Soluble TNF-α receptor and IL-1 receptor antagonist elevation in BAL in active pulmonary TB


Soluble TNF-α receptor and IL-1 receptor antagonist elevation in BAL in active pulmonary TB. ©ERS Journals Ltd 1999.

ABSTRACT: Accumulating evidence suggests that patients with active pulmonary tuberculosis (TB) have an alveolar inflammation resulting in the release of tumour necrosis factor (TNF)-α and interleukin (IL)-1β in bronchoalveolar epithelial fluid. It was proposed that the levels of these cytokines would correlate with clinical status parameters (extent of pulmonary involvement, fever, and body weight loss) and that their naturally occurring inhibitors would be concomitantly released in the local inflammatory sites.

To test this hypothesis lung epithelial lining fluid (ELF) obtained by bronchoalveolar lavage and serum were collected from 29 patients with active pulmonary TB and 15 healthy subjects to determine the levels of these variables using a sandwich enzyme-linked immunosorbent assay (ELISA).

ELF levels of TNF-α, soluble (s)TNF receptor I (RI), sTNF-receptor II (RII) and interleukin-1 receptor antagonist (IL-1RA) but not IL-1β, and their serum levels except for sTNF-RII and IL-1β were significantly higher in TB patients. Nevertheless, only ELF levels of TNF-α and IL-1β were significantly correlated with disease status.

No correlation was found between TNF-α levels and those of sTNF-RI and sTNF-RII, nor between IL-1β and IL-1RA in ELF and serum of TB patients, although there was a significant correlation between sTNF-RI and sTNF-RII levels both in ELF and serum. These findings suggest local release of tumour necrosis factor-α and interleukin-1β and a correlation with disease status. Soluble tumour necrosis factor-α receptors and interleukin-1β receptor antagonist, although increased in lung epithelial lining fluid and serum in tuberculosis patients, were not correlated with tumour necrosis factor-α or interleukin-1β or with disease status.

Studies in patients with active pulmonary tuberculosis (TB), have shown an increased amount of tumour necrosis factor (TNF)-α in the serum [1, 2], and the pleural fluid [3, 4]. Recently, CONDOS et al. [5] demonstrated elevated levels of TNF-α and interleukin (IL)-1β in bronchoalveolar lavage fluid (BALF) from patients with active pulmonary TB compared with that from healthy subjects. They also found that IL-1β levels were increased in radiographically involved lobes compared with radiographically uninvolved lobes. Levels of TNF-α were equally elevated in both involved and uninvolved lobes. On the contrary, bronchoalveolar cells released increased amounts of both TNF-α and IL-1β from the radiographically involved lobe compared with the radiographically uninvolved lobe in 24-h cell culture supernatants [5, 6].

Two types of soluble(s) TNF-α receptor forms (sTNF-Rs) with molecular masses of 55 kD (sTNF-RI) and 75 kD (sTNF-RII) have been found in human biological fluid [7–9], and tissue [10, 11]. The function of these TNF-binding proteins is thought to be to regulate the bioavailability of TNF-α in the body. Moreover the soluble form of these proteins may be one component of an autocrine and paracrine regulatory system which limits the toxic effects of systemically circulating TNF [12–14]. The elevation of sTNF-Rs in serum/plasma, reflecting a variety of inflammatory disorders, has been shown in many diseases, such as cancer [15], rheumatoid arthritis [16], sarcoidosis [17], and human immunodeficiency virus (HIV) infection [18]. In patients with active pulmonary TB and TB meningitis, the level of sTNF-Rs was elevated in the serum [19, 20] and cerebrospinal fluid (CSF) [21, 22], respectively.

IL-1 receptor antagonist (IL-1RA) a naturally occurring anti-inflammatory protein competitively blocks the binding of IL-1α and IL-1β to type I and type II IL-1 receptors, but exerts no agonist activity [23, 24]. IL-1RA is elevated in the serum of patients with a variety of conditions including sepsis [25] and autoimmune diseases [26–28]. Recently, JUFFERMANS et al. [29] described elevated levels of sTNF-RI, sTNF-RII, and IL-1RA in patients with active TB compared with control subjects and stated that the levels of sTNF-RI and IL-1RA were higher in patients with fever and anorexia.

In patients with active pulmonary TB, the local inflammation in the lower respiratory tract may cause the release of these cytokines as well as the shedding of their soluble receptors in bronchoalveolar epithelial fluid. The local levels of these inflammatory cytokines and their receptors or antagonists in BALF would be more representative of pulmonary disease, suggesting that the levels of these cytokines could be correlated with clinical course, such as extent of pulmonary involvement, fever, and body weight loss. In order to address these questions, BALF was...
collected from 29 patients with active pulmonary TB and 15 healthy subjects. The levels of the above cytokines, their receptor forms and antagonists were analysed, after normalization with urea levels to obtain lung epithelial lining fluid (ELF) measures.

**Materials and methods**

**Patient selection**

The study included 29 patients with active pulmonary TB who were referred to Chang Gung Memorial Hospital. Twenty-two patients were male and nine were female; mean age was 50±3 (22–77) (mean±SEM (range)) yrs. Pulmonary TB was diagnosed by chest radiographs, clinical pictures and at least one positive sputum smear for acid-fast bacilli and a positive sputum culture for *Mycobacterium tuberculosis*. None of the patients was immunocompromised, such as with human immunodeficiency virus (HIV) infection or immunosuppressant therapy. Fifteen healthy volunteers including 10 males and five females were studied as control subjects. All had normal chest radiographs, no symptoms of disease, and were not taking any medications.

**Bronchoscopy and bronchoalveolar lavage**

Bronchoscopy with bronchoalveolar lavage (BAL) was performed as described previously [30]. In brief, patients were pretreated with codeine phosphate (5 mg, i.m.) 30 min prior to the procedures and midazolam (2–3 mg, i.v. slowly) was given to some patients suffering from anxiety. An Olympus 5 mm fiberoptic bronchoscope (Olympus, Tokyo, Japan) was used and wedged into a forth or fifth subsegmental bronchus of patients under a local spray of xylocain. Lavage was performed using 50-mL aliquots of warmed normal saline introduced by syringe through the bronchoscopic aspiration port. In consideration of the dilution effect of the cytokines and their receptors in the BALF, a fixed volume of 300 mL saline was infused sequentially, and the return fluid was obtained through the same syringe. Any individuals who could not tolerate the whole procedure or whose returned fluid was <60% of total infused volume were excluded. In patients with pulmonary TB, BAL was performed in the radiographically involved bronchi within the first 3 days of antituberculous chemotherapy. In healthy subjects, BAL was performed in the right middle lobe bronchus. All returned fluid was filtered through four layers of sterile gauze, and then pooled and chilled immediately before experimental use. The pooled fluid was spun at 4°C at 400 × g for 15 min to pellet the cells and the supernatant was centrifuged at 80,000 × g for 30 min at 4°C to remove the surfactant-rich fraction. The resultant supernatant was concentrated 10-fold on a 10,000 molecular weight cut-off filter (Amicon, Danvers, MA, USA) under nitrogen. The concentrated supernatant was then divided into 200-μL aliquots and rapidly frozen at -70°C. Serum was obtained by drawing whole blood using standard procedures on the same day as the BAL.

**Determination of cytokines and cytokine receptor forms**

A sandwich enzyme-linked immunosorbent assay (ELISA) was used to detect the levels of cytokines and receptors in the BALF and serum. The kits for TNF-α and IL-1β were purchased from Medgenix (Fleurus, Belgium), and those for sTNF-RI, sTNF-RII as well as IL-1RA were from Quantikine™ (R&D, Minneapolis, MN, USA). The frozen aliquots from the BALF and serum were thawed at room temperature for each assay, and the samples were diluted 1:10 for sTNF-RI and sTNF-RII. The kits were used according to the manufacturer’s instructions. The minimal detectable dose using a standard curve generated with calibrator diluent was 3 pg·mL⁻¹ for TNF-α, 10 pg·mL⁻¹ for TNF-RI, 5 pg·mL⁻¹ for TNF-RII, 0.06 pg·mL⁻¹ for IL-1β and 6.5 pg·mL⁻¹ for IL-1RA.

**Standardization of cytokine levels**

The levels of cytokine in the BALF were standardized to the concentration of urea in serum and BALF to give concentrations per millilitre of ELF. Urea concentration was determined using a modified urease Berthelot reaction in microtitre plates [31]. ELF volume was then calculated using the formula: |[Urea]BALF/[Urea]Serum| × Vo = BALF. Urea concentrations were measured on unconcentrated BALF.

**Classification of patients by clinical course**

To evaluate whether the levels of the cytokines and soluble receptor forms would be correlated with the clinical course, TB patients were classified by the extent of pulmonary involvement, fever, or body weight loss. A grading of the extent of pulmonary involvement proposed by Crofton and Douglas [32] was adopted to assess the extent of disease: (1) Minimal. Minimal lesions include those which are of slight to moderate density but which do not contain demonstrable cavitation. They may involve a small part of one or both lungs, but the total extent, regardless of distribution, should not exceed the volume of lung on one side which is present above the second chondrosternal junction and the spine of the fourth or the body of the fifth thoracic body; (2) Moderately advanced. Moderately advanced lesions may be present in one or both lungs, but the total extent should not exceed the following limits: disseminated lesions or slight to moderate density which may extend throughout the total volume of one lung, or equivalent in both lungs, dense and confluent lesions which are limited in extent to one third the volume of one lung; total diameter of cavitation, if present must be <4 cm; and (3) Far advanced. Lesions more extensive than moderately advanced.

All patients received plain posterior, anterior, and lateral chest radiographs and 13 had chest computed tomographic (CT) scans in order to obtain a clearer image for patient classification. The presence of fever was defined when a patient had a core body temperature >37.5°C and body weight loss was confirmed by the patient. To avoid observer bias the radiographs and clinical courses were initially assessed independently by two pulmonary physicians prior to the laboratory studies to give an objective and consistent evaluation.

**Statistics**

Values are presented as medians (range). Comparisons between groups were made using the Wilcoxon rank-sum test for unmatched samples, [33] and the correlation
analysis, using the nonparametric Spearman test. Multivariate analysis of the correlation between cytokines and the clinical course (logistic regression) were performed. The null hypothesis was rejected at p<0.05.

Results

Levels of tumour necrosis factor-α, soluble tumour necrosis factor receptors, interleukin-1β and interleukin-1 receptor antagonist

Figures 1 and 2 show the levels of TNF-α, sTNF-Rs, IL-1β and IL-1RA in ELF and serum from patients with pulmonary TB and healthy subjects. The levels of TNF-α and sTNF-R1 were significantly increased in ELF and serum from TB patients. Median ELF and serum levels of IL-1RA level significantly increased only in the ELF, (162701.1 pg·mL⁻¹ (218.2±11130), p<0.0002 TNF-α, IL-1β and IL-1RA (2033.1±97689.6) and 33007±560751, p<0.0002 IL-1β, and sTNF-RII; 0.002 IL-1RA; and 0.004 sTNF-RI. IL-1β was undetectable in all but one control subject and in all but three TB patients.

Correlation of clinical status and cytokine levels

Figures 3, 4 and 5 show the correlation between clinical status and levels of TNF-α, sTNF-Rs, IL-1β and IL-1RA in ELF from patients with pulmonary TB. Patients with far advanced pulmonary involvement had significantly higher ELF levels of TNF-α and IL-1β than patients with moderately advanced and minimal involvement, 24242.1 (2191.7–97689.6) versus 4566.3 (2066.6–30111.9) versus 3891.1 (2033.5–5816.8) pg·mL⁻¹, respectively, for TNF-α, and 9916.3 (0–87305.4) versus 733.4 (0–3526) versus 0 pg·mL⁻¹, respectively, for IL-1β.
Regression, the ELF TNF-α level was the significant predictor for the clinical course including far advanced pulmonary involvement and fever. The BALF levels of stTNF-R1, stTNF-RII and IL-1RA in TB patients did not correlate with the disease status. There was no correlation between the serum levels of TNF-α, TNF-Rs, IL-1β and IL-1RA and the clinical status (data not shown).

Correlation between tumour necrosis factor-α and soluble tumour necrosis factor receptors

Since it is possible that increased TNF-α itself is a stimulating factor for production of soluble TNF receptors, the relationship between TNF-α and its two receptor forms was investigated. A very poor correlation was found between the levels of TNF-α and stTNF-R1 (r = 0.42) and stTNF-RII (r = 0.36) in the ELF of TB patients. The serum level of TNF-α also failed to show a good correlation with the level of stTNF-R1 (r = 0.45) and stTNF-RII (r = 0.34) in TB patients. There was no significant correlation between the levels of TNF-α and stTNF-R1 as well as sTNF-RII in the healthy subjects. The level of stTNF-R1 showed a significant correlation with stTNF-RII in the ELF (r = 0.85, p = 0.0001) and serum (r = 0.68, p = 0.001) from TB patients. A significant correlation between sTNF-R1 and stTNF-RII was also found in ELF but not in the serum from healthy subjects. No correlation was found between IL-1β and IL-1RA in ELF and serum from either TB patients or healthy subjects. There was no correlation between ELF levels and serum levels of TNF-α, stTNF-R1, stTNF-RII, IL-1β and IL-1RA.

Discussion

A set of observations are described here, which show that the ELF from TB patients presented significantly higher levels of TNF-α and its soluble receptor forms (stTNF-R1, stTNF-RII) than that of healthy control subjects. Moreover, the ELF levels of TNF-α and IL-1β were significantly correlated with the clinical status, i.e. the patient with more advanced disease, with fever or with body weight loss had significantly higher levels of TNF-α and IL-1β. Nevertheless, a correlation between the ELF levels of stTNF-R1, stTNF-RII and IL-1RA and the clinical status was not found. Also, there was no correlation between the serum levels of TNF-α, stTNF-R1, stTNF-RII, IL-1β, and IL-1RA and the clinical course. A good correlation, was not found between the levels of TNF-α and those of both sTNF-R1 and sTNF-RII in the ELF and serum of TB patients, although, there was a significant correlation between sTNF-R1 and sTNF-RII levels both in the ELF and the serum from TB patients.

Recently, two studies have described bronchoalveolar cells from the site of active pulmonary TB spontaneously releasing more TNF-α and IL-1β than cells from healthy subjects [5, 6]. One of these studies also showed increased levels of TNF-α and IL-1β in the BALF compared with those from healthy subjects [5, 6]. In the current study, the levels of TNF-α, stTNF-R1, stTNF-RII, IL-1β and IL-1RA were measured in the ELF. It was found that ELF levels of these proteins, except for IL-1β, were significantly higher in TB patients than in healthy subjects. Indeed higher levels of IL-1β were found compared to healthy subjects, 1441.5 (0–87305.4) pg·mL⁻¹ ELF, but the p-value was not significant because of the large
data variation. The positive correlation between the disease status and levels of TNF-α and IL-1β may suggest that bronchoalveolar cells are activated and release pro-inflammatory cytokines in the local epithelial fluid after exposure to *M. tuberculosis*. The release of cytokines tends to be local in nature and corresponds to the load of TB bacilli and these cytokines may reach the circulation later. Nevertheless, a significant correlation between the ELF and serum levels for each variable of TNF-α, sTNF-RI, sTNF-RII, IL-1β and IL-1RA was not found. Therefore, the reasons for the significantly increased serum levels of TNF-α, sTNF-RI and IL-1RA in this study might be complex.

It is not yet clear whether the soluble TNF-α receptor forms are TNF-α inhibitors, or whether they prolong TNF-α effects by stabilizing TNF-α. In 1990, Foley et al. [34] first described the inhibition of TNF-α toxicity caused by sera from most TB patients. The soluble forms of TNF-α receptors had inhibitory properties against TNF-α [12, 14], and were elevated in serum/plasma from patients with a variety of disorders [15, 18]. Recently, a few studies have demonstrated significantly increased levels of sTNF-RI and sTNF-RII in serum from patients with active pulmonary TB [19, 20, 29]. In the current study, significantly higher levels of sTNF-RI were found, but not sTNF-RII, in serum from TB patients. This was caused by a higher baseline of sTNF-RII in healthy subjects associated with a milder increase of this receptor in TB patients. Nevertheless, significantly higher levels of both sTNF-RI and sTNF-RII were found in the ELF. Juffermans et al. [29] also demonstrated higher serum levels of sTNF-RI and IL-1RA in TB patients with fever and anorexia. In the current study, ELF levels of TNF-α and IL-1β but not serum levels of TNF-α, sTNF-Rs, IL-1β or IL-1RA were found to be correlated with the disease status, including extent of pulmonary involvement, fever and body weight loss. One study described a significant positive correlation between serum levels of TNF-α and both sTNF-RI and sTNF-RII [20]. In this study no strong correlations were found between TNF-α and stNF-RI and stNF-RII in the ELF or in the serum from TB patients, although, there was a significant correlation between sTNF-RI and sTNF-RII levels both in the ELF and serum from TB patients. These negative findings may indicate that the regulation of the release of soluble TNF-α receptors in response to TB infection is complex. Bargetzi et al. [35] stated that in patients given an intravenous infusion of recombinant human (rh)IL-1β, a maximum 7–8-fold increase of sTNF-RI, and a 2–3-fold increase of TNF-RII were seen. These results support diversity of the increase of soluble TNF-α receptors. In this study the significant increases of sTNF-RII in the ELF but not the serum may only hint at the difference between the local (bronchoalveolar) and the systemic response of this receptor to *M. tuberculosis* infection. From these data it is not possible to deduce whether TNF-α induces its soluble receptors or whether these molecules are instead co-regulated.

IL-1β has been demonstrated to be an important cytokine in granulomatous alveolitis in a bacille Calmette-Guérin (BCG)-infected mouse [36]. Bronchoalveolar cells [5, 6] or peripheral monocytes [37–39] from patients with active pulmonary TB have been shown to release significantly greater amounts of this cytokine than did healthy subjects. In this study, although the median ELF level of IL-1β in TB patients was higher than in healthy subjects, there was a borderline p-value. However, it was found that both the ELF and serum levels of IL-1β were significantly higher in patients with more advanced disease, with fever or with body weight loss. These findings suggest that the expression and release of IL-1β may need exposure to larger amount of *M. tuberculosis* and with more severe infection. The IL-1RA level is elevated in the serum of patients with many conditions such as sepsis and autoimmune diseases [25–27]. The effects of IL-1RA on blocking receptor binding of IL-1 during the acute-phase response may serve to suppress the inflammatory consequences of early IL-1 release [28]. In this study it was found that the level of IL-1RA was high in both the ELF and serum from TB patients. Because the level of IL-1β in the serum was mostly undetectable in both healthy subjects and TB patients, no correlation was found between IL-1β and IL-1RA. However, no correlation was found in the ELF of healthy subjects or TB patients either. Arend et al. [40] stated that the production of these two proteins in human is regulated differently. The expression of IL-1RA in response to mycobacterial infection may be not related, or only partially related, to the presence of IL-1β. In conclusion, a significantly higher level of tumour necrosis factor-α was found both in the lung epithelial lining fluid and in the serum of patients with active pulmonary tuberculosis. Significant elevations of soluble tumour necrosis factor receptor I and soluble tumour necrosis factor receptor II were also found in the lung epithelial lining fluid of these patients. Higher lung epithelial lining fluid levels of interleukin-1β in tuberculosis patients were also found but the p-value was not significant because of the large data variation. Increased levels of interleukin-1 receptor antagonist were found both in the lung epithelial lining fluid and in the serum from tuberculosis patients. The lung epithelial lining fluid levels of tumour necrosis factor-α and interleukin-1β were significantly higher in patients with more advanced disease, with fever or with body weight loss. These findings suggest that tumour necrosis factor-α, soluble tumour necrosis factor receptor I, soluble tumour necrosis factor II, interleukin-1β and interleukin-1 receptor antagonist are released in local inflammatory sites and play an important role in tuberculosis infection. A further study focusing on regulation of tumour necrosis factor-α and its soluble receptor forms, interleukin-1β and interleukin-1 receptor antagonist in response to *Mycobacterium tuberculosis* infection may be important in investigating the pathogenesis and treatment of tuberculosis.

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