Production of Tumor Necrosis Factor Alpha by Monocytes from Patients with Pulmonary Tuberculosis

TETSUYA TAKASHIMA,* CHISATO UETA, IZUO TSUYUGUCHI, AND SUSUMU KISHIMOTO

Osaka Prefectural Habikino Hospital, 3-7-1, Habikino, Habikino City, Osaka, 583 Japan

We studied the production of tumor necrosis factor alpha (TNF-α) by peripheral blood monocytes taken from patients with pulmonary tuberculosis and from healthy controls. It was found that the monocytes from patients with newly diagnosed tuberculosis released significantly greater amounts of TNF-α in vitro in response to lipopolysaccharide than did those from healthy controls ($P < 0.05$). However, the monocytes from patients with chronic refractory tuberculosis released significantly lower amounts of TNF-α than did those from patients with newly diagnosed tuberculosis ($P < 0.005$). Even when the cells were primed for 24 h with 500 U of recombinant interferon gamma per ml, the same pattern of results was observed. The depressed TNF-α production by the monocytes from patients with chronic refractory tuberculosis was also shown in response to *Mycobacterium bovis* BCG. This depressed TNF-α production did not recover, even when cultured for 1 to 7 days in the sera of healthy individuals. The sera from patients with chronic refractory tuberculosis did not have any suppressive effect on the lipopolysaccharide-induced TNF-α production. Thus, it was demonstrated that the levels of TNF-α produced by monocytes were related to the disease states of pulmonary tuberculosis and that the depressed TNF-α production by monocytes in patients with chronic refractory tuberculosis might not be acquired.

Tumor necrosis factor alpha (TNF-α) is released from the cells of monocyte or macrophage lineage in response to lipopolysaccharide (LPS) or to organisms such as *Mycobacterium tuberculosis*, *Mycobacterium bovis* BCG, and *Listeria monocytogenes* (6, 12, 21, 23). In addition to tumoricidal activity, TNF-α has many biological activities and has been recognized as an important inflammatory mediator affecting a variety of cell types including polymorphonuclear cells, endothelial cells, fibroblasts, and macrophages (4, 17). Recently, it has been reported that TNF-α can activate macrophages inhibiting the intracellular multiplication of certain organisms in vitro, such as *Trypanosoma cruzi* (8) and *Mycobacterium avium* (3). Furthermore, in mice experimentally infected with *L. monocytogenes* and *M. bovis* BCG, in vivo administration of anti-TNF-α antibody inhibited the development of granuloma in the host organs, resulting in the extensive growth of organisms in vivo (13, 14, 16). The administration of TNF-α decreased the number of these organisms in the infected organs (2, 13). Thus, TNF-α has an essential role in antibacterial resistance against infections caused by facultative intracellular organisms in vivo as well as in vitro.

In the present study, we investigated the production of TNF-α by using monocytes taken from patients with pulmonary tuberculosis. It was found that the monocytes of patients with chronic refractory tuberculosis showed a depressed ability to produce TNF-α compared with the monocytes of patients with newly diagnosed tuberculosis and healthy controls. The depressed TNF-α production did not improve even when cultured in the sera of healthy individuals. The sera of patients with chronic tuberculosis did not exert any suppressive effect on the LPS-induced TNF-α production. These results suggested that the depressed TNF-α production by monocytes in chronic refractory tuberculosis might not be acquired and that this depressed

production might contribute to the development of chronic refractory tuberculosis.

MATERIALS AND METHODS

Subjects. Thirty-eight inpatients with pulmonary tuberculosis, which was confined to the lungs, were considered in this study. None of the patients had received corticosteroid or immunosuppressive therapy. The patients were divided into two groups. The first group consisted of 24 patients who had been newly diagnosed as having pulmonary tuberculosis on the basis of chest X rays and the demonstration of acid-fast bacilli in the sputum. They had been treated with an antituberculous regimen of streptomycin, isoniazid, and rifampin, and the mean medication period in this group was 1.3 months at the time when the venous blood samples were obtained. The second group consisted of 14 patients with chronic refractory tuberculosis, all of whom had clinical histories of bacterial resistance to antituberculosis drugs with attendant continuous excretion of tubercle bacilli for more than 2 years. Changes in radiographic extent of pulmonary tuberculosis, respiratory function tests such as vital capacity, and measurement of body weight were evaluated and revealed that patients in the second group were comparatively stable in their clinical courses during the last 6 months. The control group consisted of healthy hospital staff members ($n = 23$) who had had actual physical contact with tuberculous patients. All subjects in the three groups were tuberculin reactive on skin testing with 5 tuberculin units of purified protein derivative. The profile of the three groups is shown in Table 1. The three groups were studied concurrently.

Reagents. LPS (*Escherichia coli* 0111B4) was obtained from Difco Laboratories, Detroit, Mich., and polymyxin B was obtained from Sigma Chemical Co., St. Louis, Mo. *M. bovis* BCG (Tokyo strain) was grown on Ogawa egg medium and prepared as a suspension in phosphate-buffered saline. The organism was killed by heating at 60°C for 2 h. Indomethacin was obtained from Sigma, dissolved in ethanol,

---

* Corresponding author.

TABLE 1. Profile of study groups

Stimulation by:	Subject group	Age (yr) (mean ± SD)	Sex (M/F)	Months of medication (mean ± SD)
LPS	Healthy control (n = 23)	41 ± 14	14/9	
	Newly diagnosed (n = 24)	45 ± 12	17/7	1.3 ± 1.0
	Chronic (n = 14)	55 ± 11[a]	11/3	112 ± 91
BCG	Healthy control (n = 14)	42 ± 15	9/5	
	Newly diagnosed (n = 14)	45 ± 13	11/3	1.5 ± 1.3
	Chronic (n = 12)	55 ± 12[a]	9/3	109 ± 89

[a] $P < 0.05$ compared with control.

and diluted in RPMI 1640 medium to give a final concentration of 10 μg/ml. Recombinant human interferon gamma (IFN-γ) was kindly provided by Takeda Chemical Industries, Ltd. Recombinant human TNF-α (rTNF-α) was kindly provided by Fujisawa Chemical Industries, Ltd. Polyclonal rabbit anti-rTNF-α antibody was raised by immunizing rabbits with rTNF-α as described by Espevik et al. (11).

Cells and TNF-α induction. By using pyrogen-free materials, mononuclear cells were isolated by gradient centrifugation of heparinized venous blood on lymphocyte separation medium (Organon Teknika Co., Durham, N.C.), washed three times, and suspended in RPMI 1640 medium containing 10% fetal calf serum (M.A. Bioproducts, Walkerville, Md.) at 2×10^6 cells per ml. A 3-ml volume of this suspension was plated onto a plastic petri dish (60 by 15 mm; Kotai Kagaku Co Ltd., Tokyo, Japan) and incubated for 1 h at 37°C in a humidified CO_2 incubator. Nonadherent cells were removed by rinsing three times with RPMI 1640 medium containing 10% fetal calf serum. The monocyte-enriched population was then detached by further incubation at 4°C with phosphate-buffered saline containing 10% fetal calf serum and 3 mM EDTA. After 20 min, cells were recovered by gentle pipetting, washed once, and suspended in RPMI 1640 medium containing 10% heat-inactivated pooled human serum, indomethacin (10 μg/ml), penicillin G (100 U/ml), and streptomycin (100 μg/ml). This suspension was placed in a flat-bottomed 96-well microtiter plate (Falcon 3072; Becton Dickinson Labware, Oxnard, Calif.). The purity of monocytes was always greater than 90% as determined by morphological examination, nonspecific esterase staining, and immunofluorescence with anti-Leu-M3 monoclonal antibody (Becton Dickinson Immunocytometry Systems, Mountain View, Calif.).

In this experiment, cells were first stimulated with IFN-γ and then with LPS or BCG to examine the difference between primed and nonprimed monocytes for TNF-α production, using a sufficient dose of IFN-γ (500 U/ml) to provide continued stimulation. Twenty-four hours was chosen as the standard reference point for steady activation. Monocytes were primed by culturing them for 24 h in the presence of IFN-γ (500 U/ml) for the significant increase of TNF-α production. The cells were then stimulated with LPS or BCG and incubated for an additional 20 h. In the BCG experiments, polymyxin B was added at a concentration of 10 μg/ml. In one experiment, monocytes from a healthy control subject were incubated for 24 h with graded concentrations of sera taken from patients with pulmonary tuberculosis, and then the medium was replaced with the fresh

culture medium described above and stimulated with 1 μg of LPS per ml. The supernatants were harvested, centrifuged, and stored at −20°C until TNF-α activity was assayed.

Bioassay for TNF-α activity. The monocyte culture supernatants were assessed for TNF-α activity by measuring their cytotoxicity on L929 cells as described by Espevik and Nissen-Meyer (11). Briefly, L929 cells ($3 \times 10^4/100$ μl) were cultured in a flat-bottomed, 96-well microtiter plate for 18 h at 37°C in a humidified CO_2 incubator in the presence of 1 μg of actinomycin D per ml, with subsequent twofold dilutions of test samples. After incubation, 10 μl of MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H tetrazolium bromide; Dojin, Kumamoto, Japan] at a concentration of 5 mg/ml in phosphate-buffered saline was added, and the cells were further incubated for 4 h at 37°C. After the supernatants were decanted from the wells, 100 μl of isopropanol with 0.04 N HCl was added to all of the wells. The plates were enumerated by reading on an Immuno Reader NJ 2000 (Inter Med), by using a test wavelength of 570 nm and a reference wavelength of 630 nm. A standard preparation of rTNF-α was always tested in parallel. For characterization of TNF-α-induced cytotoxicity, half of the supernatant samples were incubated with rabbit anti-rTNF-α antiserum and half of them were incubated with normal rabbit serum prior to the addition of the supernatants to the target cell cultures. The detection limit of TNF-α in the supernatant was about 50 pg/ml.

Statistical analysis. Data in the table and figures are expressed as means ± standard deviations (SDs). Statistical analysis was performed by use of Student's t test, and the results were considered significantly different at $P < 0.05$.

RESULTS

Kinetics of LPS-induced TNF-α production. Monocytes from a healthy individual were cultured for 24 h with or without IFN-γ (500 U/ml) and then stimulated with 10 μg of LPS per ml (Fig. 1). The TNF-α activity in the culture supernatants linearly increased and reached a plateau at 12 h after LPS stimulation. This response was significantly enhanced when the cells were primed with IFN-γ (500 U/ml). As previously observed by others (19, 21), the TNF-α production by IFN-γ-primed monocytes was even further enhanced in the presence of indomethacin (10 μg/ml).

Dose responses of LPS and BCG stimuli for TNF-α production by monocytes with or without preincubation with IFN-γ. After preincubating the monocytes for 24 h in the presence or absence of IFN-γ (500 U/ml), the graded doses of LPS and BCG were used to induce TNF-α production. The representative results of the graded doses on the monocytes of healthy individuals are shown in Fig. 2. In our experiment, IFN-γ (500 U/ml) alone had no measurable effect on the monocytes for producing TNF-α (data not shown). LPS was able to induce the production of considerable amounts of TNF-α in the supernatants of monocyte cultures in a dose-dependent manner, and this response was synergistically enhanced by preincubation of the monocytes with IFN-γ (Fig. 2). BCG also induced the release of TNF-α, and this response was maximal at a dose of 100 μg of BCG per ml. The possibility of LPS contamination in BCG preparations was excluded because the BCG-induced TNF-α production was not diminished in the presence of 10 μg of polymyxin B per ml. In contrast to the LPS stimulation, the preincubation of monocytes with IFN-γ did not augment the BCG-induced TNF-α production (Fig. 2). These data suggest that LPS and BCG may stimulate the monocytes to produce TNF-α by different mechanisms.
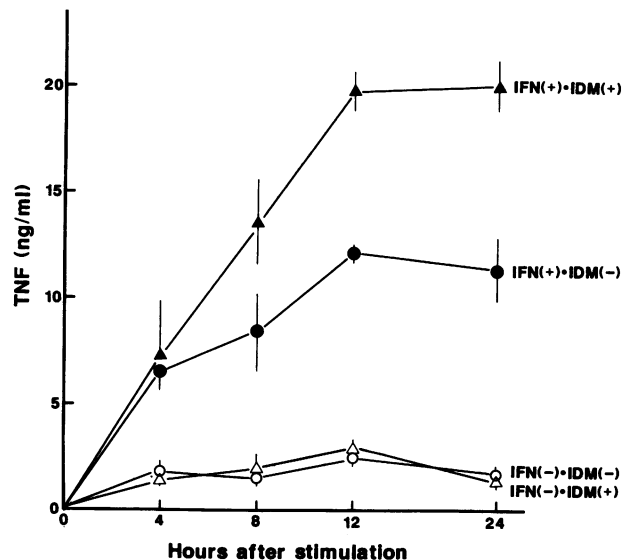
FIG. 1. Kinetics of LPS-induced TNF-α production by monocytes in the presence (▲, △) or absence (●, ○) of indomethacin (10 μg/ml), and the effect of preincubation for 24 h with (▲, ●), or without (△, ○) IFN-γ (500 U/ml). Results are expressed as the mean ± SD.

LPS-induced TNF-α production by monocytes from patients with pulmonary tuberculosis and from healthy controls. The levels of TNF-α activity were examined in the culture supernatants of monocytes from patients with pulmonary tuberculosis and healthy controls (Fig. 3). Spontaneous TNF-α release was observed in two cultures from healthy control subjects (n = 23) and in three patients with newly diagnosed tuberculosis (n = 24) (mean ± SD: 0.04 ± 0.15 and 0.04 ± 0.12 ng/ml, respectively).

The monocytes from the newly diagnosed tuberculosis group produced significantly greater amounts of TNF-α than those from the healthy control group (13.2 ± 11.5 and 7.0 ± 5.9 ng/ml, respectively; $P < 0.05$) in response to LPS (10 μg/ml) without IFN-γ preincubation. However, the amounts

of TNF-α produced by the cells from the chronic refractory tuberculosis group (5.4 ± 4.2 ng/ml) were lower than those of the newly diagnosed tuberculosis and healthy control groups. The difference between the newly diagnosed tuberculosis group and the chronic refractory tuberculosis group was statistically significant ($P < 0.005$).

When the cells were preincubated for 24 h with IFN-γ (500 U/ml), the level of TNF-α produced by the monocytes from the newly diagnosed tuberculosis group was higher than that of the healthy control group, although the difference was not significant (23.1 ± 16.4 and 16.0 ± 13.7 ng/ml, respectively; $P > 0.1$). On the other hand, the TNF-α production by monocytes taken from chronic refractory tuberculosis patients (9.9 ± 8.8 ng/ml) was still lower than that by monocytes from newly diagnosed tuberculosis patients and healthy controls, and this difference was statistically significant when compared with that of newly diagnosed tuberculosis patients ($P < 0.005$).

BCG-induced TNF-α production. We studied TNF-α production in response to BCG (100 μg/ml). The monocytes from newly diagnosed tuberculosis patients did not show any increased TNF-α production compared with those from healthy controls (4.2 ± 3.7 and 3.6 ± 3.2 ng/ml, respectively; $P > 0.5$) (Fig. 4A). However, the level of TNF-α produced by the cells from the chronic refractory tuberculosis group was significantly lower than that of the healthy control and newly diagnosed tuberculosis groups (1.1 ± 0.9 ng/ml; $P < 0.02$ and $P < 0.01$, respectively). In this case, preincubation with IFN-γ had no enhancing effect on the BCG-induced TNF-α production of all three groups, and the same pattern of results was observed (Fig. 4B).

TNF-α production by monocytes cultured for 1 to 7 days. To evaluate the recovery of TNF-α production, the monocytes from patients with chronic refractory tuberculosis were cultured for 1 to 7 days in the pooled human sera from healthy donors and then exposed to LPS (10 μg/ml) for the last 20 h. The levels of TNF-α in the culture supernatants had increased on days 2 and 4 of culture in all three groups as compared with the levels on day 1 (Fig. 5). However, the lower ability of TNF-α production by monocytes from
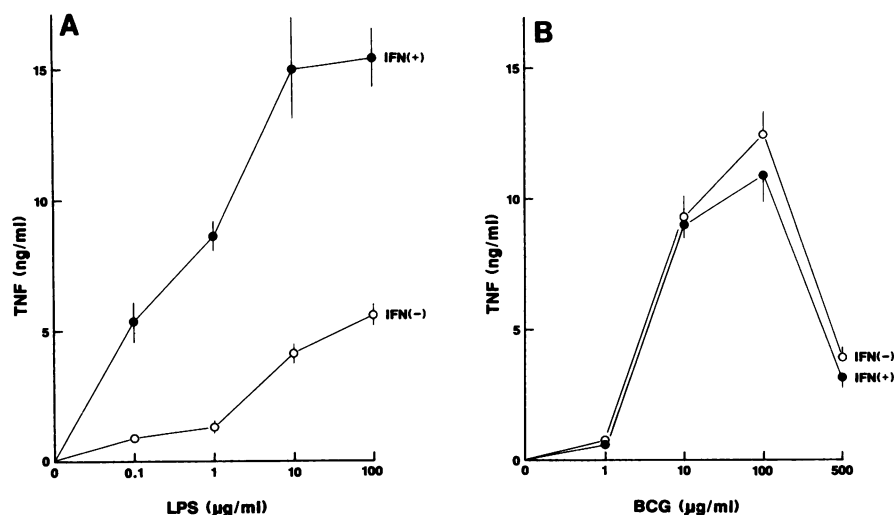

FIG. 2. Dose-response curves for LPS- (A) and BCG- (B) induced TNF-α production by monocytes obtained from healthy controls, preincubated for 24 h in the presence (●) or absence (○) of IFN-γ (500 U/ml). Each point represents the mean production for triplicate cultures (±SD) of one representative experiment.
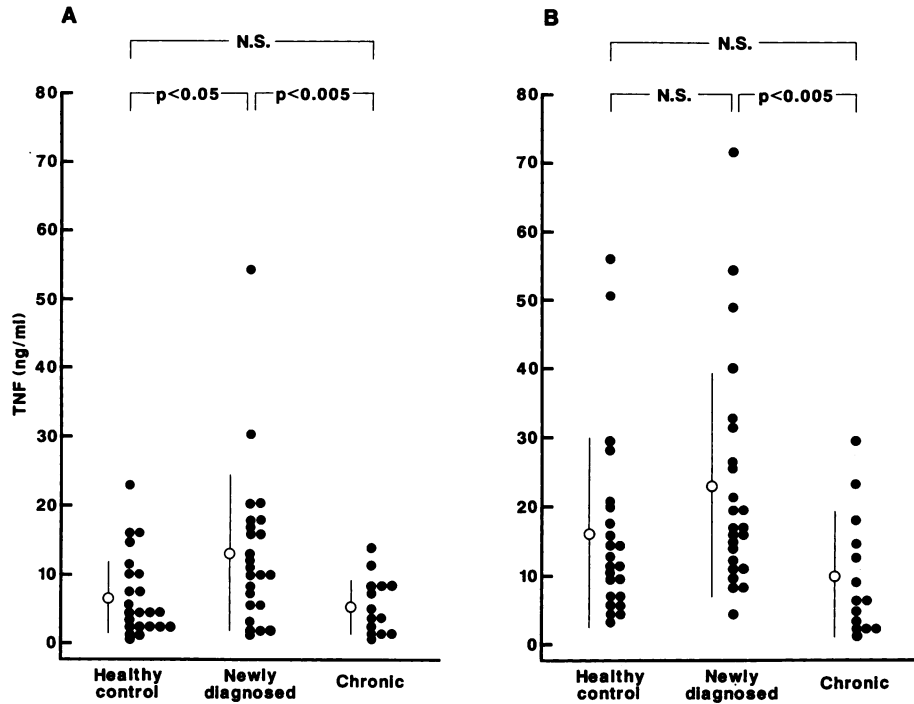
FIG. 3. Levels of TNF-α induced by LPS (10 μg/ml) with (B) or without (A) preincubation with IFN-γ (500 U/ml). Vertical bars represent the mean values ± SD.

patients with chronic refractory tuberculosis did not recover throughout incubation.

Effect of sera from patients with tuberculosis on LPS-induced TNF-α production by monocytes from a healthy donor. There was a possibility that monocytes from patients

with chronic refractory tuberculosis had already been under some suppressive effects in vivo. Monocytes from a healthy donor were incubated for 24 h in sera from patients with tuberculosis and then stimulated with 1 μg of LPS per ml. TNF-α production was enhanced by preincubation of mono-
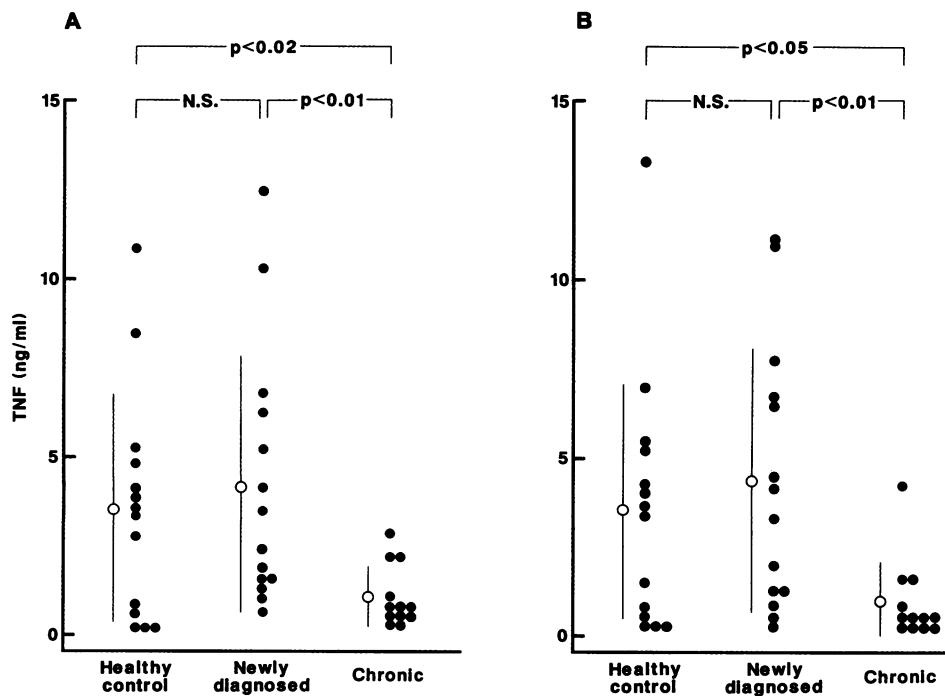

FIG. 4. Levels of TNF-α induced by BCG (100 μg/ml) with (B) or without (A) preincubation with IFN-γ (500 U/ml). Vertical bars represent the mean values ± SD.
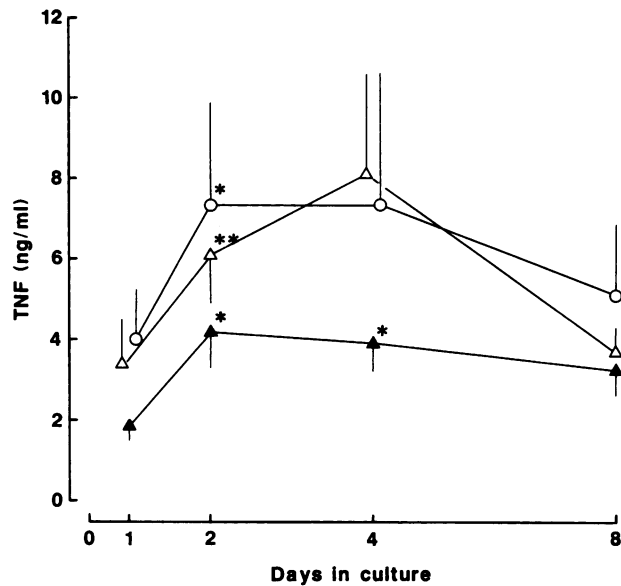
FIG. 5. TNF-α released from monocytes that had been cultured in the heat-inactivated pooled human serum (10%) from healthy donors. The cells were exposed to LPS (10 μg/ml) for the last 20 h in culture: healthy control (n = 6; O), newly diagnosed tuberculosis (n = 10; △), and chronic refractory tuberculosis (n = 10; ▲). Results are expressed as the mean ± standard error of the mean. Single asterisk, P < 0.05; double asterisk, P < 0.02 compared with day 1.

cytes with sera from all three groups in a dose-dependent manner (Fig. 6). At a serum concentration of 40%, the monocytes released higher amounts of TNF-α in the cases of both newly diagnosed (1.79 ± 1.27 ng/ml, mean ± SD) and chronic refractory tuberculosis (1.35 ± 0.90) compared with healthy controls (0.74 ± 0.20; P < 0.05 and P = 0.07, respectively). However, there was no statistical difference between the newly diagnosed and chronic refractory tuberculosis groups (P = 0.4).

Correlation between levels of TNF-α produced by monocytes and age of subjects. The mean age of patients with chronic refractory tuberculosis was significantly higher than the mean ages for both the healthy control and newly diagnosed tuberculosis groups (Table 1). For this reason, we investigated the correlation between the levels of TNF-α produced by monocytes and the age (in years) of the subjects (Fig. 7). There was no significant correlation between the levels of LPS- or BCG-induced TNF-α production and the age of the subjects.

DISCUSSION

It is well known that TNF-α is released from the cells of monocyte or macrophage lineage in response to LPS (6). TNF-α can also be released by a variety of infectious organisms themselves, including M. bovis BCG, M. tuberculosis, or L. monocytogenes (12, 21, 23). In this study, both LPS and BCG were able to stimulate the monocytes to produce TNF-α, although preincubation of monocytes with IFN-γ enhanced LPS- but not BCG-induced TNF-α production. The dose-response curves for TNF-α production by BCG differed from those for LPS (Fig. 2), and the amounts of TNF-α induced by BCG were much smaller than those induced by LPS (Fig. 3 and 4). It has been shown that TNF-α production can be induced when the monocyte phagocytoses particles, such as silica or insoluble immune complexes (5,
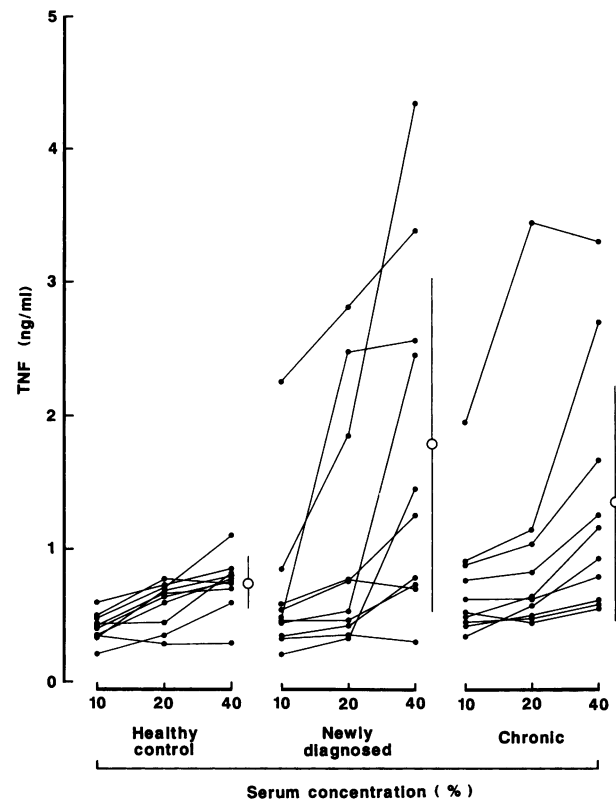

FIG. 6. Effect of preincubation with sera from tuberculosis patients on TNF-α production by monocytes taken from a healthy donor. After incubating for 24 h with the indicated serum concentration, the medium was replaced with RPMI 1640 medium which contained 10% PHS and indomethacin (10 μg/ml) and then stimulated with 1 μg of LPS per ml. The cells preincubated with 10, 20, and 40% of autologous serum released 0.36, 0.62, and 0.74 ng of TNF-α per ml, respectively. Vertical bars represent the mean values ± SD of the results at a serum concentration of 40%.

7). The effects of BCG and silica on TNF-α production by monocytes are similar. Thus, it seems likely that BCG may also trigger the monocyte activation to produce TNF-α through a phagocytic process by means of a different mechanism than that used by LPS.

In this study, the monocytes from newly diagnosed tuberculosis patients produced significantly more TNF-α than those from healthy controls in response to LPS. When the cells were primed with a T-cell-derived lymphokine such as IFN-γ, the significant difference disappeared. However, even when primed, the cells from patients with chronic refractory tuberculosis had a significantly lower ability to produce TNF-α compared with those from patients with newly diagnosed tuberculosis (Fig. 3). This was further confirmed in the BCG-induced TNF-α production, where the monocytes from the chronic refractory tuberculosis group produced significantly lower amounts of TNF-α than those from the newly diagnosed tuberculosis and healthy control groups (Fig. 4). We believe that this is the first time that increased or decreased TNF-α production in the various disease states of tuberculosis has been considered. Our findings suggest the important clinical implication of TNF-α in the pathogenesis of mycobacterial infections.

The patients with newly diagnosed tuberculosis had been treated with streptomycin, isoniazid, and rifampin. However, the patients with chronic refractory tuberculosis had
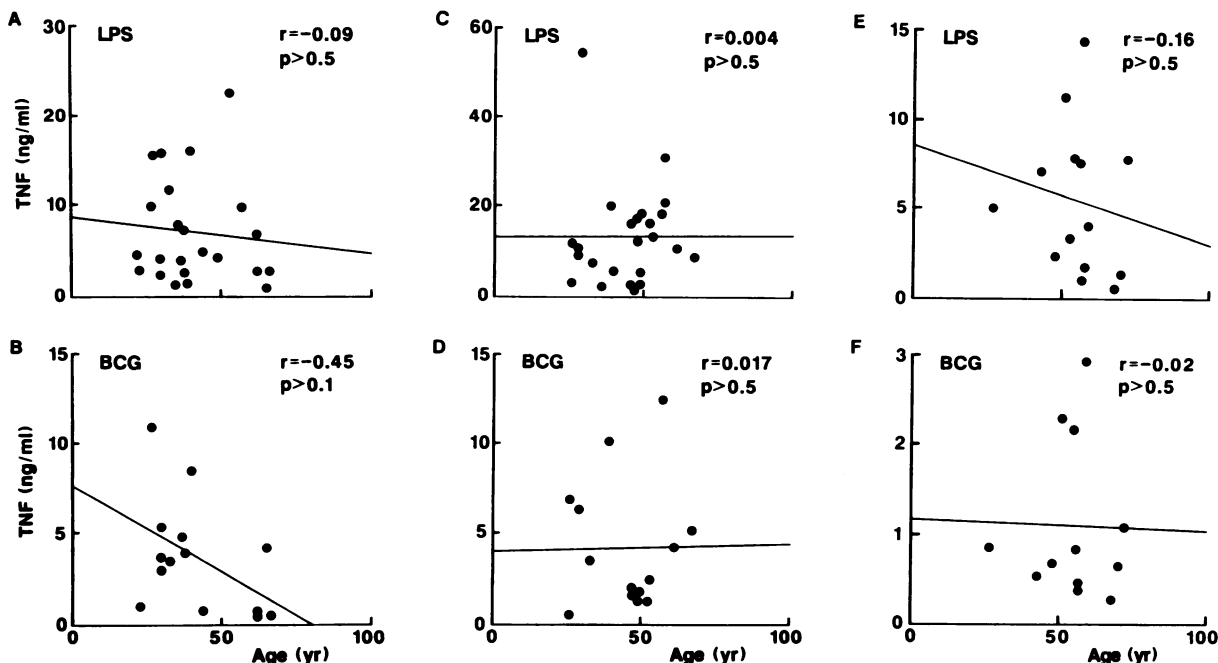
FIG. 7. Correlation between the levels of TNF-α produced by monocytes in response to LPS (10 μg/ml) or BCG (100 μg/ml) and the age (in years) of subjects. Subject groups: healthy controls (A and B), newly diagnosed tuberculosis patients (C and D), and chronic refractory tuberculosis patients (E and F).

not received any antituberculosis drugs because they had tubercle bacilli which are resistant to almost all drugs commonly used in tuberculosis treatment. Therefore, we investigated the effects of isoniazid and rifampin on LPS-induced TNF-α production by using monocytes from healthy controls. Although monocytes were cultured with doses ranging from 0.01 to 10 μg of isoniazid per ml or from 1.0 to 100 μg of rifampin per ml, LPS-induced TNF-α production was not affected by these drugs (data not shown). Weiss et al. have reported that T-cell and interleukin-4 may down-regulate the release of monokine by monocytes (24). This would seem to suggest that cells from patients with chronic refractory tuberculosis may have been under such suppressive effects. However, no suppressive effects by the sera from tuberculosis patients on LPS-induced TNF-α production were found in our study (Fig. 6). In addition, the low production of TNF-α by the monocytes derived from chronic refractory tuberculosis patients did not recover even when cultured in the sera of healthy individuals for 1 to 7 days (Fig. 5). These results suggest that antituberculosis drugs or serum factors were not responsible for the depressed TNF-α production in tuberculosis patients.

It is widely known that mycobacterial infections should be controlled by the cell-mediated immunity when sensitized T-cells and macrophages cooperate (15, 18). It is also thought that even in cases where antituberculosis drugs are used, the host must have sufficient antituberculous immunity to eradicate residual drug-resistant bacilli. Hence, it is reasonable to assume that impaired host immunity may be responsible for the development of chronic refractory tuberculosis. We formerly reported that the peripheral blood lymphocytes taken from patients with chronic refractory tuberculosis expressed a significantly depressed proliferative response to purified protein derivative, compared with those from cases of newly diagnosed tuberculosis, and that this response recovered to some degree with the addition of

interleukin-2 (22). This proved that depressed T-cell-mediated immunity was evident in patients with chronic refractory tuberculosis. Recently it has been shown that T-cell-derived lymphokines, such as IFN-γ and interleukin-2, are potent activators of TNF-α production by macrophages (10, 21). At first glance, it may seem that the increased or decreased TNF-α production by monocytes is simply a reflection of the level of T-cell-mediated immunity against mycobacterial infections. However, it seems unlikely that the depressed TNF-α production observed in chronic refractory tuberculosis was simply the result of depressed T-cell-mediated immunity against infections, because the depressed TNF-α production was demonstrated in response to both LPS and BCG even when primed with IFN-γ, with no suppressive effects on the production of TNF-α detected in the sera in these patients.

Recently, it has been reported that TNF-α can activate human macrophages to inhibit the intracellular multiplication of M. avium complex in vitro (3). It has also been shown that TNF-α plays a critical role in granuloma formation and bacillary elimination in experimental M. bovis BCG and L. monocytogenes infections in mice (13, 14). Thus, evidence is accumulating that TNF-α is an important macrophage-activating factor for antibacterial resistance against infections caused by facultative intracellular organisms. It has also been shown that there is a relation between HLA phenotype and production of TNF-α by monocytes in man (1, 20), and that the capacity of TNF-α production in Biozzi mouse lines is correlated to genetic resistance to various infections (9). Taken together, this suggests the possibility that TNF-α production by the cells of monocyte or macrophage lineage may be genetically controlled and that impaired function of the cells of monocyte or macrophage lineage, such as the depressed TNF-α production indicated in this study, may exist in the background of patients who develop chronic refractory mycobacterial infections.

3292 TAKASHIMA ET AL.

LITERATURE CITED

1. **Bendtzen, K., N. Morling, A. Fomsgaard, M. Svenson, B. Jakobsen, N. Odum, and A. Svejgaard.** 1988. Association between HLA-DR2 and production of tumour necrosis factor and interleukin 1 by mononuclear cells activated by lipopolysaccharide. Scand. J. Immunol. **28:**599–606.

2. **Bermudez, L. E., P. Stevens, P. Kolonoski, M. Wu, and L. S. Young.** 1989. Treatment of experimental disseminated *Mycobacterium avium* complex infection in mice with recombinant IL-2 and tumor necrosis factor. J. Immunol. **143:**2996–3000.

3. **Bermudez, L. E., and L. S. Young.** 1988. Tumor necrosis factor, alone or in combination with IL-2, but not IFN-γ is associated with macrophage killing of *Mycobacterium avium* complex. J. Immunol. **140:**3006–3013.

4. **Beutler, B., and A. Cerami.** 1987. Cachectin: more than a tumor necrosis factor. N. Engl. J. Med. **316:**379–385.

5. **Borm, P. J. A., N. Palmen, J. J. M. Engelen, and A. Buurman.** 1988. Spontaneous and stimulated release of tumor necrosis factor-alfa (TNF) from blood monocytes of miners with coal workers' pneumoconiosis. Am. Rev. Respir. Dis. **138:**1589–1594.

6. **Carswell, E. A., L. J. Old, R. L. Kassel, S. Green, N. Fiore, and B. Williamson.** 1975. An endotoxin-induced serum factor that causes necrosis of tumors. Proc. Natl. Acad. Sci. USA **72:**3666–3670.

7. **Debets, J. M. H., C. J. Van Der Linden, I. E. M. Dieteren, J. F. M. Leeuwenberg, and W. A. Buurman.** 1988. Fc-receptor cross-linking induces rapid secretion of tumor necrosis factor (cachectin) by human peripheral blood monocytes. J. Immunol. **141:**1197–1201.

8. **de Titto, E. H., J. R. Catterall, and J. S. Remington.** 1986. Activity of recombinant tumor necrosis factor on *Toxoplasma gondii* and *Trypanosoma cruzi*. J. Immunol. **137:**1342–1345.

9. **Dockrell, H. M., J. Taverne, R. Lelchuk, P. Depledge, I. N. Brown, and J. H. L. Playfair.** 1985. Macrophage functions in Biozzi mice. Immunology **55:**501–509.

10. **Economou, J. S., W. H. McBride, R. Essner, K. Rhoades, S. Golub, E. C. Holmes, and D. L. Morton.** 1989. Tumour necrosis factor production by IL-2-activated macrophages *in vitro* and *in vivo*. Immunology **67:**514–519.

11. **Espevik, T., and J. Nissen-Meyer.** 1986. A highly sensitive cell line, WEHI 164 clone 13, for measuring cytotoxic factor/tumor necrosis factor from human monocytes. J. Immunol. Methods **95:**99–105.

12. **Havell, E. A.** 1987. Production of tumor necrosis factor during murine listeriosis. J. Immunol. **139:**4225–4231.

13. **Havell, E. A.** 1989. Evidence that tumor necrosis factor has an important role in antibacterial resistance. J. Immunol. **143:**2894–2899.

14. **Kindler, V., A. P. Sappino, G. E. Grau, P. F. Piguet, and P. Vassalli.** 1989. The inducing role of tumor necrosis factor in the development of bactericidal granulomas during BCG infection. Cell **56:**731–740.

15. **Mackaness, G. B.** 1969. The influence of immunologically committed lymphoid cells on macrophage activity *in vivo*. J. Exp. Med. **129:**973–992.

16. **Nakane, A., T. Minagawa, and K. Kato.** 1988. Endogenous tumor necrosis factor (Cachectin) is essential to host resistance against *Listeria monocytogenes* infection. Infect. Immun. **56:**2563–2569.

17. **Nathan, C. F.** 1987. Secretory products of macrophages. J. Clin. Invest. **79:**319–326.

18. **North, R. J.** 1974. T cell dependence of macrophage activation and mobilization during infection with *Mycobacterium tuberculosis*. Infect. Immun. **10:**66–71.

19. **Renz, H., J. H. Gong, A. Schmidt, M. Nain, and D. Gemsa.** 1988. Release of tumor necrosis factor-α from macrophages. Enhancement and suppression are dose-dependently regulated by prostaglandin E_2 and cyclic nucleotides. J. Immunol. **141:**2388–2393.

20. **Repo, H., M. Jaattela, M. Leirisalo-Repo, and M. Hurme.** 1988. Production of tumour necrosis factor and interleukin 1 by monocytes of patients with previous *Yersisia* arthritis. Clin. Exp. Immunol. **72:**410–414.

21. **Rook, G. A. W., J. Taverne, C. Leveton, and J. Steele.** 1987. The role of gamma-interferon, vitamin D_3 metabolites and tumor necrosis factor in the pathogenesis of tuberculosis. Immunology **62:**229–234.

22. **Shiratsuchi, H., Y. Okuda, and I. Tsuyuguchi.** 1987. Recombinant human interleukin-2 reverses in vitro-deficient cell-mediated immune response to tuberculin purified protein derivative by lymphocytes of tuberculous patients. Infect. Immun. **55:**2126–2131.

23. **Valone, S. E., E. A. Rich, R. S. Wallis, and J. J. Ellner.** 1988. Expression of tumor necrosis factor in vitro by human mononuclear phagocytes stimulated with whole *Mycobacterium bovis* BCG and mycobacterial antigens. Infect. Immun. **56:**3313–3315.

24. **Weiss, L., N. Haeffner-Cavaillon, M. Laude, J. M. Cavaillon, and M. D. Kazatchkine.** 1989. Human T cells and interleukin 4 inhibit the release of interleukin 1 induced by lipopolysaccharide in serum-free cultures of autologous monocytes. Eur. J. Immunol. **19:**1347–1350.