Original Article

# Cytokines and acute phase serum proteins as markers of inflammatory regression during the treatment of pulmonary tuberculosis*

## Citocinas e proteínas de fase aguda do soro como marcadores de regressão da resposta inflamatória ao tratamento da tuberculose pulmonar

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## Abstract

**Objective:** To evaluate the pattern of pro-inflammatory cytokines, anti-inflammatory cytokines and the acute phase response (APR) as markers of the response to treatment of pulmonary tuberculosis. **Methods:** Twenty-eight patients with pulmonary tuberculosis were evaluated at three time points: pretreatment (T0), treatment month 3 (T3) and treatment month 6 (T6). Levels of interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), interleukine-10 (IL-10) and transforming growth factor-beta (TGF-β) were determined using ELISA in the supernatant of peripheral blood mononuclear cell and monocyte culture. Levels of total protein, albumin, globulins, C-reactive protein (CRP), alpha-1-acid glycoprotein (AAG) and erythrocyte sedimentation rate (ESR) were also determined. All of these parameters were also evaluated, only once, in a group of healthy controls. **Results:** In relation to controls, patients presented cytokine levels and APR that were higher at T0, lower at T3 and either lower (TNF-α, IL-10, TGF-β, AAG and ESR) or normal (IFN-γ and CRP) at T6. **Conclusions:** For individuals with negative smear sputum microscopy, CRP, AAG and ESR are potential markers of pulmonary tuberculosis and of the need for treatment; CRP (T0 > T3 > T6 = reference) can also be a marker of treatment response. In the patients, the Th0 profile (IFN-γ, IL-10, TNF-α and TGF-β), inducer of and protector against inflammation, predominated at T0, whereas the Th2 profile (IL-10, TNF-α and TGF-β), protecting against the harmful pro-inflammatory effect of the remaining TNF-α, predominated at T6. The behavior of IFN-γ (T0 > T3 > T6 = controls) suggests its use as a marker of treatment response.


**Keywords:** Acute-phase proteins; Cytokines; Mycobacterium tuberculosis; Tuberculosis/therapy.


## Resumo

**Objetivo:** Analisar o padrão de citocinas pró- e antiinflamatórias e da resposta de fase aguda (RFA) como marcadores de resposta ao tratamento da tuberculose pulmonar. **Métodos:** Determinação dos níveis de interferon-gama (IFN-γ), *tumor necrosis factor-alpha* (TNF-α, fator de necrose tumoral-alfa), interleucina-10 (IL-10) e *transforming growth factor-beta* (TGF-β, fator transformador de crescimento-beta), pelo método ELISA, em sobrenadante de cultura de células mononucleares do sangue periférico e monócitos, assim como dos níveis de proteínas totais, albumina, globulinas, alfa-1-glicoproteína ácida (AGA), proteína C reativa (PCR) e velocidade de hemossedimentação (VHS) em 28 doentes com tuberculose pulmonar, em três tempos: antes (T0), aos três meses (T3) e aos seis meses (T6) de tratamento, em relação aos controles saudáveis, em um único tempo. **Resultados:** Os pacientes apresentaram valores maiores de citocinas e RFA que os controles em T0, com diminuição em T3 e diminuição (TNF-α, IL-10, TGF-β, AGA e VHS) ou normalização (IFN-γ e PCR) em T6. **Conclusões:** PCR, AGA e VHS são possíveis marcadores para auxiliar no diagnóstico de tuberculose pulmonar e na indicação de tratamento de indivíduos com baciloscopia negativa; PCR (T0 > T3 > T6 = referência) pode também ser marcador de resposta ao tratamento. Antes do tratamento, o perfil Th0 (IFN-γ, IL-10, TNF-α e TGF-β), indutor de e protetor contra inflamação, prevaleceu nos pacientes; em T6, prevaleceu o perfil Th2 (IL-10, TNF-α e TGF-β), protetor contra efeito nocivo pró-inflamatório do TNF-α ainda presente. O comportamento do IFN-γ (T0 > T3 > T6 = controle) sugere sua utilização como marcador de resposta ao tratamento.


**Palavras-chave:** Proteínas da fase aguda; Citocinas; Mycobacterium tuberculosis; Tuberculose/terapia.

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## Introduction

In cases of infection with *Mycobacterium tuberculosis*, the interaction of T cells with infected macrophages is a critical factor of protective immunity against the bacillus, and the cytokines produced by these cells are important mediators of the immune and inflammatory responses.[1]

After being phagocytosed, the bacilli induce macrophages, dendritic cells and T cells to secrete the cytokine tumor necrosis factor-alpha (TNF-α), which is important for controlling the active infection due to its role in local inflammation and macrophage activation, as well as being an important factor in the immunopathology of the disease.[1,2] Interferon gamma (IFN-γ) activates the macrophages, which start to produce reactive nitrogen and oxygen intermediates. The reactive nitrogen and oxygen intermediates inhibit mycobacterial growth and promote the death of mycobacteria.[1] However, the bacillus survives, multiplies in the macrophage and induces the recruitment of T lymphocytes, which are great producers of IFN-γ and TNF-α, to form a granuloma at the site of the infection.[1]

When tuberculosis is in activity, we can observe a decrease in the Th1 response, together with an increase in the production and activity of Th2 cytokines such as interleukin (IL)-10, which inhibits cell proliferation and IFN-γ production, affecting the microbicidal mechanisms of macrophages, as well as antigen presentation. Such cytokines also have an effect contrary to that of TNF-α, protecting against tissue damages by regulating inflammation and apoptosis. Macrophage production of these cytokines is stimulated by the mycobacterial cell wall components.[3]

Another macrophage-produced cytokine, transforming growth factor-beta (TGF-β), suppresses the Th1 profile and participates in the induction of fibrosis.[4] At low concentrations, TGF-β is chemotactic for monocytes and induces secretion of IL-1α as well as of TNF-α.[5] In the chronic phase of tuberculosis, the production of TGF-β peaks, promoting deactivation of macrophages, as well as inhibiting the expression and function of IFN-γ receptors.[6,7]

Pro-inflammatory cytokines promote the disappearance of mycobacteria, being useful as markers of inflammatory process activity and of treatment response. Such cytokines are also inducers of the acute phase response (APR)—the systemic correspondent of inflammation—which results in an increase in the hepatic synthesis and serum levels of acute phase proteins. In addition, pro-inflammatory cytokines can be serially quantified, which makes them useful in the diagnosis and follow-up of patients.[8]

Other markers include C-reactive protein (CRP), which is considered the most sensitive and specific APR marker, since its plasma concentration directly reflects the intensity of the pathological process, and the erythrocyte sedimentation rate (ESR), a nonspecific marker that changes during the infectious process, indicating the inflammation intensity and the treatment response, making it useful for monitoring the progress of inflammatory diseases such as tuberculosis.[9,10]

Induction of the APR is a result of the activity of inflammatory cytokines. A change in the levels of its markers in the peripheral blood, together with clinical, epidemiological and imaging data, indicates active disease, even with negative sputum smear microscopy results. This makes the therapeutic test more reliable and allows the effect of antituberculous treatment to be determined. In Brazil, 26.7% of all patients with suspected pulmonary tuberculosis (PTB) are treated without diagnostic confirmation, the treatment being based solely on clinical, radiological and APR marker findings characteristic of granulomatous processes.[11]

The aim of the present study was to evaluate the effect that treatment has on the inflammatory process in patients with PTB. To that end, we determined total proteins, albumin, globulins, alpha-1-acid glycoprotein (AAG), CRP, ESR and serum parameters, as well as levels of the cytokines TNF-α, IFN-γ, IL-10 and TGF-β, in the supernatant of peripheral blood mononuclear cells (PBMCs) and monocytes.

## Methods

This study was approved by the Ethics in Research Committee of the Botucatu School of Medicine, *Universidade Estadual Paulista* – UNESP, São Paulo State University (protocol 1825/2005).

### *Studied groups*

As controls, 20 blood donors from the Blood Bank of the UNESP *Hospital das Clínicas da Faculdade de Medicina de Botucatu* (HC-FMB, Botucatu School of Medicine *Hospital das Clínicas*) were studied. The mean age of the controls was 38.5 years (range, 24-56 years), and all were male. They were evaluated only once, to establish the standard of normality for the cytokines studied.

Twenty-eight PTB patients were evaluated, of which 13 were from HC-FMB and 15 were from the Infectious Diseases Division of the State Health Department in Bauru, São Paulo. Of those 28 patients, 23 were male and 5 were female. Among the men, the mean age was 54.3 years (range, 21-77 years), compared with 43.4 years (range, 29-74 years) among the women. The patients were enrolled in the study at the time of diagnosis, after evaluation of clinical parameters, determination of APR markers and imaging studies. All of the patients were classified as having moderate PTB. The inclusion criteria were being at least 18 years of age and having been diagnosed with PTB based on sputum smear microscopy or culture positivity for *M. tuberculosis* (or having been given a presumptive diagnosis based on clinical and epidemiological profiles, as well as biochemical, hematological and imaging test results, compatible with active tuberculosis, despite a negative sputum smear microscopy result). All patients who also had another active granulomatous disease or HIV/AIDS were excluded from the study. All studied variables were determined at three time points: pretreatment (T0), treatment month 3 (T3) and treatment month 6 (T6).

All patients with PTB were treated for six months and were considered clinically cured at the end of the treatment.

## Cell culture

Peripheral blood samples (20 mL each) were collected from the controls at a single time point and from the patients at the three study time points. The PBMCs were obtained through Histopaque® gradient separation.[12] The lymphocyte- and monocyte-rich ring was rinsed in RPMI 1640 culture medium (Gibco Laboratories, Grand Island, NY, USA) for 5 min at 200 rpm. Subsequently, the cell suspension was re-suspended in supplemental RPMI 1640 culture medium with 2 mM of L-glutamine (Sigma-Aldrich, St. Louis, MO, USA), 40 µg/mL of gentamicin and 10% of inactive human autologous serum (complete cell culture medium). Cell identity and viability were determined by counting after staining with Turk liquid for PBMCs and neutral red for monocytes. The cell suspension was then distributed in 24-well culture plates (Nunc, Life Tech Inc, Maryland, MA, USA) at $2 \times 10^6$/mL. The PBMC culture was incubated with or without a stimulus. To isolate the monocytes, after 1 h of incubation at 37°C in an atmosphere of 5% $CO_2$, the nonadherent cells were eliminated by rinsing the plates with RPMI 1640 culture medium. After adherence, the cells were re-incubated in the complete cell culture medium, with or without stimuli.

## Cytokine quantification

The levels of TNF-α, IFN-γ, TGF-β and IL-10 were determined with commercial kits (R & D Systems,

**Table 1** – Comparison of biochemical variables (total proteins, albumin, globulin, C-reactive protein and α1-acid glycoprotein) and hematological variables (erythrocyte sedimentation rate) at three time points during the specific treatment of patients with pulmonary tuberculosis.

| | Acute phase markers | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | TP[a] (g/dL) | Alb[b] (g/dL) | Glob[a] (g/dL) | CRP[b] (g/dL) | AAG[a] (mg/dL) | | ESR[a] (mm/h) | |
| | | | | | M | F | M | F |
| T0 | (n = 21) (ref.: 6.3-8.2) | (n = 21) (ref.: 3.5-5.0) | (n = 21) (ref.: 2.8-3.2) | (n = 20) (ref. ≤ 1.00) | (n = 16) (ref.: 30-50) | (ref.: 40-120) | (n = 11) (ref. ≤ 10) | (ref. ≤ 20) |
| T0 | 7.92 ± 0.59 | 3.90 (3.40; 4.60) | 3.92 ± 0.72 | 3.65 (1.70; 6.60) | 115.40 ± 46.08 | 127.80 ± 92.78 (n = 5) | 37.64 ± 28.61 | 65.00 ± 31.23 (n = 5) |
| T3 | 7.49 ± 0.68 | 4.20 (3.75; 4.40) | 3.45 ± 0.49 | 0.95 (0.30; 1.60) | 67.44 ± 26.69 | 66.20 ± 53.60 (n = 5) | 18.32 ± 13.38 | 20.00 ± 12.98 (n = 5) |
| T6 | 7.67 ± 0.69 | 4.30 (3.85; 4.55) | 3.49 ± 0.43 | 0.15 (0.00; 0.40) | 62.18 ± 21.35 | 43.98 ± 7.57 (n = 4) | 10.59 ± 7.41 | 19.33 ± 13.65 (n = 4) |
| ρ | 0.059 | 0.331 | 0.001* | <0.0001** | <0.0001* | 0.278 | 0.0016* | 0.025 |

TP: total proteins; Alb: albumin; Glob: globulins; CRP: C-reactive protein; AAG: α1-acid glycoprotein; ESR: erythrocyte sedimentation rate; n: number of evaluated patients; ref.: reference value; T (0, 3 and 6): study's time points, in 0, 3 and 6 months of treatment, respectively; and p: significance level of the applied test. [a]Summary in mean and standard deviation; e [b]Summary in median and quartile. *T0 > T3; T0 > T6. **T0 > T3 > T6.

Billings, MT, USA) using the ELISA technique, with a detection limit of 5 pg/mL for each cytokine, in the supernatant obtained from the PBMC and monocyte cultures, in the absence or presence of 8 µg/mL of phytohemagglutinin or 20 µg/mL of lipopolysaccharide, respectively, after 24 h of incubation at 37 °C, in an atmosphere of 5% $CO_2$. Aliquots of these materials were stored at −80°C for later determination of cytokine levels.

### *Statistical analysis*

The APR results were analyzed using ANOVA, Friedman test, Bonferroni test, Shapiro-Wilk test, Student's t-test and Dunn's post-test. The cytokine results were analyzed using ANOVA, Bonferroni test and Student's t-test. The linear relationship between APR and cytokines at each time point was studied using Spearman and Pearson correlations. All tests were applied with significance level of $\alpha = 0.05$.[13]

## Results

### *Inflammatory markers of the acute phase*

In the patients, inflammatory markers of the acute phase were evaluated at three time points. At T0, serum levels of the following markers, all expressed as mean and standard deviation, were above the reference (ref.) values in 21 of the 28 patients: globulins (g/dL): 3.92 ± 0.72 (ref., 2.8-3.2; 95% CI: 3.59-4.25); AAG (mg/dL) in women: 127.8 ± 92.78 (ref., 40-120; 95% CI: 12.87-242.73); ESR (mm/h) in women: 65 ± 31.23 (ref., ≤ 20; 95% CI: 26.31-103.69) and in men: 37.6 ± 28.61 (ref., ≤ 10; 95% CI: 18.49-56.79). Among the patients, total proteins, albumin and AAG (in the men) did not differ from the standard of normality (data not shown). In 22 (81.48%) of the 27 patients evaluated, CRP was above the ref. value (≤ 1.00 g/dL) at T0. Although the levels of all markers feel over the course of the treatment, significant differences between the time points were observed only for CRP levels. As shown in Table 1, this decrease (expressed as median and quartiles) was significant ($p < 0.0001$) in 20 of the 28 patients: T0: 3.65 (1.70; 6.60) > T3: 0.95 (0.30; 1.60) > T6: 0.15 (0.00; 0.40).

Cytokine production in the supernatant of PBMC and monocyte cultures was determined at only one time point in the controls and at three time points in the patients. Of the 28 patients, 21 were evaluated at T0, T3 and T6, whereas the remaining 7 patients were evaluated only at T0 and T3. In patients, the TNF-α levels (pg/mL, mean and standard deviation), with and without stimulus, respectively, were as follows: T0 (485.30 ± 16.80; 601.90 ± 193.10) > T3 (343.20 ± 38.00; 472.40 ± 190.10) > T6 (219.30 ± 113.00; 321.40 ± 158.90) ($p < 0.01$). These values were all signifi-

**Table 2 -** Production of tumor necrosis factor-alpha, interferon-gamma, interleukin-10 and transforming growth factor-beta by peripheral blood mononuclear cells and monocytes of control individuals, at three time points during the specific treatment of patients with pulmonary tuberculosis.

| | Cytokines | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | TNF-α | | IFN-γ | | IL-10 | | TGF-β | |
| Stimulus | LPS | | PHA | | LPS | | LPS | |
| | − | + | − | + | − | + | − | + |
| Control (n = 20) | 125.45 ± 33.04 | 204.20 ± 64.60 | 247.35 ± 56.31 | 316.30 ± 222.80 | 9.40 ± 7.08 | 16.80 ± 9.86 | 176.30 ± 54.73 | 458.45 ± 117.03 |
| T0 (n = 21) | 485.30 ± 16.80 | 601.90 ± 193.10 | 532.20 ± 202.20 | 646.70 ± 222.80 | 60.33 ± 23.11 | 81.05 ± 25.58 | 636.60 ± 191.40 | 837.50 ± 180.90 |
| T3 (n = 21) | 343.30 ± 138.10 | 472.40 ± 190.10 | 440.00 ± 216.30 | 523.80 ± 229.70 | 42.57 ± 21.21 | 56.81 ± 24.58 | 492.50 ± 185.10 | 676.80 ± 175.10 |
| T6 (n = 21) | 219.30 ± 113.10 | 321.40 ± 158.90 | 295.60 ± 147.20 | 392.60 ± 191.10 | 27.52 ± 18.48 | 39.86 ± 23.82 | 318.30 ± 135.70 | 476.10 ± 200[a] |
| ρ | < 0.05* | < 0.05* | < 0.05** | < 0.05** | < 0.05* | < 0.05* | < 0.05* | < 0.05** |

TNF-α: tumor necrosis factor-alpha; IFN-γ: interferon-gamma, IL-10: interleukin-10; TGF-β: transforming growth factor-beta; LPS: lipopolysaccharide; PHA: phytohemagglutinin; −: cell culture without stimulus; +: cell culture with stimulus; Control: control group reference values; and T (0, 3 and 6): study time points, at 0, 3 and 6 months of treatment, respectively. [a]n = 20. *Control < T6 < T3 < T0 ($p < 0.02$). **Control = T6 < T3 < T0 ($p < 0.01$).

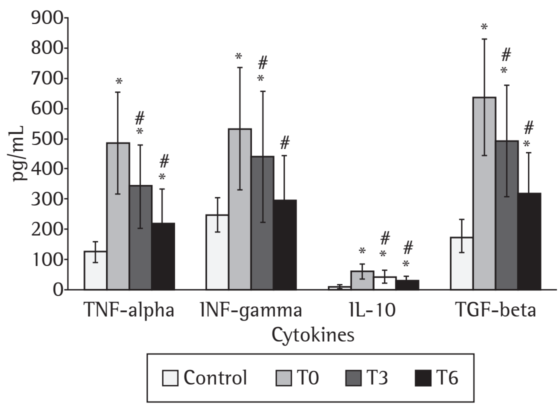


**Figure 1 -** Mean and standard deviation of tumor necrosis factor-alpha (TNF-alpha), interferon-gamma (IFN-gamma), interleukin-10 (IL-10) and transforming growth factor-beta (TGF-beta) in supernatant of peripheral blood mononuclear cells and peripheral blood monocyte cultures, without stimulus, of controls (n = 20), at one time point, and of patients with pulmonary tuberculosis (n = 21) before treatment (T0), after three months of treatment (T3) and at the end of treatment (T6). Significant differences (r < 0.05) between controls and patients (Student's t-test) indicated by * and between time points (T0, T3 and T6; ANOVA and Bonferroni test) indicated by * and between time points (T0, T3 and T6; ANOVA and Bonferroni test) indicated by #.

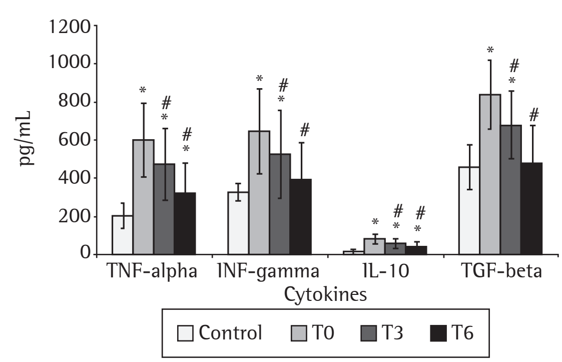


**Figure 2 -** Mean and standard deviation of tumor necrosis factor-alpha (TNF-alpha), interferon-gamma (IFN-γ), interleukin-10 (IL-10) and transforming growth factor-beta (TGF-beta) in supernatant of peripheral blood mononuclear cells and peripheral blood monocyte cultures, with stimulus (phytohemagglutinin: IFN-gamma; lipopolysaccharide: TNF-alpha, IL-10 and TGF-beta), of controls (n = 20), at one time point, and of patients with pulmonary tuberculosis (n = 21 for TNF-alpha, IFN-gamma and IL-10; and n = 20 for TGF-beta) before treatment (T0), after three months of treatment (T3) and at the end of treatment (T6). Significant differences (r < 0.05) between controls and patients (Student's t-test) indicated by * and between time points (T0, T3 and T6; ANOVA and Bonferroni test) indicated by #.

cantly higher ($p < 0.02$) than were those obtained in the controls (125.45 ± 33.04; 204.20 ± 64.60). The levels of IFN-γ (pg/mL), with and without stimulus, respectively, were as follows: T0 (532.20 ± 202.20; 646.70 ± 222.80) > T3 (440.00 ± 216.30; 523.80 ± 29.70) > T6 (295.60 ± 147.20; 392.60 ± 191.10) ($p < 0.01$). At T0 and T3, these levels were higher than were those obtained in the controls (247.35 ± 56.31; 316.30 ± 46.03) ($p < 0.001$), although the T6 values were equal those obtained in the controls. The levels of IL-10 production (pg/mL), with and without stimulus, respectively, were as follows: T0 (60.3 ± 23.11; 81.05 ± 24.58) > T3 (42.57 ± 21.21; 58.81 ± 24.58) > T6 (27.52 ± 18.48; 39.86 ± 23.82). These levels were all significantly higher ($p < 0.01$) than were those obtained in the controls (9.40 ± 7.08; 16.80 ± 9.86) ($p < 0.001$). The levels of TGF-β (pg/mL), with and without stimulus, respectively, were as follows: T0 (636.60 ± 191.40) > T3 (492.50 ± 185.10; 676.80 ± 1 74.10) > T6 (318.30 ± 135.70; 476.10 ± 200) ($p < 0.01$). At all time points, except T6, with stimulus, the levels of TGF-β in the patients were significantly higher than were those found for the controls (176.30 ± 54.73; 458.45 ± 117.03) ($p < 0.001$). (Table 2; Figures 1 and 2).

## Discussion

Several studies have demonstrated the existence of a strong APR in patients with PTB, through the increase in plasma concentrations of CRP and in ESR.[14,15] In the present study, initial levels of CRP, AAG and ESR were also found to be above the ref. values, indicating their possible use as auxiliary markers in the presumptive diagnosis of the disease, in cases presenting negative sputum smear microscopy results, together with clinical and epidemiological profiles suggestive of active disease, making the therapeutic test more reliable.

In addition to disease activity markers, APR markers have also been studied as evaluators of the specific treatment effect in PTB. There are reports suggesting that ESR normalization is a marker of good response to treatment in subacute, chronic diseases such as tuberculosis.[16,17] Various authors have demonstrated increased levels of the markers APR, CRP, AAG and ESR in the initial phase, all of which decrease during the treatment.[14,15] Our results show that patients with PTB also present increased pretreatment CRP levels, when compared with the levels found after three and six months of therapy, which suggests the usefulness of this marker in clinical practice to evaluate the response to the treatment. Some authors have suggested the use of AAG, haptoglobin, alpha-1 antitrypsin and sialic acid as sensitive biochemical indicators of prognosis and monitoring of tuberculosis treatment response.[15]

Various authors have demonstrated the importance of cytokines as markers of tuberculosis activity or of response to the specific treatment. When treatment is effective, there is Th1 response recovery, with subsequent bacillus containment.[18,19]

Although IFN-γ is recognized as playing a major role in the formation of granuloma induced by *M. tuberculosis*, the results of studies evaluating the behavior of this cytokine in patients are not homogeneous. These discrepancies result from differences in the methodology used in the studies or the phases of disease evolution in which the cytokine was quantified. Some studies have also demonstrated the relationship between the production of certain cytokines and the severity of the disease.[20-23]

In one study,[14] the production of IFN-γ by PBMCs, stimulated by *M. tuberculosis*, was found to be lower than normal before the treatment, progressively increasing to levels similar to those found at the end in the controls. Different from the results above, in the present study, the production of IFN-γ by cells, stimulated or not, was higher among patients than among controls, before treatment and after three months of treatment, with a progressive decrease until reaching normal values at the end of therapy. However, in the study mentioned above,[14] the patients had moderate to advanced disease, and IFN-γ was quantified in PBMC supernatant after six days of incubation, whereas the patients in the present study had moderate tuberculosis, and the supernatant was obtained after 24 h.

Other authors did not find significant differences in the IFN-γ values obtained for patients, although they were lower in comparison with those obtained for controls.[19] Nevertheless, other studies have demonstrated high pretreatment levels of IFN-γ in the serum of patients with active tuberculosis.[24,25] Another study[24] demonstrated that serum IFN-γ levels were significantly higher in patients before treatment. The highest levels were presented by individuals with fever, although the levels normalized after the treatment. A slight decrease in IFN-γ production during treatment was also demonstrated in another study.[25] Although variable, IFN-γ levels in the patients with PTB were lower after two months of treatment than at the onset of the treatment.[25] Finally, a number of authors have observed that PBMCs submitted to various antigenic stimuli present high production of IFN-γ before and after treatment in patients with tuberculosis.[20-22]

Another cytokine that participates in the formation of granulomas is TNF-α, levels of which are high in the pleural fluid, plasma or monocyte culture of patients with tuberculosis, before or after the onset of the treatment, when compared with chronic patients or individuals without tuberc. There is evidence to suggest that TNF-α is necessary at the beginning of the inflammatory process in order to limit the multiplication of mycobacteria.[18,19,26] Other studies demonstrated that high TNF-α initial levels in PTB decreased significantly during the treatment, while the inflammatory process decreased at the same time.[14,26] In the present study, TNF-α production by monocytes, stimulated or not, was also lower in the controls than in the patients before treatment, after three months of treatment and at the end of treatment. Although cytokine production in the patients was always higher than in the controls, it decreased significantly during the treatment, and the final levels were lower than the baseline levels. These higher than normal levels after treatment, also found in another study,[20] suggest that TNF-α, in addition to its important role in the immunopathogenesis of the disease, plays a protective role.

Levels of the anti-inflammatory cytokine IL-10 are higher in cases of tuberculosis.[27] This effect is useful, because it reduces the pro-inflammatory activity of IFN-γ and TNF-α, as well as providing some protection against Th1 profile-induced tissue destruction. High levels of this cytokine are also found in healthy contacts of patients with tuberculosis, as well as in tuberculin reactors without the disease.[18,27,28] Although IL-10 levels are higher during the phase of great activity of the inflammatory process—pretreatment—than during or after the end of the specific therapy, these levels always remain above normal during treatment.[24]

In another study,[20] high initial production of IL-10 by PBMCs of patients with PTB remained unchanged with the treatment. In the present study, the production of IL-10 by monocytes, stimulated or not, was also high before, during and after the treatment when compared with normal values, although the production of this cytokine decreased during the treatment. These results are in agreement with those of other studies.[24,26] In one study mentioned previously,[14] the cytokine levels were found to be equivalent to those of the control group at the end of therapy. Again, the divergences in the results are likely related to the differences in the methodology used by the authors, the clinical status of the patients studied, the period of evolution of the disease or treatment and other factors.[24,28]

The monocytes of patients with active tuberculosis produce more TGF-β than do those of controls. The same behavior is observed in contacts of patients with tuberculosis and in treated and cured PTB patients.[23,29] High levels of TGF-β are also found in the pleural fluid.[18] The data obtained in the present study are in agreement with those presented in the literature, since levels of TGF-β were found to be significantly higher in the patients, with or without stimulus, before treatment, after three months of treatment and at the end of treatment. Although the final levels were higher than those observed for the controls, there was a significant decrease in the production of TGF-β during treatment.

Other authors found no differences in between patients and controls in terms of serum levels of TGF-β.[26] Since such studies did not demonstrate that treatment has a relevant effect on TGF-β levels, they suggested that the action of TGF-β depends on its concentration. The differences found in the TGF-β behavior in tuberculosis, in studies of in vitro cell stimulation, can be attributed to the isolation and culture techniques employed, which are not always the same.[26]

Despite the fact that pro-inflammatory cytokines are known to induce the production of APR markers, in the present study, the decrease in some of these markers during the therapy did not correlate with that of the cytokines evaluated.[30] This may be explained by the sample size and the heterogeneity of the evaluated population. Further studies involving larger patient samples are needed in order to improve understanding of the mechanism of the relationship between the APR and cytokines.

In the present study, the patients with PTB presented, prior to treatment, a Th0 profile, in which a Th1 profile cytokine (IFN-γ) coexisted with a Th2 profile cytokine (IL-10). In this phase, the production of TGF-β, a fibrosis inducer and regulatory cytokine, as well as of TNF-α, a pro-inflammatory cytokine essential to the formation and maintenance of granuloma, was also high. The balance between the pro-inflammatory and anti-inflammatory activities persisted during the treatment until T6, when the patients evolved to Th2 profile, with normalization of IFN-γ levels, likely to protect from the effects of the Th1 profile pro-inflammatory activity and ensure appropriate cicatrization, with development of fibrosis.

The higher levels of globulin, AAG, CRP and ESR in patients at T0, in agreement with the findings of other studies, suggest their use in aiding the presumptive diagnosis of tuberculosis, together with the patient clinical and epidemiological history, even in individuals with negative sputum smear microscopy results. We found CRP to be a useful marker of the effect of treatment and of the involution of inflammation, since its levels decreased during the tuberculosis treatment and normalized by end of the therapy. Levels of IFN-γ, which were higher at the beginning, decreased during treatment and normalized at the end of the treatment, showed the same utility.

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