Circulating profile of Th1 and Th2 cytokines in tuberculosis patients with different degrees of pulmonary involvement

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Abstract

To investigate whether differences in the degree of pulmonary tuberculosis lesions could be accompanied by changes in the pattern of circulating cytokines, 29 untreated tuberculosis patients showing mild (n = 10), moderate (n = 5) or advanced (n = 14) pulmonary disease, and 12 age-matched healthy controls (mean ± S.D., 36 ± 15 years) were studied. ELISA methods for the evaluation of interferon-γ, interleukin-2, interleukin-4, and interleukin-10 indicated that all patients had increased serum levels of the four cytokines in relation to controls. Mean titers of interferon-γ and interleukin-2 in mild and moderate patients appeared higher than in those with advanced disease, whereas moderate and advanced patients showed the higher levels of IL-4 in comparison to mild cases. Raised levels of interleukin-10 were more prevalent in advanced disease, and statistically different from those in mild patients. This cytokine pattern may help to explain findings wherein mild tuberculosis is characterized by preserved cellular immune responses while advanced disease is accompanied by an impairment of such parameters.

Keywords: Tuberculosis; Interferon-gamma; Interleukin; Severity

1. Introduction

Human tuberculosis (TB) is a clear example in which the immune response to Mycobacterium tuberculosis, its etiologic agent, is involved not only in the protection against disease but also in tissue damage occurring during disease. The majority of individuals exposed to the bacillus becomes infected but progression to disease is prevented because of the effective immune response, although living mycobacteria are not fully eliminated. In those prone to develop the disease, pulmonary TB is the most frequent clinical form exhibiting a variable degree of organ involvement. This spectrum of clinical manifestations ranges from a few foci affecting the upper parts of the lungs to important necrosis which usually disintegrates producing lung cavities or erodes blood vessels to cause hemorrhaging [1]. Since the adequate functioning of T lymphocytes is essential for both protective immunity and granuloma formation in response to M. tuberculosis infection [2], it is sensible to assume that this clinical outcome is largely dependent on the...
type of immune response which develops during infection. T cells can now be divided into at least two different subsets on the basis on their cytokine production. Th1 cells secrete interleukin-2 (IL-2), interferon-gamma (IFN-γ) and lymphotoxins, whereas Th2 cells produce IL-4, IL-5, IL-6 and IL-10. Functionally, Th1 cells have mainly been associated with cell-mediated immune responses (delayed type hypersensitivity reactions and macrophage activation) while Th2 cells are the principal stimulators of humoral immunity [3]. In addition, Th1 and Th2 cell types regulate each other via their cytokine synthesis, which is important in the final balance of host resistance against pathogens [4].

Studies of stimulated peripheral T cells from TB patients with several mycobacterial antigens failed to demonstrate a clear dichotomy in the profile of secreted cytokines, while IL-4-secreting cells appeared significantly increased in relation to controls [5]. Since cytokine responses to in vitro stimulation may not reflect the actual situation in vivo, and partly because of the variations in type and extent of lung TB lesions, we now wished to ascertain whether the different clinical presentation of pulmonary TB was accompanied by a particular pattern of serum cytokine profile.

2. Materials and methods

2.1. Subjects

The study population consisted of 29 (22 men and 7 women) inpatients admitted to Carrasco Hospital in Rosario, whose diagnosis of TB was based on clinical and radiological data along with the identification of tubercle bacilli in sputum. The patients were aged 18–69 with a mean age of 36 years (standard deviation = 15). Pulmonary TB patients were classified according to the extent and type of X-ray findings into three groups: mild (n = 10), patients with a single lobe involved, and without visible cavities; moderate (n = 5), patients presenting unilateral involvement of two or more lobes with cavities, if present, reaching a total diameter no greater than 4 cm; advanced (n = 14), bilateral disease with massive involvement and multiple cavities. The control populations consisted of 12 sex- and age-matched healthy volunteers living in the same area. None of the subjects had serological evidence of HIV infection. Permission to conduct this study was obtained from the Ethics Committee of the Facultad de Ciencias Medicas of the Universidad Nacional de Rosario, and all subjects gave their consent to participate.

2.2. Cytokine measurements

Serum samples were obtained before the initiation of antituberculous treatment, and stored at −70°C until use. Commercially available enzyme-linked immunosorbent assay kits (ELISA, Genzyme Diagnostics, Massachusetts, USA) were used for assaying IL-2 (detection limit 100 pg ml⁻¹), IFN-γ (detection limit 100 pg ml⁻¹), IL-4 (detection limit 100 pg ml⁻¹) and IL-10 (detection limit 5 pg ml⁻¹). Briefly, serum samples were added to the wells of microtiter plates precoated with anti-IL-2, anti-IFN-γ, anti-IL-4 and anti-IL-10 monoclonal antibodies. After incubation at 37°C for 2 h, the unbound components from the samples were removed by washing. Second anti-human IL-2, IFN-γ, IL-4 and IL-10 rabbit antibodies were added and incubated for 1 h at 37°C. Subsequently, a third antibody, goat anti-rabbit IgG conjugated with peroxidase, was added and incubated for 1 h at 37°C. Finally, the reaction was developed after adding the substrate and stop solution and measured with an ELISA reader at 450 nm. Samples were assayed in duplicate and results are expressed as the average absorbance of the two readings.

2.3. Statistical analysis

Differences in cytokine results among groups were evaluated with the Kruskal-Wallis and Mann-Whitney tests. Correlations between cytokine serum levels were analyzed with Spearman’s rank test. Values of P < 0.05 were considered significant.

3. Results

Serum concentrations of Th1 cell type cytokines in TB patients and controls are shown in Fig. 1. Compared to controls, the three groups of TB patients
had increased serum levels of IFN-γ and IL-2 ($P < 0.0001$, both cases). Further analysis among patient groups revealed that mean concentrations of IFN-γ and IL-2 were even higher in mild and moderate patients with statistically significant differences compared to patients with advanced disease ($P < 0.001$).

As stated in Section 2, sera were also investigated for the presence of IL-4 and IL-10. Fig. 2 indicates that TB patients had raised serum concentrations of IL-4 in comparison to controls ($P < 0.001$). In addition, moderate and advanced patients showed the highest levels with significant differences with regard to mild cases ($P < 0.002$ and $P < 0.01$, respectively). A search for the presence of IL-10 yielded lower concentrations in relation to IL-4, although values in patient groups were raised above those recorded in controls ($P < 0.001$, Fig. 2). Raised levels of IL-10 were more prevalent in cases with advanced TB, and statistically significant when compared to mild patients ($P < 0.005$, Fig. 2).

Attempts were also made to determine whether serum concentrations of studied cytokines showed some degree of relationship among them. As depicted in Fig. 3, there was a significant correlation between serum levels of IL-2 and IFN-γ ($r = 0.62, P < 0.01$).

4. Discussion

The human immune response to *M. tuberculosis* is involved in protection against the disease but can be at the same time detrimental and largely responsible for the lesions seen during TB. Hence, the different clinical picture of pulmonary TB is the result of a complex series of interactions among immunocompetent cells and their secreted cytokines. Results from the present study indicate that significant amounts of the four cytokines can be detected in the three groups of TB patients, but each group exhibited a particular pattern of circulating cytokines.
Raised concentrations of IFN-γ and IL-2, which were detected in mild cases, suggest a predominant activity of CD4+ Th1 cells, a T cell subset playing a critical role in the acquisition of resistance to \( M. \) \( \text{tuberculosis} \) \([2,6]\). T lymphocyte activation following mycobacterial antigen recognition on antigen presenting cells induces a variety of responses in the CD4+ Th1 cells including cytotoxic activity on infected macrophages and synthesis of IFN-γ and IL-2. In turn, IFN-γ stimulates human macrophages to produce TNF and 1,25-dihydroxyvitamin D, both of which facilitate mycobacterial inhibition \([7–9]\), probably due to the generation of reactive oxygen and nitrogen intermediates, as well as control of intracellular iron levels \([10]\). On the other hand, IL-2 is essential for the expansion of \( M. \) \( \text{tuberculosis} \)-specific T cells and may act to recruit other T cell subsets, γ T cells and CD8+ T cells, which may exert an additional role in the immune response to this pathogen \([2]\). It follows that immune mechanisms in a milieu where Th1 activities are prevalent may serve to produce a much less severe form of disease, as seen in mild TB.

Equally raised amounts of IFN-γ and IL-2 could still be detected in the moderate group, but these patients also showed significantly increased levels of IL-4, which seems compatible with a mixed profile of circulating cytokines. Furthermore, advanced disease was characterized by an opposite pattern of serum cytokines consisting of lower concentrations of IFN-γ and IL-2, in the presence of higher values of IL-4 and IL-10. These findings are in keeping with in vitro evidence wherein TB patients with progressive disease failed to generate IL-2 and IFN-γ in response to stimulation with mycobacterial antigens \([11–13]\). Likewise, predominant secretion of IL-4 by mycobacteria reactive T cells has already been reported in TB, while such studies gave no indication as to whether this was correlated with the severity of disease \([5,14]\). Data from our study suggest that a preferential expansion of IL-4 and IL-10 producing cells is more likely to occur in the severe form of pulmonary TB. IL-4 and IL-10 are well known for their downregulating activities on Th1 responses \([4,15]\), as well as their macrophage deactivating effects \([16,17]\). Hence, the presence of abundant amounts of IL-4 and IL-10 constitutes an important element in the aggravation of TB because of the counteractive effects both cytokines may exert on the protective immune response against \( M. \) \( \text{tuberculosis} \). Besides this detrimental effect, Th2 type cytokines may also be playing a regulatory role addressed to reduce lung lesions. Working in conjunction, IL-4 and IL-10 have been shown to inhibit in vitro IFN-γ-induced macrophage cytotoxicity and nitric oxide (NO) production \([18]\), a compound that, in addition to its microbicidal effects \([19]\), also participates in the pathophysiology of several diseases accompanied by inflammation and tissue damage \([20]\).

Factors determining this shift in the profile of circulating cytokines are poorly understood, although the presence of locally operating influences may account for this process. Macrophages are sites of habitation by mycobacteria and seem to exert important influences in the differentiation of Th1-like and Th2-like cells, via the costimulatory signal delivered to a precursor T helper cell \([21]\). Furthermore, mycobacterial products released at the site of infection may trigger macrophages and other cell types to secrete cytokines which may play a role in the differentiation of specialized Th subsets. To what extent such a shift in cytokine production represents the activation of two well differentiated types of immunocompetent cells or a particular modulation of the same Th cell population remains unknown. Although cytokine production profiles of human Th1 and Th2 cell clones are reminiscent of those of murine counterparts, there is evidence that the range of cytokines secreted is less polarized \([22,23]\). Whatever the case, the significant correlation between circulating levels of IFN-γ and IL-2 suggests that synthesis of both cytokines is closely regulated and likely produced by the same cell population, that is Th1 cells.

The differences in the pattern of circulating cytokines reported here can help to explain data recorded in our \([24,25]\) and other laboratories \([12,26]\), in which mild tuberculosis is characterized by preserved cellular immune responses whereas advanced disease is accompanied by impaired cell-mediated immune parameters and augmented humoral responses.

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References


