Production of Tumor Necrosis Factor- α by Alveolar Macrophages from Patients with Pulmonary Tuberculosis

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In order to ascertain the role of TNF- α in pulmonary tuberculosis, we determined the TNF- α productivity of alveolar macrophages (AMs) obtained by bronchoalveolar lavage (BAL), along with the level of TNF- α in the serum of patients with tuberculosis including pulmonary, miliary, and endobronchial tuberculosis, healthy controls, and pulmonary diseases such as diffuse interstitial lung disease (DILD) and pneumonia.

AMs from patients with pulmonary tuberculosis did not produce a larger amount of TNF- α than did those from the healthy control subjects. However, among the patients with pulmonary tuberculosis, the AMs from the fresh and reactivated groups produced a larger amount of TNF- α than those from the inactive group. AMs from patients showing positivity in culture produced a larger amount of TNF- α than those showing negativity.

The average level of serum TNF- α in patients with pulmonary tuberculosis was slightly higher than that of the healthy control group. Among patients with pulmonary tuberculosis, significantly increased levels of serum TNF- α were noted in the reactivated group compared to those of the fresh and inactive group. Patients with moderate to far-advanced infiltration on their chest X-rays, showed a significantly higher level of serum TNF- α than those with minimal involvement on the chest X-ray. Smokers from the healthy control group showed a significantly higher level of serum TNF- α than non-smokers from the same group.

These results suggest that an increase in the production of TNF- α may correspond with the severity of pulmonary tuberculosis.

Key Words : Pulmonary tuberculosis, Tumor necrosis factor- α , Bronchoalveolar lavage, Immunoradiometric assay

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INTRODUCTION

Tumor necrosis factor- α (TNF- α), also referred to as cachectin, is a monokine produced

by activated monocytes and macrophages. Although at first TNF- α was described by its cytotoxicity for certain tumor cell lines (Carswell *et al.*, 1975), it is now recognized as having a broad range of activities associated with acute and chronic inflammatory states (Cerami and Beutler, 1988; Stephens *et al.*, 1988). TNF- α inhibits lipoprotein lipase and lipid-specific enzymes with a resulting loss of fatty tissue and hyperlipoproteinemia (Torti *et al.*, 1985). In addition, it is capable of acting as a growth stimulus for mesenchymal cells (Vilcek *et al.*, 1986). If recombinant TNF- α is introduced to animals, it induces anorexia and weight loss. However, the role of TNF- α in patients with cachexia due to chronic diseases is unclear.

It has been recognized that activated mononuclear phagocytes are a major source of TNF- α , and the alveolar macrophages (AMs) are pulmonary representatives of the mononuclear phagocytic system in the lung. The AMs accumulate in the inflammatory sites of the lung, release several molecules that fight cancer cells, and induce local fibrotic reaction. TNF- α is also one of these molecules, and the AMs are significantly more potent TNF- α producers than are their blood counterparts (Martinet *et al.*, 1988).

Pulmonary tuberculosis is a chronic inflammatory disease that shows necrosis and fibrosis of the lung parenchyme and is commonly accompanied by cachexia in terminal cases. In addition, the production of TNF- α is greatly influenced by whether or not it is primed with a priming agent such as *Bacillus Calmette-Guerin* (BCG) (Vogel *et al.*, 1980). Peritoneal macrophages sensitized with BCG can produce TNF- α by triggering with 1/100 parts of lipopolysaccharide (LPS), i.e., 1/100 of the amount of LPS used to induce TNF- α production in unsensitized peritoneal macrophages (Weinberg *et al.*, 1978). In the case of pulmonary tuberculosis patients, since it can be assumed that they are sensitized with *M. tuberculosis*, the triggering of TNF- α release from the resulting activated macrophage populations might explain much of the pathogenesis of the weight loss and tissue damage that characterize this disease.

In this study, we determined the TNF- α productivity of AMs obtained by bronchoalveolar lavage (BAL) and the amount of TNF- α in serum from pulmonary tuberculosis patients.

Table 1. Age, Sex, and Smoking History of Patients

Group	No. of Pts Examined	Sex		Age (mean $\pm$ S.D.)	Smoking	
		M	F		Yes	No
Control	14	10	4	42.2 $\pm$ 11.6	6	8
Tuberculosis						
Pulmonary tbc [★]	36	21	15	33.7 $\pm$ 13.5	7	29
fresh ^a	17	10	7	32.5 $\pm$ 13.1	5	12
reactivated ^b	9	5	4	33.7 $\pm$ 15.0	0	9
inactive ^c	10	6	4	34.4 $\pm$ 14.8	2	8
Miliary tbc	2	0	2	20.0 $\pm$ 0.0	0	2
Endobronchial tbc	2	0	2	20.0 $\pm$ 0.0	0	2
Other pulmonary diseases						
DILD ^{★★}	5	3	2	60.0 $\pm$ 7.2	2	3
Pneumonia	4	2	2	42.0 $\pm$ 11.5	1	3

★tbc : tuberculosis

★★DILD : diffuse interstitial lung disease

a fresh : pulmonary tbc without previous history of the same disease and less than 6 months of antituberculous treatment

b reactivated : pulmonary tbc with previous history of the same disease and less than 6 months of antituberculous treatment

c inactive : pulmonary tbc with more than 6 months of antituberculous treatment

MATERIALS AND METHODS

Study population

Patients diagnosed as pulmonary, miliary, or endobronchial tuberculosis were included in this study. Persons with normal chest X-ray findings or with mild inactive calcified lesions on the chest X-ray but with no other abnormal physical findings were regarded as healthy controls. Other pulmonary diseases such as diffuse interstitial lung disease (DILD) and pneumonia were also included in this study.

According to the previous tuberculosis history, along with disease activities determined by sputum studies and chest X-rays, pulmonary tuberculosis patients were subdivided into three groups: fresh, reactivated, and inactive.

Fresh group: These were patients who either showed a positive result on sputum smear or culture or had an active pulmonary lesion on the chest X-ray. In addition, they did not have a previous history of the same disease and had less than 6 months of antituberculous treatment.

Reactivated group: These were patients who either showed a positive result on sputum smear or culture, or had an active pulmonary lesion on the chest X-ray among those with a previous antituberculous treatment.

Inactive group: These were patients either with a negative result of sputum smear or culture, or with inactive or nonexistent pulmonary lesions as determined by a chest X-ray among those who were within one year after chemotherapy of 6–12 months.

Even though a person had previous history of pulmonary tuberculosis, if he had normal chest X-ray findings or calcified lesions without clinical symptoms and more than 1 year had passed since treatment, he was classified as a normal control. The age, sex, and smoking histories of patients are summarized in Table 1.

BAL

After the patients were premedicated with atrophine sulfate and demerol as in general bronchoscopy, the bronchoscope was inserted, and local anesthesia was done with 1% lidocaine. The bronchoscope was fixed on the left mid or lingular lobe in the control group and on the lesion site in patients with pulmonary tuberculosis and other pulmonary diseases. However, in case of difficulty in per-

forming the BAL due to excess secretions or bleeding, disease-free sites were selected. Lavage was performed using 30ml of 37°C and 0.9% NaCl solutions each time. A total of 150ml of 0.9% NaCl solution was used for each patient.

After fluid recovery, a small amount was taken for determination of cell number, viability, and differential counting. Lavage cell viability, assessed by trypan blue exclusion, was always more than 90%.

Purification of AMs

The purification of AMs was done by adhering the lavage cells on gelatin-coated flasks as described by Freundlich and Avdalovic(1983).

Preparation of gelatin-coated flasks was as follows: 2 percent gelatin solution was filtered with Whatman No. 2 filter paper, 0.8 and 0.45 μ m filter, sequentially. Ten ml of filtered gelatin solution was added to a 25cm³ culture flask, and placed at 37°C in an incubator for 2 hours. After removing the gelatin solution, the flasks were dried overnight.

Prior to the purification of AMs from the BAL fluid, the human pooled plasma was added to the already prepared gelatin-coated flask, left for 45 minutes, and washed 3 times with isotonic saline.

The BAL fluid was filtered with multi-layered sterile gauze and centrifuged(Kleinhenz et al., 1986). The cellular components were resuspended in a complete medium (RPMI 1640 (Hazleton, Lenexa KS) supplemented with 10% fetal calf serum (CSL, Australia), 2mM L-glutamine (Hazleton, Lenexa KS), 100 IU of penicillin/ml and 100 μ g of streptomycin/ml), and the cells were allowed to adhere on gelatin-coated culture flasks at 37°C with 5% CO₂. After 45 minutes, the nonadherent cells were removed with PBS, while the adherent cells were detached by a 5-minute exposure to 4.5 mM EDTA. Enriched AMs preparation contained more than 95% AMs, as determined by nonspecific esterase-stained(Li et al., 1973) cytocentrifuge preparations. Cell viability was more than 98%, tested by the trypan blue method.

Induction of TNF- α production by AMs

Adherent mononuclear cells were cultured in 24-well culture plates at 2x10⁶ cells/ml. As the AMs excreted various engulfed substances to the medium, such as carbon, the media were changed after 24 hours, the supernatants were collected, clarified by centrifugation, and stored frozen at -20°C until tested.

In order to determine the optimal concentration of LPS, various concentrations of LPS (0, 0.1, 1, and 10 $\mu\text{g/ml}$) were studied prior to the experiment, and 10 $\mu\text{g/ml}$ was chosen as the level that would provide maximal stimulation without cytotoxicity.

Determination of TNF- α in serum and culture supernatant using immunoradiometric assay (IRMA)

The levels of TNF- α in serum and AMs culture supernatants were measured by using TNF-IRMA kit (Medgenix Co., Brussels, Belgium). In brief, serum or supernatants for determination and ^{125}I -labeled-anti-TNF- α were added to anti TNF- α -coated tubes and incubated at room temperature for 18 hours. After aspirating the complete contents, the tubes were washed twice with 20% tween 20 solution and then aspirated again. The tubes were counted in a gamma counter for 60 seconds. The standard curve was prepared by cpm on the ordinate against the standard concentration on the abscissa using semi-log graph paper. The equation for calculating the concentration of sample was made by computer-assisted polynomial linear regression statistics.

Statistical analysis

The Student's t-test and Mann-Whitney U test were used to determine the significance of the data.

RESULTS

Number and types of cells in lavage fluid and recovery rate of lavage fluid

The mean total number of cells recovered, lavage cell differential (by cyto centrifugation preparation), and the recovery rate of the lavage fluid differed slightly according to the group (Table 2).

In tuberculosis patients, the mean recovery rate of the lavage fluid was similar to the healthy controls; however, the mean total number of cells in the lavage fluid was less than in the healthy controls.

The proportion of lymphocytes in the cells of the lavage fluid was higher in the tuberculosis patients when compared with the healthy control subjects.

TNF- α production by AMs

The amount of TNF- α produced by AMs showed great variations from 1.7 to 646.8 ng/ml. The average amounts of TNF- α produced by AMs were 304.8 ng/ml in control, 330.3 ng/ml in tuberculosis, 96.2 ng/ml in DILD, and 175.1 ng/ml in pneumonia.

Fig. 1. Individual values of TNF- α produced by alveolar macrophages from tuberculosis patients after stimulation with 10 $\mu\text{g/ml}$ of LPS. Supernatant was harvested after 24 hours of culture. The amount of TNF- α was determined by immunoradiometric assay.

a, b and c are described in the legends of Table 1.

* DILD : diffuse interstitial lung disease

Fig. 2. Individual values of TNF- α in pulmonary tuberculosis patients.

a, b and c are described in the legends of Table 1.

* DILD : diffuse interstitial lung disease.

Table 2. Recovered Fluid Volume and Number and Types of Cells in Bronchoalveolar Lavage Fluid

	Control (n=14)	Tuberculosis (n=33)	DILD ^a (n=5)	Pneumonia (n=4)
Recovered fluid volume (%) ^a	48.6 $\pm$ 3.7 ^b	45.6 $\pm$ 2.0	33.6 $\pm$ 2.9*	42.6 $\pm$ 3.8
No. of cells ($\times 10^4$ cells/ml)	18.6 $\pm$ 6.2	17.0 $\pm$ 3.1	21.9 $\pm$ 12.5	20.0 $\pm$ 6.8
Cell differential(%)				
macrophage	85.8 $\pm$ 1.7	80.1 $\pm$ 2.5	52.6 $\pm$ 15.7**	38.3 $\pm$ 12.3
lymphocyte	10.7 $\pm$ 1.0	17.2 $\pm$ 2.3	19.8 $\pm$ 11.3	43.0 $\pm$ 14.0
neutrophil	2.6 $\pm$ 0.9	2.4 $\pm$ 0.5	21.4 $\pm$ 15.3	2.1 $\pm$ 0.8

^a Recovered fluid volumes(%)=[aspirated fluid(ml)/injected fluid(ml)] $\times$ 100

^b Values are mean $\pm$ S.E.

* p=0.035 vs control by Mann-Whitney U test

** p=0.0272 vs control by Mann-Whitney U test

Table 3. TNF- α Concentration in Serum and in Culture Supernatant of Alveolar Macrophages from Tuberculosis Patients

Group	Supernatant(ng/ml)		Serum(pg/ml)	
	No. of Exam.	TNF- α ^d	No. of Exam.	TNF- α
Control	13	304.8 $\pm$ 64.4	13	5.4 $\pm$ 0.6
Tuberculosis				
Pulmonary tbc*	32	330.3 $\pm$ 59.2	38	7.4 $\pm$ 1.1
fresh ^d	14	361.6 $\pm$ 91.1	17	5.8 $\pm$ 0.8
reactivated ^d	8	326.4 $\pm$ 64.3	9	13.3 $\pm$ 3.6*
inactive ^d	9	157.9 $\pm$ 44.3	10	4.6 $\pm$ 0.6
Miliary tbc	2	100.2 $\pm$ 98.5	2	43.7 $\pm$ 39.4
Endobronchial tbc	2	233.7 $\pm$ 192.8	2	188.2 $\pm$ 183.8
Other pulmonary diseases				
DILD	4	96.2 $\pm$ 44.3	4	6.9 $\pm$ 1.9
Pneumonia	4	175.1 $\pm$ 80.4	4	8.1 $\pm$ 0.8

^a, ^b and ^c are described in the legends of Table 1.

^d Values are mean $\pm$ S.E.

*p=0.0032 vs control, p=0.0008 vs fresh, and p=0.0026 vs inactive, by Mann-Whitney U test.

monia patients(Table 3). The AMs from the patients with pulmonary tuberculosis did not produce a larger amount of TNF- α than did those from the healthy control subjects. Among the patients with pulmonary tuberculosis, the AMs from the fresh and reactivated groups produced a larger amount of TNF- α than those from the inactive group(Fig. 1).

Level of TNF- α in serum

In the healthy control group, the mean amount of TNF- α in the serum was 5.3 pg/ml. The pulmonary tuberculosis patient had 7.1 pg/ml, DILD 6.9

pg/ml, and the pneumonia patient had 8.1 pg/ml of TNF- α in serum.

The average level of serum TNF- α in patients with pulmonary tuberculosis was slightly higher than that of the healthy control group. Among the patients with pulmonary tuberculosis, significantly increased levels of serum TNF- α were noted in the reactivated group compared with those of the fresh and the inactive groups.

As the productivity of TNF- α by AMs and the level of TNF- α in serum was prominent in the reactivated group of pulmonary tuberculosis, we attempted to explore the relationship between the pro-

Fig. 3. Scattergram of TNF- α amount in serum and in culture supernatant of alveolar macrophages from total subjects.

Table 4. Bronchoalveolar Lavage Fluid Findings and Amount of TNF- α Produced by Alveolar Macrophages According to the Lavage Site in Tuberculosis Patients

	Healthy Control	Tuberculosis	
	(n=11)	Diseased Site(n=10)	Disease-free Site(n=24)
Recovered fluid volume (%) ^a	49.8 $\pm$ 4.2 ^b	46.7 $\pm$ 3.8	46.4 $\pm$ 2.2
Cell differential(%)			
macrophage	85.6 $\pm$ 1.9	70.6 $\pm$ 6.2*	79.5 $\pm$ 2.7
lymphocyte	10.5 $\pm$ 1.1	25.8 $\pm$ 5.6**	17.9 $\pm$ 2.5
neutrophil	2.8 $\pm$ 1.0	3.0 $\pm$ 1.1	2.3 $\pm$ 0.6
TNF- α level (ng/ml)			
supernatant	283.4 $\pm$ 56.0	230.3 $\pm$ 75.2	306.3 $\pm$ 55.6

^a Values are mean $\pm$ S.E.

^b Recovered fluid volume (%)=[aspirated fluid(ml)/injected fluid(ml)] $\times$ 100

*p=0.0486, and **p=0.0166 vs those of control by Mann-Whitney U test.

Table 5. TNF- α Production by Alveolar Macrophages According to Bacteriological and Radiological Findings in Pulmonary Tuberculosis Patients

TNF- α level	Mycobacteria in Culture		Chest X-ray Involvement	
	Negative	Positive	Minimal	Moderate to Far-advanced
Serum (pg/ml)	7.2 $\pm$ 1.2 ^a (n=19)	8.5 $\pm$ 2.1 (n=15)	5.4 $\pm$ 0.6 (n=20)	9.7 $\pm$ 2.2* (n=17)
Supernatant (ng/ml)	260.6 $\pm$ 46.7 (n=17)	346.4 $\pm$ 96.6 (n=13)	246.1 $\pm$ 53.6 (n=14)	273.6 $\pm$ 50.1 (n=16)

^a Values are mean $\pm$ S.E.

* p=0.0488 vs minimal involvement by Student's t-test

ductivity of TNF- α and the level of TNF- α in serum. However, the amount of TNF- α produced by AMs did not have any relationship to the level of TNF- α in serum(Fig. 3).

Relationship among the amount of TNF- α produced by AMs and the lavage site, the bacteriological findings, and the radiological findings.

Significantly larger proportions of lymphocytes and smaller macrophages were found in the lavage fluid obtained from the lesion site than from the disease-free site of pulmonary tuberculosis patients (Table 4). The AMs from the lesion site did not produce a larger amount of TNF- α than those from the disease-free site; however, those from patients with positivity in the sputum culture showed a higher productivity of TNF- α than those with ne-

Table 6. TNF- α Concentration in Serum and in Culture Supernatant of Alveolar Macrophages According to Smoking History in the Controls

TNF- α level	Smoker	Non-smoker
Serum (pg/ml)	6.9 $\pm$ 1.0 ^a *	4.4 $\pm$ 0.5 (n=8)
Supernatant (ng/ml)	202.7 $\pm$ 49.3	392.3 $\pm$ 104.3 (n=7)

^a Values are mean $\pm$ S.E.

* p=0.0404 vs non-smoker by Mann-Whitney U test

gativity (Table 5).

The levels of serum TNF- α were not related to the culture positivity of sputum AFB in the patients with pulmonary tuberculosis. However, the patients with moderate to far-advanced chest infiltration showed a significantly higher level of serum TNF- α than those with minimal involvement by chest X-ray (Table 5).

In addition, we evaluated the production of TNF- α by AMs from smokers and the serum level of TNF- α in smokers. Smokers from the healthy control group showed significantly higher levels of serum TNF- α than non-smokers from the same group (Table 6).

DISCUSSION

Until recently, TNF- α has generally been quantified by potency tests of in vitro cytotoxic activity against sensitive cell lines such as L-929 derived from mouse fibroblast, WEHI 164 clone 13 (Aggarwal et al., 1985; Espevik and Nissen-Meyer, 1986). In spite of the absence of TNF- α in the culture supernatants by means of in vitro cytotoxicity assay, low levels of TNF- α gene transcription could be detected in normal macrophages (Sariban et al., 1988). In this study, we used IRMA for determination of TNF- α . Since the sensitivity and specificity of IRMA depends on the affinity of TNF- α specific monoclonal antibody, IRMA is highly reproducible and its detection limit is at the picogram level. In our studies, we could not detect the TNF- α in human serum by means of the bioassay technique. In the case of IRMA, however, the mean concentration of TNF- α in the serum of the healthy control was detected as 5.3 pg/ml.

It is well-known that macrophages play an important role in the pathogenesis of chronic granulomatous diseases, including pulmonary tuberculosis. In order to induce the release of TNF- α , certain

sensitizing agents such as BCG, zymosan, and *Corynebacterium parvum* are required. *Mycobacterium tuberculosis* can also act as a TNF-inducing agent like LPS. In contrast to lymphocytes that react to specific antigens, macrophages can act as nonspecific defenders. If antigens such as *M. tuberculosis* come into the respiratory tract, they are phagocytosed by AMs, and the phagocytosed macrophages activate the circuit of immune response by stimulating T lymphocyte. Macrophages, which are activated directly by antigens, and/or indirectly by soluble mediators released from activated T lymphocytes, release various chemical mediators, such as complement protein, lysosomal enzyme, neutral protease, lysozyme, and TNF- α (Männel et al., 1980; Bates, 1982; Nathan, 1987). As the role of TNF- α in immunological and inflammatory responses is unveiled, changes in the amount of TNF- α are now being reported in acute inflammatory diseases (Gran et al., 1987; Waage et al., 1987; Furukawa et al., 1988). However, the role of TNF- α in chronic inflammatory diseases is still unclear.

On the basis of the above information, we suspected that TNF- α may have some role in the pathogenesis of pulmonary tuberculosis and measured the quantity of TNF- α in serum and in culture supernatants of AMs from patients with pulmonary tuberculosis.

The mean level of TNF- α in the serum of the pulmonary tuberculosis patients was 7.1 pg/ml, which was slightly higher than in the normal controls. Among the subgroups of pulmonary tuberculosis, elevation of TNF- α was most prominent in the reactivated group. The moderate to far-advanced pulmonary tuberculosis patients showed a higher level of TNF- α in their serum than the minimally involved ones. In addition, the more severely infiltrated patients mainly belonged to the reactivated group. Rook et al. (1987) suggested that the necrosis in tuberculosis might result from the triggering of TNF release from the activated macro-

phages. More severely infiltrated patients with the higher level of serum TNF- α , found in this study, suggested that TNF- α may participate in the formation of granulomatous lesions and tissue necrosis of pulmonary tuberculosis.

In order to ascertain whether the increased level of TNF- α in the serum of the pulmonary tuberculosis patients was due to the increased TNF release or to the increased number of AMs at the lesion site, we counted the number of AMs in the lavage fluid and determined the productivity of TNF- α by AMs stimulated with LPS. Since the number of AMs from tuberculosis patients showed slightly lower than that from the normal controls, it seems that the increased level of TNF- α in patients' serum does not result from changes in the cell number. Despite the increased blood level of TNF- α in reactivated group, the productivity of TNF- α did not increase markedly compared with the control group. In addition, there was no noticeable relationship between the level of TNF- α in serum and the amount of TNF- α produced by AMs in this experiment. Beutler *et al.* (1985) reports that the prolonged stimulation of macrophages with LPS maintains high serum levels of TNF- α ; however, in the case of a single, massive inoculation of LPS, the increase is temporary. In a chronic disease like pulmonary tuberculosis, in contrast with an acute disease like endotoxic shock, an increase in serum TNF- α may result from localized, prolonged activation of macrophages directly by *M. tuberculosis* or indirectly by the activated immune system without activating all the macrophages systemically.

It is noteworthy that the level of TNF- α is significantly higher in cigarette smokers' serum than in the non-smokers'. Animal studies reveal that the humoral and cellular immunities mediated by T lymphocytes are augmented by short-term exposure to smoking but decrease in chronic exposure. Depression of T lymphocyte function, a prominent increase in the number of AMs, a three-fold increment in metabolism, and an increase of lysozymes are noted in chronic smokers (Holt, 1987). It is reported that the activation of macrophages and the resulting increased release of cytotoxic mediators by macrophages may be the cause of tissue damage in smokers. The significant increase of TNF- α in smokers' serum shown in our study suggests that TNF- α also may be involved in tissue damage.

In this study, we found that the serum TNF- α levels were higher in patients with reactivated pul-

monary tuberculosis and miliary tuberculosis and that serum TNF- α levels were significantly higher in cigarette smokers. These data suggest the possibility that *M. tuberculosis* and a nonspecific irritant, such as cigarette smoke, induce the activation of AMs and that the secretion of TNF- α by these activated AMs is an important predisposing factor for tissue damage in pulmonary tuberculosis.

REFERENCES

- Aggarwal BB, Kohr WJ, Hass PE : *Human necrosis factor, production, purification, and characterization. J Biol Chem* 260 : 2345-2354, 1985.
- Bates JH : *Tuberculosis : susceptibility and resistance. Am Rev Respir Dis* 125(Koch centennial suppl) : 20-24, 1982.
- Beutler BA, Milsark IW, Cerami A : *Cachectin/tumor necrosis factor : production, distribution and metabolic fate in vivo. J Immunol* 135 : 3972-3977, 1985.
- Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, Williamson B : *An endotoxin-induced serum factor that causes necrosis of tumors. Proc Natl Acad Sci USA* 72 : 3666-3670, 1975.
- Cerami A, Beutler B : *The role of cachectin/TNF- α in endotoxic shock and cachexia. Immunology Today* 9 : 28-31, 1988.
- Espevik T, Nissen-Meyer J : *A highly sensitive cell line, WEHI 164 clone 13, for measuring cytotoxic factor/tumour necrosis factor from human monocytes. J Immunol Methods* 95 : 99-104, 1986.
- Freundlich F, Avdalovic N : *Use of gelatin/plasma coated flasks for isolating human peripheral blood monocytes. J Immunol Methods* 62 : 31-37, 1983.
- Furukawa S, Matsubara T, Jujoh K, Yone K, Sugawara T, Sasai K, Kato H, Yabuta K : *Peripheral blood monocyte/macrophages and serum tumor necrosis factor in Kawasaki disease. Clin Immunol Immunopath* 48 : 247-251, 1988.
- Gran GE, Fajardo LF, Piguet PF, Allet B, Lambert PH, Vassalli P : *Tumor necrosis factor(cachectin) as an essential mediator in murine cerebral malaria. Science* 237 : 1210-1212, 1987.
- Holt PG : *Immune and inflammatory function in cigarette smokers. Thorax* 42 : 241-249, 1987.
- Kleinhenz ME, Fujiwara H, Rich EA : *Interleukin-1 production by blood monocytes and bronchoalveolar cells in sarcoidosis. Ann New York Acad*

- Sci* 465 : 91-97, 1986.
- Li CY, Lam KW, Yam LT : Esterases in human leukocytes. *J Histochem Cytochem* 21 : 1-12, 1973.
- Männel DN, Moore RN, Mergenhagen SE : Macrophages as a source of tumoricidal activity (tumor-necrotizing factor). *Infect Immun* 30 : 523-530, 1980.
- Martinet Y, Yamauchi K, Crystal RG : Differential expression of the tumor necrosis factor/cachectin gene by blood and lung mononuclear phagocytes. *Am Rev Respir Dis* 138 : 659-665, 1988.
- Nathan CF : Secretory products of macrophages. *J Clin Invest* 79 : 319-326, 1987.
- Rook GAW, Taverne J, Leveton C, Steele J : The role of gamma-interferon, vitamin D3 metabolites and tumor necrosis factor in the pathogenesis of tuberculosis. *Immunology* 62 : 229-234, 1987.
- Sariban E, Imamura K, Luebbers R, Kufe D : Transcriptional and posttranscriptional regulation of tumor necrosis factor gene expression in human monocytes. *J Clin Invest* 81 : 1506-1510, 1988.
- Stephens KE, Ishizaka A, Larrick JW, Raffin TA : Tumor necrosis factor causes increased pulmonary permeability and edema. *Am Rev Respir Dis* 137 : 1364-1370, 1988.
- Torti FM, Dieckmann B, Beutler B, Cerami A, Ringold GM : A macrophage factor inhibits adipocyte gene expression : an in vitro model of cachexia. *Science* 229 : 867-869, 1985.
- Vilcek J, Palombella VJ, Henriksen-De Stephano D, Swenson C, Feinman R, Hirai H : Fibroblast growth enhancing activity of TNF and its relationship to other polypeptide growth factors. *J Exp Med* 163 : 632-643, 1986.
- Vogel SN, Moore RN, Sipe JD, Rosenstreich DL : BCG-induced enhancement of endotoxin sensitivity in C3H/HeJ mice I. in vivo studies. *J Immunol* 124 : 2004-2009, 1980.
- Waage A, Halstensen A, Espevik T : Association between tumor necrosis factor in serum and fatal outcome in patients with meningococcal disease. *Lancet* 8529 : 355-357, 1987.
- Weinberg JB, Chapman HA, Hibbs JB : Characterization of the effects of endotoxin on macrophage tumor cell killing. *J Immunol* 121 : 72-80, 1978.