Interleukin 1ß (IL-1ß), IL-10, Tumor Necrosis Factor-α, and Cellular Proliferation Index in Peripheral Blood Mononuclear Cells in Patients with Ankylosing Spondylitis

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ABSTRACT. ***Objective.*** To evaluate cytokine production and cellular proliferation index (CPI) in peripheral blood mononuclear cells (PBMC) of patients with ankylosing spondylitis (AS), and their association with clinical variables.

Methods. In a cross sectional study we compared the production of tumor necrosis factor-α (TNF-α), interleukin 1ß (IL-1ß), and IL-10 and CPI in response to phytohemagglutinin (PHA) in PBMC of 27 patients with AS and 24 healthy controls. We also assessed clinical characteristics including disease activity index (BASDAI) and functional index (BASFI).

Results. Levels of IL-1ß were higher in patients with AS (median 242 pg/ml) than in controls (median 65 pg/ml); $p = 0.002$. No differences were observed in median levels of TNF-α or IL-10 between AS and controls. Patients had a reduction in CPI (1.2 in AS vs 1.8 in controls; $p < 0.001$). A positive correlation was observed between IL-10 production and age (rho = 0.34, $p = 0.01$). A borderline negative correlation was observed between CPI and age (rho = –0.26, $p = 0.07$).

Conclusion. Patients with AS had high production of IL-1ß compared with controls and a poor response in CPI. These findings may explain the lack of response for microbial antigens mediated by the innate immune response. (J Rheumatol 2002;29:522–6)

Key Indexing Terms:
ANKYLOSING SPONDYLITIS CYTOKINES
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Ankylosing spondylitis (AS) is the prototype of the seronegative spondyloarthropathies (SpA) associated to HLA-B27[1]. It is generally accepted that both genetic and autoimmune factors can play a significant role on the pathogenesis of AS. Nevertheless, only a few studies evaluate the role of cytokines in the pathophysiology of AS. Correlation has been described between serum levels of interleukin 6 (IL-6) and disease activity variables in AS such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR)[2]. But these findings have not been supported by other authors[3]. Macrophage derived cytokines such as tumor necrosis factor-α (TNF-α) and IL-1ß may have a different role in AS. It is well recognized that these 2 cytokines have a central role in rheumatoid arthritis (RA), triggering the production of matrix metalloproteases (MMP) with subsequent subchondral erosion[4-6]. The percentage of TNF-α positive cells in peripheral blood mononuclear cells (PBMC) from HLA-B27 positive patients with AS has been observed to be substantially lower compared to HLA-B27 negative controls[7-9]. This low TNF-α was associated with the TNF1/1 genotype at position –308, whereas the presence of TNF2 allele (TNF1/2) was associated with high production of TNF-α, similar to that observed in HLA-B27 nega-

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tive controls[8]. Nevertheless, the biological effects of these polymorphisms in the production of TNF-α mediated by TNF genes do not confer an independent effect for AS susceptibility in healthy subjects[9]. One study examining synovial biopsies from sacroiliac joints in AS described abundant message for TNF-α mRNA, supporting its pathogenic role in the disease[10]. Using microarrays of 588–1176 cDNA to generate gene expression profiles in patients with SpA, one study observed that the gene for TNF-α is activated in both PBMC and synovial fluid cells, suggesting that TNF-α has a pathogenic role in SpA similar to that observed in RA[11]. An open pilot study described improvement in disease activity variables in patients with SpA following treatment with infliximab, suggesting that blockade of TNF-α can be a useful strategy in treatment of AS[12]. IL-1ß is a major proinflammatory mediator in several inflammatory diseases; however, its precise role or clinical association in AS has not been confirmed. Elevated plasma levels of IL-10 have been associated with the disease activity in AS[13]. On the other hand, the reactivity of PBMC from patients with AS in response to bacterial antigens suggests that abnormalities in this response can provide a clue to the pathogenesis of the disease[14].

We analyzed cytokine production in PBMC and the proliferative response of mononuclear cells in the presence of phytohemagglutinin (PHA) in patients with AS. We also assessed the association between the production of cytokines and clinical variables measured using the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI)[15] and Bath Ankylosing Spondylitis Functional Index (BASFI)[16], to establish the importance of these cytokines in the clinical picture of AS.

MATERIALS AND METHODS

Study design. This was a cross sectional study comparing levels of cytokines and cellular proliferation index (CPI) in PBMC from patients with AS compared with healthy controls.

Clinical setting. Consecutive patients with AS were selected from an outpatient rheumatology clinic in a secondary care center.

Patient population. Patients diagnosed with AS according to the 1966 New York criteria[17] were included in the study if they met the following criteria: age 18–45 years, disease duration > 1 year, with radiographic evidence for bilateral sacroiliitis grade II or higher. All patients were selected independently of disease activity status. Patients were excluded if they had any of the following: evidence of infectious process, pregnancy, impaired renal function (creatinine clearance < 60 ml/min), elevated hepatic enzyme levels (twice the upper limit of normal), or any surgical procedure within 3 months before starting the study.

Control selection. Controls were selected from volunteer workers and students at the University of Guadalajara, with the following inclusion criteria: age 18–45 years and absence of any known disease, pregnancy, or infectious process within one week of the clinical examination. Controls were excluded if they were taking any kind of drugs or if they had received any surgical procedure 3 months before starting the study.

Clinical assessment. All patients were evaluated by the same investigator (AGG) at the time of study. Clinical variables assessed included age, sex, disease duration, history of drug use, and current therapy. Disease activity was evaluated using the BASDAI[15] and function using the BASFI[16].

Laboratory assessment. The following variables were assessed: ESR, CRP, and complete blood cell count. At the clinical evaluation a venous blood sample was taken (15 ml collected in tubes containing heparin).

Cellular immune response. The evaluation of cellular response proliferation to a mitogenic stimulus was carried out by a modification of the original Mossman technique[18,19] using PBMC. PBMC were obtained through a density gradient (Lymphoprep™). Once we identified cell viability, they were adjusted to 1×10^6/ml in RPMI-1640 culture medium (Gibco-BRL™). Mononuclear cell (MNC) cultures were done using 8×10^5 cells/well of culture box and stimulated with and without PHA 10 mg/ml. After 72 h dimethyl-tetrazolium (Sigma) and extraction buffer (20% sodium dodecyl sulfate, 50% dimethyl formamide) were added. The plate was incubated 24 h and then read at 570/630 nm. The result was expressed as cellular proliferation index (CPI): the optical densities (OD) in stimulated MNC divided by the results of OD in MNC without stimulation with PHA.

Cytokine expression in PBMC supernatants. PBMC were obtained as described and cultured in the presence of PHA 10 mg/ml for 24 h. Then supernatants were collected and held at –70°C until determinations of IL-1ß, IL-10, and TNF-α were done by ELISA (Amersham) following the manufacturer's instructions.

Statistical analysis. The nonparametric Mann-Whitney U test was used to compare differences for medians in cytokine levels (IL-10, IL-1ß, TNF-α) and CPI between patients with AS and controls. Spearman correlation test was used for evaluating the strength of association (rho) between cytokine values and clinical variables in patients with AS. Clinical variables in the correlation analyses were age, disease duration, ESR, CRP, BASDAI, and BASFI. Statistical significance was set at the level of p = 0.05. All analyses were performed using SPSS version 10.0.

RESULTS

Twenty-seven patients with AS were compared with 24 healthy controls. The median for age in patients with AS was higher than controls, but this difference did not achieve statistical significance (37 vs 29 years, respectively; p = 0.08); as well there were no statistical differences for proportions of women with AS (9/27, 67%) and controls (7/24, 71%) (p = 0.8). Patients with AS had a median for BASDAI of 4.2 (range 1.4 to 8.4) and a median for BASFI of 4.0 (range 0.3 to 8.4).

Cytokine determination. Cytokine levels in PBMC supernatant from patients and controls are shown in Table 1. The median level for IL-1ß was higher in patients (242 pg/ml) than in controls (65 pg/ml) (p = 0.002). Neither TNF-α nor

Table 1. Comparison in cytokine levels and cellular proliferation index (CPI) between patients with AS and healthy controls. Values are expressed in medians (range).

Cytokine, pg/ml	AS, n = 27	Controls, n = 24	p
IL-β	242 (0–2609)	65 (0–676)	0.002
IL-10	7.3 (0–169)	34 (0–103)	> 0.17
TNF-α	0 (0–1235)	0 (0–203)	> 0.2
CPI	1.22 (1.07–1.92)	1.80 (1.62–2.00)	< 0.001

Comparisons by Mann-Whitney U test.

IL-10 were increased in AS. The expression of these 2 cytokines was similar to levels in controls. Figure 1 shows the comparison for IL-1ß among patients and controls.

Cellular proliferation index. Patients with AS had a reduction in their CPI in response to PHA. The median CPI in AS patients was 1.2 compared to 1.8 in controls ($p < 0.001$) (Table 2). Figure 2 shows the comparison for CPI among patients and controls.

Associations between cytokine levels and clinical variables in AS. A significant Spearman correlation coefficient was observed between IL-10 and age (rho = 0.34, p = 0.01). Older patients with AS had higher IL-10 levels.

A borderline insignificant correlation was also observed between CPI and age (rho = –0.26, p = 0.07). No other statistical correlation was observed between levels of cytokines and clinical variables including the BASDAI or BASFI indices.

DISCUSSION

Many studies of cytokine expression in SpA are devoted to reactive arthritis[20-22]. However, several reports have evaluated cytokines in AS[2,3,7-11,13]. Two studies described a low detection of particular polymorphism of TNF-α in those patients with AS who were HLA-B27+[7,8]. On the other hand, a high expression of TNF-α mRNA has been found in synovial biopsies from AS patients[10]. Recently, 2 studies have reported the efficacy of anti-TNF-α in AS[12,23]. The role of other proinflammatory cytokines such as IL-1ß in AS is not clear. IL-1ß has a major role in the genesis of joint destruction in RA[4-6]. IL-1ß and TNF-α have similar proinflammatory actions, mediated by the same transcriptional factors[24]. IL-1ß also mediates innate immune responses against pathogens, one of the most important being cytokine release by macrophages[25]. We observed an increase in IL-1ß levels in patients with AS compared with healthy controls.

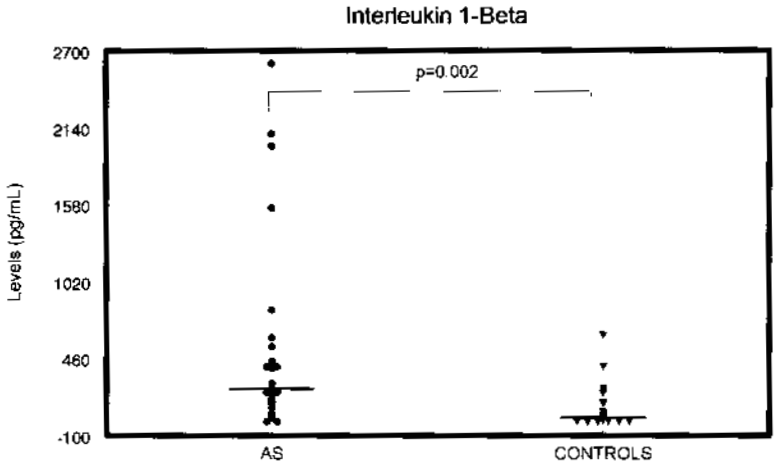

Figure 1. Levels of interleukin-1ß in patients with AS and healthy controls. Horizontal bars indicate the medians.

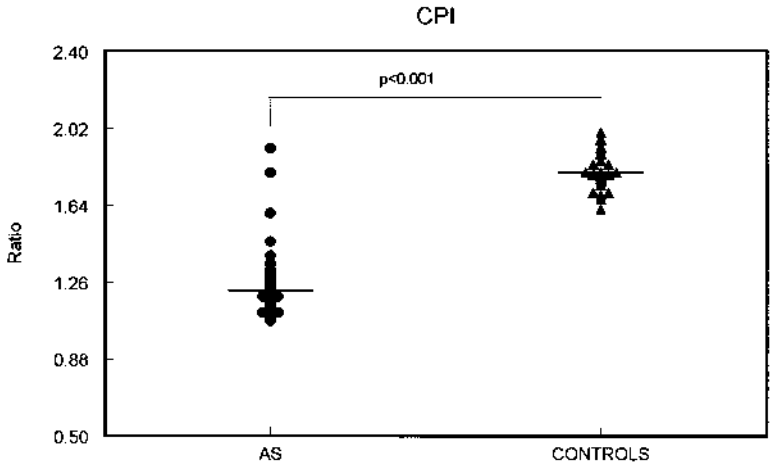

Figure 2. Cellular proliferation index (CPI) in patients with AS and healthy controls. Horizontal bars indicate the medians.

However, in our patients we were unable to find a parallel increment in TNF-α corresponding to the observed increment in IL-1ß. In addition, levels of IL-1ß did not correlate with the BASFI or BASDAI scores. Other authors report no association of the serum concentration of IL-1ß and clinical variables of disease activity[2]. However, these authors did find an association of some clinical severity variables, ESR, and CRP with IL-6, suggesting that this last cytokine is also involved with disease activity[2]. In one study investigating the presence of cytokine messenger RNA in sacroiliac joint biopsy specimens using *in situ* hybridization, no message for IL-1ß was found in the 3 patients examined by this technique[10]. Instead, they found a high amount of TNF-α mRNA. This information seems to contradict our findings; however, only 3 biopsy specimens were evaluated[10], and due to that small sample size the influence of chance cannot be excluded for explaining either the negative results for IL-1ß or the positive results for TNF-α.

With respect to the TNF-α, our results are in accord with authors who described a poor expression of this cytokine in HLA-B27+ patients with AS[7,8]. These authors found lower T cell production of TNF-α in the PBMC of HLA-B27+ patients with AS compared with healthy HLA-B27– controls[7,8]. Interestingly, HLA-B27+ controls also had lower T cell production than HLA-B27– controls, suggesting that low production of TNF-α can be linked to the susceptibility of HLA-B27+ subjects to develop AS.

These findings are in contrast with reports that the gene for TNF-α is activated in PBMC and synovial fluid cells in patients with AS[11]. Differences in methods for assessing TNF-α as well as differences in the clinical characteristics of the study population could explain these contradictory findings. Unfortunately this last study[11] has not been published in an extensive form that would allow better evaluation of the results.

Other indirect evidence for the role of TNF-α in AS is the response observed with infliximab in 2 studies, where disease activity measures improved in patients with SpA[12,23]. This controversy requires further investigation.

Since the cellular immune response seems to be important in the pathogenesis of reactive arthritis and AS, we decided to evaluate the CPI in AS compared with healthy controls. In our results we observed a reduction in the CPI in response to PHA in patients with AS. However, we were unable to find a statistical correlation of the CPI with disease activity variables in AS. The lower CPI in AS and the increment of IL-1ß expression, as well as the normal expression of TNF-α and Il-10, can be explained by many factors including differences in methods for cytokine determination. In comparison with our approach, the flow cytometry cytokine staining of stimulated cells constitutes a more specific method, having the additional advantage of determining which cells are involved in the production of specific cytokines. Using this approach, a low T cell production for TNF-α in HLA-B27+ patients with AS has been observed[7,8].

In SpA a positive correlation has been reported between plasma levels of IL-10 and duration of morning stiffness and level of pain[13]. We did not observe a correlation between IL-10 and BASDAI or BASFI results, and only observed a positive correlation between IL-10 and age — in this correlation older patients had higher IL-10 levels.

We hypothesize that in a very early stage of AS, cytokine expression and the cellular immune response for conventional mitogens may be exacerbated in response to a permanent antigenic stimulus, and that this feature can disappear through the years of the disease. Further studies evaluating the cytokine profile in patients with recent onset of disease may answer these questions. Studies following cytokine levels through periods of exacerbations and remissions are also needed. We are monitoring a group of AS patients in a longitudinal study to evaluate the importance of these cytokines for clinical outcome and their changes with treatment.

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