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Serum concentrations of cytokines in patients with active tuberculosis (TB) and after treatment

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SUMMARY

During TB cytokines play a role in host defence. To determine the cytokine pattern during various disease stages of TB, serum levels of IL-12, interferon-gamma (IFN-γ), IL-4, IL-6 and IL-10 were measured in 81 patients with active TB, 15 patients during therapy and 26 patients after anti-tuberculous therapy as well as in 16 persons who had been in close contact with smear-positive TB and in 17 healthy controls. IFN-γ was elevated during active TB when compared with healthy controls, declining during and after treatment. IL-12 (p40 and p70) serum levels were not significantly higher in patients with active TB compared with any of the other groups. IL-4 levels were low in all groups. IL-6 and IL-10 serum levels were elevated in patients with active TB and during treatment. In patients with active TB serum levels of IFN-γ and IL-6 were higher in patients with fever, anorexia and malaise. IL-12 levels were higher in patients with a positive smear. Cytokine levels did not correlate with localization of TB (pulmonary *versus* extrapulmonary), or skin test positivity. Cytokines directing a Th1 response (IL-12) or a Th2 response (IL-4) were not elevated in sera of this large group of patients with pulmonary and extrapulmonary TB. In patients with active TB, cytokines that were elevated in serum were IFN-γ, IL-6 and IL-10.

Keywords tuberculosis cytokines

INTRODUCTION

Despite advances in therapy, TB remains responsible for 2–3 million deaths annually. If world-wide control of TB does not improve, 90 million new cases and 30 million deaths are expected from 1990 to 1999 [1]. Of persons infected with *Mycobacterium tuberculosis*, 5–10% develop tuberculous disease; most people become healthy tuberculin reactors.

Resistance to mycobacterial infections is mediated by macrophages, T cells, and their interaction, and is dependent on the interplay of cytokines produced by each cell [2]. $CD4^+$ T cells are considered to consist of two subsets: Th1 cells, characterized by the production of interferon-gamma (IFN-γ) and IL-2, and Th2 cells, characterized by the production of IL-4, IL-5 and IL-6. However, when cytokine levels are measured in serum, it is not possible to determine the origin of these cytokines. Therefore, we will not refer to measured cytokines as Th1 or Th2 cytokines.

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IL-12 has been implicated as an important factor in the pathogenesis of T cell-mediated pathology because it drives antigen-naive Th cells towards development to Th1 cells [3]. Although IL-6 is considered a type 2 cytokine, it is associated with protection against *M. tuberculosis* [4]. In addition, mice deficient in either IFN-γ or IL-12 are highly susceptible to infection with *M. tuberculosis* [5,6]. Moreover, vaccination experiments in mice showed that either high doses of *M. vaccae* (shown to induce a Th2 response) or use of a TB subunit vaccine in an adjuvant that induced a Th2 response increased the susceptibility of the animals to a subsequent challenge with *M. tuberculosis* [7,8]. In a model of *in vitro* mycobacterial infection, peripheral blood monocytes of patients with TB were shown to have increased numbers of IL-4-secreting T cells compared with skin test-positive controls, but no significant difference in IFN-γ-producing cells [9]. In another study diminished expression of IL-2 and IFN-γ was found, while no change in IL-4, IL-10 and IL-13 could be detected [10].

Knowledge of systemic levels of different cytokines in patients with TB is limited. In the present study we sought to determine serum concentrations of IFN-γ, IL-12, IL-4, IL-6, and IL-10 in

patients with active pulmonary or extrapulmonary TB, and to compare those with levels in patients with TB during and after treatment, TB contacts and healthy subjects. In addition, the relation of cytokine levels and clinical symptoms in patients with TB was studied.

PATIENTS AND METHODS

Study population

Sera were obtained from 81 patients with active TB before treatment. Mean age was 35 years (range 15–86 years), and 68% were male. Of these patients, 45 had pulmonary TB and 36 had extrapulmonary TB. Extrapulmonary sites included pleural ($n=11$), lymph nodes ($n=9$), soft tissue ($n=2$), meninges ($n=3$), gastrointestinal ($n=2$), bone and joints ($n=6$) and disseminated disease ($n=3$). Sera were also obtained from 15 patients with TB who had been treated for at least 2 weeks, but had not yet completed therapy at the time of blood sampling (mean age 39 years, range 22–86 years) and from 26 patients who had completed anti-tuberculous therapy (mean age 48 years, range 20–79 years). Of these 122 patients, 64 attended the Academic Medical Centre, a tertiary referral hospital, and 58 the Municipal Health Service, a regional reference centre for TB. There was no significant difference in age or ethnic origin between patient groups. The ethnic origin comprised European (43%), Asian (24%), African (17%), and South-American (16%). Records of all patients with active TB were reviewed for clinical data as fever (rectal temperature > 38°C), anorexia, skin test, bacille Calmette–Guérin (BCG) vaccination, direct microscopy, and malaise.

Furthermore, sera were obtained from 16 persons who had been in close contact with patients with smear-positive TB (mean age 35 years, rang 14–71 years) and from 17 healthy Dutch male army recruits (all skin test-negative and mean age 20 years, range 18–23 years).

Assays

IFN-γ and IL-4 were measured with an ELISA (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, CLB, Amsterdam, The Netherlands) according to the instructions of the manufacturer. The detection limits of the assays were 5·0 pg/ml and 1·0 pg/ml, respectively.

Concentrations of IL-12 p40 were determined by sandwich ELISA using IL-12 p40-specific MoAb C11.79 (2 μg/ml) as coating antibody, biotinylated anti-human IL-12 p40 MoAb C8.6 (0·5 μg/ml) as detecting antibody and human recombinant IL-12 as standard. Human rIL-12 was kindly provided by Dr M. K. Gately (Hoffmann La Roche Inc., Nutley, NJ); the hybridomas producing the IL-12 p40-specific MoAbs C11.79 and C8.6 were kindly provided by Dr G. Trinchieri (The Wistar Institute, Philadelphia, PA). The detection limit of the assay was 30 pg/ml. Concentrations of IL-12 p70 were measured using a mouse anti-human IL-12 p70 MoAb (2 μg/ml) as capturing antibody, biotinylated goat anti-human IL-12 p70 (150 μg/ml) as detecting antibody and recombinant human IL-12 p70 as standard (all from R&D Systems, Abingdon, UK). The detection limit was 4·9 pg/ml.

IL-6 and IL-10 were measured by ELISA (Pharmingen, San Diego, CA) according to the instructions of the manufacturer. Detection limits were 8·2 and 24·7 pg/ml, respectively.

Statistical analysis

Pair-wise analysis using Wilcoxon rank sum test for unmatched samples was done to compare differences in cytokine levels between groups of patients. $P<0{\cdot}05$ was considered significant.

RESULTS

The mean IFN-γ serum level in patients with active TB was 22 pg/

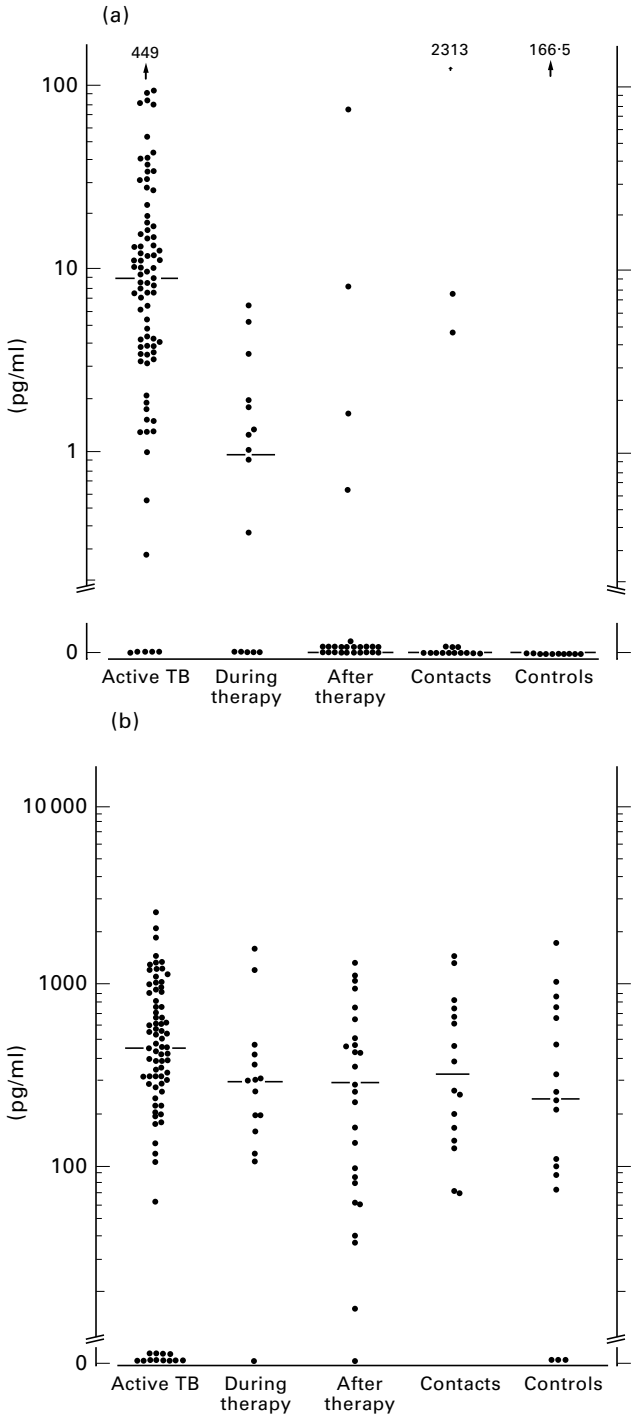

Fig. 1. Serum levels of IFN-γ (a) and IL-12 (b) p40 determined in patients with TB and control subjects. A logarithmic scale has been used. Each point represents one person. The median value for each group has been indicated by a horizontal line.

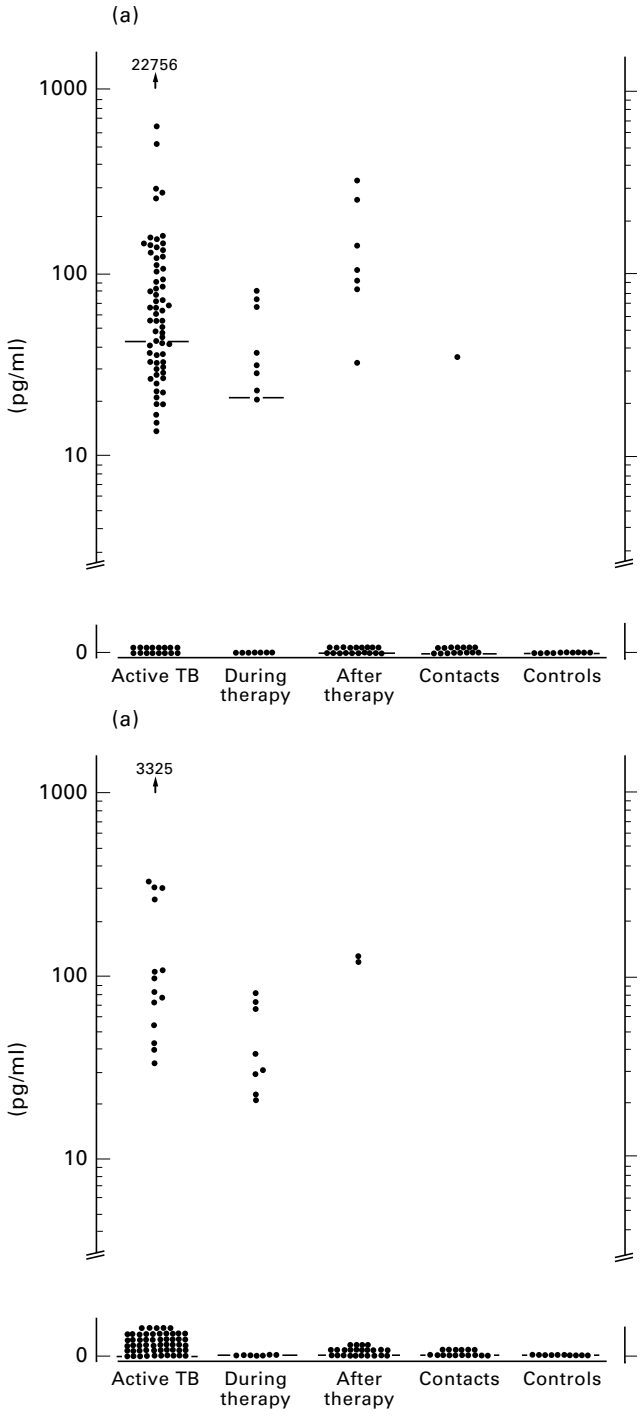

Fig. 2. Serum levels of IL-6 (a) and IL-10 (b) determined in patients with TB and control subjects. A logarithmic scale has been used. Each point represents one person. The median value for each group has been indicated by a horizontal line.

ml (s.e.m. 5·8 pg/ml), which was significantly higher than in patients during therapy ($P < 0{\cdot}001$), in patients after treatment ($P < 0{\cdot}001$), in contacts ($P < 0{\cdot}001$) and in healthy control persons ($P < 0{\cdot}001$) (see Fig. 1). There was no significant difference between patients with TB after treatment and contacts compared with control subjects.

IL-12 p40 serum levels were not significantly higher in patients with active TB compared with any of the other groups (Fig. 1). IL-12 p70 serum levels were not detectable in patient or control groups (data not shown).

The association between clinical data and serum IFN-γ and IL-12 p40 levels was determined in patients with active TB. No significant association was found between serum levels of these cytokines and localization of TB (pulmonary or extrapulmonary), skin test positivity, or prior BCG vaccination (data not shown).

The mean IFN-γ serum level was significantly elevated in patients with active TB and fever (46 pg/ml) compared with such patients without fever (29 pg/ml; $P = 0{\cdot}001$), and tended to be higher in patients with anorexia (43 pg/ml) and malaise (43 pg/ml) than in patients without these symptoms (33 and 33 pg/ml). Mean IL-12 serum levels were significantly lower in patients with a positive smear in direct microscopy (39 pg/ml) compared with patients without a positive smear (52 pg/ml; $P = 0{\cdot}041$).

IL-4 serum levels were low (< 20 pg/ml) in all groups, and no difference in IL-4 concentrations between groups was found (data not shown).

The mean IL-6 serum level in patients with active TB was 355 pg/ml (s.e.m. 280 pg/ml), which was significantly higher than in patients during therapy ($P = 0{\cdot}01$), in patients after treatment ($P = 0{\cdot}001$), in contacts ($P < 0{\cdot}001$) and in control persons ($P < 0{\cdot}001$) (see Fig. 2). IL-6 levels were significantly elevated in patients with TB during and after treatment compared with controls ($P < 0{\cdot}05$) (Fig. 2).

The mean IL-10 serum level in patients with active TB was 68 pg/ml (s.e.m. 42 pg/ml), but significantly higher than in patients during therapy ($P < 0{\cdot}001$), in patients after treatment ($P < 0{\cdot}001$), in contacts ($P < 0{\cdot}001$) and in control persons ($P < 0{\cdot}001$) (see Fig. 2). IL-10 levels were significantly elevated in patients with TB during treatment compared with controls ($P = 0{\cdot}005$) (Fig. 2).

In patients with active TB, no significant association was found between serum levels of IL-4, IL-6 and IL-10 and localization of TB (pulmonary or extrapulmonary), skin test positivity, or prior BCG vaccination (data not shown).

IL-6 was significantly higher in patients with anorexia (mean 45 pg/ml) compared with patients without anorexia (29 pg/ml; $P = 0{\cdot}002$) and was elevated in patients with malaise (42 pg/ml) and fever (41 pg/ml) compared with patients without these symptoms (32 and 34 pg/ml, respectively), although this was not statistically significant.

DISCUSSION

Previous studies that sought to determine the cytokine balance at the systemic level in patients with active TB used the '*ex vivo*' cytokine production capacity of isolated peripheral blood mononuclear cells (PBMC) or $CD4^+$ T cells after stimulation. These investigations have yielded conflicting results, reporting increased, unchanged or decreased cytokine production [9–11]. Although the explanation for these apparent discrepancies is unclear at present, it is conceivable that differences in isolation and culture techniques, and differences in the stimulus used are involved. In addition, it should be noted that the '*ex vivo*' stimulated production of cytokines does not necessarily provide insight into the actual status of the cytokine network *in vivo*. Indeed, in patients with severe bacterial infections, much of the observed organ injury is considered to be related to enhanced

in vivo production of proinflammatory cytokines, while PBMC isolated from those patients produce significantly less cytokines upon stimulation than cells from healthy controls [12]. Therefore, in the present study we chose to measure cytokine levels in serum.

IL-12 is a cytokine produced by phagocytic and antigen-presenting cells, it is an important activator of IFN-γ production by T cells and natural killer cells and provides an early switch in the differentiation of $CD4^+$ T cells. Hence, IL-12 seems an important factor determining the outcome of TB [6,13]. In the present study in patients with active TB, IL-12 p40 concentrations were not different from those in contacts and healthy controls. Of considerable interest, however, was the fact that patients with positive smears had significantly lower p40 serum levels than any of the other study groups. Considering the essential role of IL-12 in a protective immune response to TB in mice [6], and the fact that positive smears are associated with a higher mycobacterial burden [14], it is tempting to speculate that also in man IL-12 production plays a role in anti-mycobacterial host defence.

IL-4 levels were low in all groups tested. Although levels < 20 pg/ml may still be biologically active, there was no significant difference between patients with TB and control persons. This is in contrast to the studies in which PBMC of 11 of 18 patients with TB had an elevated mRNA expression of IL-4 [15] or in which an increase of IL-4-secreting T cells was found compared with skin test-positive controls [9]. However, others found that PBMC from patients with TB did not produce significant quantities of IL-4, whereas either control subjects had a diminished production [16] or no change in IL-4 expression could be detected [10]. This suggests that an altered balance between cytokines is implicated in tuberculous disease, rather than elevation or decrease of just one cytokine.

IL-6 is usually thought of as a type 2 cytokine, but it may also be produced by type 1 cells [17]. Although it has been shown that IL-6 promotes the intracellular growth of mycobacteria in monocytes [18,19], it was found that IL-6 seems critical in resistance to TB [4]. In this study we showed that IL-6 serum levels were elevated in the vast majority of patients with active TB. In addition, the positive correlation between serum IL-6 as well as IFN-γ and clinical symptoms suggests that IL-6 may be implicated in host defence against TB.

Cytokines play an important role in the pathogenesis of TB. We demonstrate here that cytokines directing a Th1 response (IL-12) or a Th2 response (IL-4) are not elevated in sera of a large group of patients with active pulmonary or extrapulmonary TB. Cytokines that were elevated in serum are IFN-γ, IL-6, and IL-10.

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