with medium (lane *1*), PPD 10 μg/ml (lane *2*), 65 kd 10 μg/ml (lane *3*), and LPS 1 μg/ml (lane *4*). Densitometric analysis at 0.75 μg (row 3) shows a sixfold difference between lanes *1* and *2*, 3.5-fold difference between lanes *1* and *3*, and 3.9-fold difference between lanes *1* and *4*.

ent monocytes obtained from a skin test nonreactor were cultured for an additional 3 h in the presence or absence of stimuli (10 × 10 adherent cells for each condition). Total RNA was then isolated and dot blot analysis for IL 2R performed (Fig. 6). PPD induction of monocyte IL 2R message was strong, whereas r65kd and LPS were comparable in this respect. A small amount of IL 2R message also was seen when monocytes were left in culture without stimulatants (Fig. 6, lane *1*). This could be the result of induction of IL 2R mRNA by adherence to plastic, and accumulation of the message during subsequent culturing. In comparison, RNA obtained from uncultured monocytes of the control individual (Fig. 5 *A*, lane *1*) was negative for IL 2R message.

Release of soluble IL 2R in culture by adherent cells from TB patients. In addition to expression of membrane IL 2R, a soluble released form of IL 2R has recently been demonstrated in supernatants of both activated T and B lymphocytes (20) and LPS or IFN stimulated monocytes. We therefore analyzed supernatants of adherent cells obtained after 72 h of culture from patients and healthy donors for soluble IL 2R by an enzyme immunoassay. Fig. 7 shows the results for 13 TB patients and six skin test–reactive controls. Despite variable content of IL 2R in culture supernatants, TB patients had a three-fold higher activity as compared to controls ($P < 0.05$). Moreover, the levels of IL 2R in supernatants of adherent monocytes from TB patients doubled when cells were stimulated with PPD for 72 h in vitro (143±32 to 325±47 U/ml of IL 2R; $P < 0.02$) (Fig. 8). Three patients with newly diagnosed pulmonary infection due to mycobacteria other than *M. tuberculosis*, three patients with cavitary TB, three healthy skin test nonreactors, and three healthy skin test reactors also were studied for the release of soluble IL 2R by adherent cell cultures. As seen in Table II, culture supernatants prepared from patients with atypical mycobacteriosis did not contain large amounts of IL 2R. Moreover PPD stimulated release of IL 2R to comparable levels in these patients and in healthy skin test reactors and nonreactors.

Discussion

Activation of circulating human peripheral blood monocytes results in the expression of the gene for IL 2R followed by the appearance of IL 2R on the cell surface (11, 12). Concomitantly, a soluble form of IL 2R can be identified in culture supernatants of monocytes (11). The cytokine, interferon gamma (IFNγ), and the bacterial products, lipopolysaccharide (LPS) and muramyl dipeptide (MDP) are comparably potent in induction of IL 2R on monocytes from healthy individuals; the kinetics of induction by these stimuli differ however and thus their effects are additive (12). Both superoxide generation by monocytes and cytotoxicity against the extracellular proto-
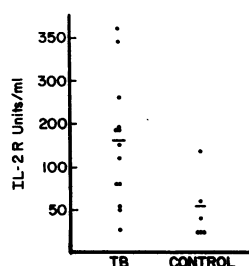
Figure 7. Soluble IL 2R levels in supernatants of adherent cells of patients with TB and skin test reactive controls. Adherent cells were cultured at 1 × 10⁶/ml for 72 h. Cell-free supernatants were assessed for soluble IL 2R on a commercial ELISA assay.
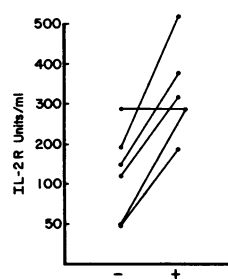
Figure 8. Soluble IL 2R levels in supernatants of PPD-stimulated (*right*) and unstimulated (*left*) adherent cells from patients with TB. 72-h culture supernatants were assessed for soluble IL 2R on an ELISA assay.

zoa, *Giardia lamblia*, are enhanced once IL 2R bearing monocytes are cocultured with IL 2 (12). Furthermore, recent studies have demonstrated that the intracellular killing of *Mycobacterium avium complex* organisms by monocytes is potentiated when IL 2 is present along with tumor necrosis factor (TNF) in cell cultures (35). These observations suggest that expression of IL 2R by activated monocytes and interaction of such receptors with IL 2 might have implications in microbicidal host defenses. An alternate outcome of such an interaction, however, may be a limitation in the availability of IL 2 for T cells leading to depression of T cell immune responses.

This study demonstrates the spontaneous expression of IL 2R by circulating blood monocytes in a disease state. Alveolar macrophages obtained from patients with active pulmonary sarcoidosis display Tac positivity (13) and it has been suggested that such activated tissue macrophages may be involved in the pathogenesis of lung injury, potentially by production of oxygen radicals. In TB, previous data indicate that blood monocytes are activated or primed for activation by in vitro stimuli by numerous criteria including synthetic capacity and effector function (1–5). Here we report that blood monocytes freshly isolated from patients with active pulmonary TB also express functional IL 2R molecules on their surface without in vitro induction.

Table II. Soluble IL 2R (Units per Milliliter) of Adherent Cell Cultures of Patients and Healthy Donors

Patient	—	PPD	LPS
(1) *M. kansasii*, MA	0.0	140	—
(2) *M. kansasii*, FA	5	80	—
(3) *M. scrofulaceum*, MA	5	30	—
(4) *M. tuberculosis* MA	170	550	—
(5) *M. tuberculosis* FA	450	450	—
(6) *M. tuberculosis* MA	300	530	—
(7) Healthy PPD+	0.0	45	—
(8) Healthy PPD+	0.0	30	150
(9) Healthy PPD+	0.0	50	250
(10) Healthy PPD−	0.0	100	310
(11) Healthy PPD−	0.0	50	60
(12) Healthy PPD−	0.0	90	—

Soluble IL 2R (units per milliliter) of adherent cell cultures without stimulation, or in response to 100 µg/ml PPD or 10 µg/ml LPS as assessed by an enzyme immunoassay. Patients (*1–3*) with atypical mycobacterial disease, and patients (*4–6*) with tuberculosis are identified by pathogen isolated and the extent of radiographic involvement (22). FA, far advanced; MA, moderately advanced.

In our previous studies of human TB, we have shown that circulating adherent cells are suppressive both of PPD-induced IL 2 expression and blastogenesis (6, 7). The nature of this suppressor cell was further identified as an OKMI-reactive, nonerythrocyte rosetting, radiosensitive adherent monocyte (36). In this study, we again observed that IL 2 activity of PPD-induced PBMC supernatants was significantly lower in patients as compared to healthy controls and that removal of adherent monocytes, markedly enhanced T cell supernatant IL 2 activity (Fig. 1). Absorption of purified IL 2 by adherent cells was observed in each patient (Fig. 2). This was not due to the minimal T cell contamination of this cell population since complement lysis of OKT3 reactive cells did not affect the IL 2 absorbing capacity of adherent cells, and nonadherent T cells of patients did not remove IL 2 from culture medium. Removal of IL 2 by adherent monocytes from TB subjects would explain low IL 2 activity and low blastogenic responses of PPD-stimulated cultures of their PBMC. Metabolism of IL 2 by monocytes could not, however, be the sole basis for depressed PPD-induced T cell functions in TB for several reasons. First, some patients both in the present and in the previous studies (7) did not have a significant rise in PPD-induced IL 2 generation of their T cell cultures as compared to PBMC. Additionally, the IL 2 activity of PPD-stimulated T cells remained significantly lower in patients than in skin test reactive controls. Thus, it appears that a defect in IL 2 generation by T cells is operative in addition to the modulatory effects of monocytes. Furthermore, purified IL 2 did not correct the blastogenic defect of PBMC of patients (7). These observations argue for additional regulatory mechanisms besides IL 2 consumption by monocytes as contributing to depressed responsiveness to PPD in human infection with $M.$ $tuberculosis.$

The presence of IL 2R was confirmed on adherent cells from patients by reactivity to the monoclonal antibody, anti–Tac, using two techniques FACS analysis and APAAP (Table I and Figure 3). Levels of anti–Tac reactivity (mean 30%) by FACS analysis were comparable to the reported levels of Tac expression by IFNγ stimulated healthy monocytes (11). In this regard, APAAP cytostaining proved to be more sensitive and was useful in morphologic identification of the reactive cells, which were 98% monocytes. The affinity for binding of radioiodinated IL 2 to adherent cells from patients with tuberculosis was studied next. We found similar numbers of high affinity IL 2R (84 sites per cell) as described for dually (LPS plus IFNγ) stimulated monocytes from healthy donors (87 sites per cell) (11). The number of low-affinity receptors on monocytes from TB patients (5,252 sites per cell) was similar to LPS, or IFNγ activated monocytes (6–7,000 sites per cell) reported previously.

It was still possible, however, that the IL 2R present on adherent cells from TB patients did not originate from monocytes, but were the product of another mononuclear subpopulation perhaps adsorbed to monocytes while they were in transit through the inflamed pulmonary tissues, containing activated alveolar macrophages and/or lung-recruited T cells. These possibilities were excluded by demonstrating expression of the gene for IL 2R by monocytes using a cDNA probe that encodes for the β chain of the receptor. Thus, it appears rather, that blood monocytes from subjects with TB are activated in vivo to express IL 2R. This phenomenon parallels the other amplified capacities of such monocytes.

The finding of elevated soluble IL 2R in supernatants of cultured adherent cells from patients with newly diagnosed TB (Fig. 7) and the further increase in levels upon stimulation with tuberculin PPD (Fig. 8) raises several interesting issues. First, it appears that in TB, similar to other pathological disorders associated with active cellular immune responses, such as sarcoidosis (17), rheumatoid arthritis (18), renal allograft rejection (37), and some lymphomas (38), soluble IL 2R may be a marker of disease activity. The cellular source of soluble IL 2R may, however, differ in these conditions. For example in sarcoidosis, it appears that both T cells and monocytes contribute to the content of soluble IL 2R in bronchoalveolar lavage (17). Second, augmentation of released IL 2R in the presence of PPD might imply that enhanced soluble IL 2R at local sites of infection such as the pulmonary tissue could limit T cell responses. We have previously shown that PPD stimulates release of both IL 1 (1) and TNF (39) by monocytes. Here we report that PPD and other mycobacterial products (65 kD antigen) can induce monocyte IL 2R gene expression (Fig. 6). Thus, it is possible that direct stimulatory action of mycobacterial constituents on monocytes is a factor in the depressed response of mononuclear cells from patients with TB to PPD. Moreover, it appears that monocytes from TB patients in particular are capable of releasing significant amounts of IL 2R as compared to patients with parenchymal lung disease of the same extent but due to other mycobacterial agents, again reflecting the extent of dysregulation of cellular immune parameters in human TB.

We speculate that the observed constitutive expression of functional IL 2R on monocytes from TB patients and the increased release of these receptors after exposure to mycobacterial products may be key factors in the phenomenon of antigen-specific suppression by adherent cells and the associated hypoergy on tuberculin skin testing (6). These phenomena and similar events conceivably occurring at local sites of infection may contribute to the immunopathogenesis of tuberculosis.

Acknowledgments

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