Interleukin-1-beta, Interleukin-10, and Tumor Necrosis Factor-alpha in Chinese Patients with Ankylosing Spondylitis

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**Background/Purpose.** To evaluate the relationship between the level of cytokines and the clinical findings in patients with ankylosing spondylitis (AS).

**Methods.** The study enrolled 81 AS patients and 49 healthy adults. The serum levels of Interleukin (IL)-\(\beta\), IL-10, and tumor necrosis factor (TNF)-\(\alpha\) were determined and compared between patients and control subjects. We also assessed the correlation between the production of cytokines and clinical parameters of the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and Bath Ankylosing Spondylitis Functional Index (BASFI).

**Results.** Mean serum IL-\(\beta\) level was significantly higher in AS patients than in control subjects (195.5 \(\pm\) 72.4 pg/mL vs 96.3 \(\pm\) 32.8 pg/mL; \(p < 0.001\)). Mean serum TNF-\(\alpha\) level was also significantly higher in patients with AS than in controls (85.3 \(\pm\) 42.1 pg/mL vs 34.3 \(\pm\) 12.8 pg/mL; \(p = 0.02\)). However, no significant differences were observed in levels of IL-10 between patients and controls. Furthermore, serum IL-\(\beta\) and TNF-\(\alpha\) levels in the AS patients positively correlated with the parameters in the BASDAI (\(p = 0.007\), \(r = 0.32\) and \(p = 0.001\), \(r = 0.41\), respectively). There was also a positive correlation between erythrocyte sedimentation rate and TNF-\(\alpha\) (\(p = 0.01\); \(r = 0.31\)).

**Conclusion.** Patients with a high BASDAI have higher levels of circulating TNF-\(\alpha\) and IL-\(\beta\) than patients with a low BASDAI or healthy individuals, suggesting that proinflammatory cytokines may play an important role during active inflammation. (Mid Taiwan J Med 2009;14:10-5)

**Key words**
IL-\(\beta\), IL-10, TNF-\(\alpha\), AS

**INTRODUCTION**
Ankylosing spondylitis (AS) is the prototype of the seronegative spondyloarthropathies (SpA), autoimmune diseases that are associated with the gene HLA-B27. It is generally accepted that both genetic and autoimmune factors play a significant role in the pathogenesis of AS [1].

Cytokines are soluble proteins that play specific roles in inflammatory response. They control the interaction between cells of the immune system in natural and specific immune reactions [2]. Interleukin (IL)-\(\beta\) is secreted by all nucleated cells, including macrophages, monocytes, B cells, fibroblasts, chondrocytes and keratinocytes, but are secreted primarily by macrophages. IL-1 participates in both local and systemic onset of acute and chronic inflammation. The biologic activation by IL-1 of cells such as
chondrocytes and fibroblasts is mediated through association with a special cell-surface protein, IL-1 receptor (IL-1R). IL-10 is produced under different conditions of immune activation by the T helper 0 (Th0) and T helper 2 (Th2) cell subsets [3], as well as by monocytes, macrophages and B cells. IL-10 inhibits the production by macrophage/monocytes of numerous inflammatory mediators, including IL-1α, IL-6, IL-8, granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF) and tumor necrosis factor-α (TNF-α) [4].

Th1 cells secrete proinflammatory cytokines such as interferon-γ (IFN-γ) and TNF-α, while Th2 cells secrete anti-inflammatory cytokines such as IL-10 and IL-4. The role of impaired Th1 response, which has been observed in patients with AS, remains to be clarified [5,6]. Rudwaleit and Braun demonstrated that the susceptibility of individuals carrying the HLA-B27 gene to AS was associated with low T-cell production of TNF-α and interferon-γ (IFN-γ) [7,8]. In contrast, Vazquez-Del et al. reported that IL-1β levels were significantly higher in AS patients than in healthy controls [9].

In this study, we analyzed cytokine production in patients with AS and in normal individuals. In addition, we also assessed the relationship between the production of cytokines and clinical parameters of the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) [10] and Bath Ankylosing Spondylitis Functional Index (BASFI) [11], to establish the importance of these cytokines in AS.

**PATIENTS AND METHODS**

A total of 81 patients (16 women, 65 men; mean age, 34.5 ± 10.8 years; range, 24 to 75 years) in whom a diagnosis of AS had been made according to the New York criteria from July 2006 to July 2007 were enrolled in the study (AS group) [12]. In addition, 49 unrelated healthy individuals living in central Taiwan were enrolled to serve as the control group (11 women and 38 men; mean age, 32.1 ± 8.5 years; range, 21 to 65 years). Informed consent to participate in this study was obtained from all patients and healthy individuals. All patients were evaluated by the same investigator. The age, sex, education level, medications, and the duration of the disease were noted. Disease Activity was evaluated by the BASDAI and functional status was measured by the BASFI [10,11]. The levels of pain, fatigue, and morning stiffness were measured on a 0-cm to 10-cm visual analogue scale (VAS). Erythrocyte sedimentation rate (ESR) was measured in 51 patients and C-reactive protein (CRP) was measured in 30 patients. The blood sample was centrifuged at 1600 rpm for 10 min to isolate the serum. Following this procedure, the serum was frozen at −70°C. The cytokine levels were determined using commercial human IL-1 and IL-10 enzyme-linked immunoabsorbant assay (ELISA) kits (Biosource, San Diego, California) and a TNF-α kit (Endogen, Woburn, MA, USA). The ELISA was performed according to the manufacturer’s instructions. All samples were examined in duplicate. The intervals for determination were 8 to 470 pg/mL for IL-1β, 6.5 to 350 pg/mL for IL-10 and 15.6 to 864 pg/mL for TNF-α.

Spearman’s coefficient was used to assess the correlation between disease activity parameters and serum cytokine levels. For paired samples, differences were tested by the Mann–Whitney U and Wilcoxon Rank Sum W tests and Kruskal–Wallis Kruskal–Wallis one-way ANOVA test. Results are expressed as mean ± standard deviation (SD). A p value of less than 0.05 was considered significant.

**RESULTS**

There were no significant differences in demographic data between the patients and healthy individuals (Table 1). The levels of IL-1β, IL-10, and TNF-α between the two groups are shown in Table 2. The mean serum IL-1β level in the AS group was significantly higher than that in the control group (195.5 ± 72.4 pg/mL vs 96.3 ± 32.8 pg/mL; p < 0.001). The mean serum TNF-α level in patients with AS was also significantly higher than that in controls (85.3 ± 42.1 pg/mL vs 34.3 ± 12.8 pg/mL; p = 0.02). However, no
significant differences in levels of IL-10 were observed between the AS and control groups (20.2 ± 12.3 pg/mL and 31.2 ± 15.2 pg/mL; \( p = 0.32 \)).

The association between clinical parameters and serum cytokine levels is shown in Table 3. When the levels of IL-1\(\beta\), IL-10, and TNF-\(\alpha\) were compared with the BASDAI and BASFI, we found that serum IL-1\(\beta\) levels in the AS patients positively correlated with BASDAI (\( p = 0.007, r = 0.32 \)). There was also a positive correlation between TNF-\(\alpha\) level and BASDAI (\( p = 0.001, r = 0.41 \)). However, no correlation was found between cytokine levels and BASFI, fatigue, morning stiffness, pain and chest expansion. There was no association between CRP and IL-1\(\beta\), IL-10, and TNF-\(\alpha\) levels as shown in Table 3.

A correlation was found between ESR and TNF-\(\alpha\) (\( p = 0.01, r = 0.31 \)), but no correlation between ESR and IL-1\(\beta\) or IL-10 was noted.

### DISCUSSION

IL-1\(\beta\) and TNF-\(\alpha\) directly induce the expression of matrix metalloproteinases (MMPs) in many cells, including synoviocytes and chondrocytes. Many researches have demonstrated that cartilage degradation is mediated by MMPs, aggrecanases, and other proteinases. IL-1\(\beta\) and TNF-\(\alpha\) play a major role in the pathogenesis of joint destruction in rheumatoid arthritis (RA) [13,14]. They have similar proinflammatory actions and are mediated by the same transcription factors [15].

In this study, we demonstrated that serum levels of IL-1\(\beta\) and TNF-\(\alpha\) were significantly...
higher in patients with ankylosing spondylitis than in healthy subjects; however, no significant differences in levels of IL-10 were observed between AS and control groups. We found a correlation between the levels of IL-1β and BASDAI as well as a correlation between TNF-α levels and BASDAI.

Gratacos et al [16] were one of the first groups to evaluate serum cytokines in AS. In their study, they could not find detectable levels of IL-1β in the AS group or in control subjects who had noninflammatory back pain. These authors reported no association between serum concentrations of IL-1β and clinical variables of disease activity. Sonel et al [17] and Bal A et al [18] obtained similar results. Sonel et al [17] could not find a significant difference in IL-1β levels between healthy individuals and patients with spondyloarthropathy (SpA). In addition, they were unable to show any difference in IL-1β levels between patients with active AS and those with inactive AS. In contrast to the above-mentioned studies, Vazquez-Del Mercado et al [9] showed that levels of IL-1β were significantly higher in AS patients than in healthy subjects; however, they did not find any relationship between IL-1β levels and BASFI and BASDAI scores. We found a significant difference in IL-1β levels between patients with AS and healthy subjects in this study, and we found that the BASDAI of AS correlated with the serum levels of IL-1β.

Pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β have been shown to be associated with T and B cell differentiation, lymphocyte and monocyte chemotaxis, and induction of acute phase proteins. Gratacos et al [16] demonstrated that TNF-α levels were higher in active AS patients and higher in inactive AS patients than in controls. Sonel et al [17] reported higher levels of TNF-α in their SpA group than in their control group. Bal A et al [18] and Toussirot et al [19] reported a trend toward increased TNF-α levels in patients with AS. The results from all of these studies, including ours, show raised serum levels of TNF-α in SpA. In contrast, Vazquez-Del Mercado et al [9] found no significant differences in TNF-α levels between AS patients and control subjects. In our study, we found a correlation between TNF-α levels and BASDAI. We believe that TNF-α reflects disease activity in Chinese AS patients. The differences between these studies may be due to ethnic factors or medication effects. All of the AS patients in our study were using NSAID, sulfasalazine, or a combination of both. We believe that NSAID, or sulfasalazine or both might have affected the clinical parameters; however, we were unable to compare the clinical parameters between patients taking medication and those who were not taking medication because 78% of our patients were taking a combination regimen of NSAID and sulfasalazine.

Gratacos et al [16] did not find an association between TNF-α levels and ESR and CRP. In contrast, our findings supported those reported by Bal et al [18] in which there was a relationship between TNF-α levels and ESR. In recent years, many studies have shown that anti-TNF therapies are effective in improving clinical (BASFI, BASDAI) and laboratory disease activity parameters (CRP, ESR) [20]. Gorman et al showed rapid, significant, and sustained improvement in AS patients treated with the anti-TNF-α agent etanercept [21]. Brandt et al [22] and Calin et al [23] demonstrated a similar finding.

In conclusion, the results from this study suggest that IL-1β and TNF-α play important roles in the pathogenesis of AS. Moreover, these cytokines can be used as markers of disease activity in the diagnosis and treatment of ankylosing spondylitis.

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REFERENCES
華人僵直性脊椎炎血中介白質-1β、介白質-10及腫瘤壞死因子-α之研究

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背景/目的  評估僵直性脊椎炎病患及正常人血中細胞激素與臨床表現之相關性。

方法  本研究共收入81位僵直性脊椎炎病患，及49位正常健康成人。進行檢測及比較其血中介白質-1β、介白質-10及腫瘤壞死因子-α。同時也評估病人這些細胞激素和臨床指標之關連性，如：僵直性脊椎炎疾病活動度指標巴斯量表（BASDAI）及僵直性脊椎炎功能性指標巴斯量表（BSAFI）。

結果  僵直性脊椎炎患者血中介白質-1β值明顯高於正常人（195.5 ± 72.4 pg/mL及96.3 ± 32.8 pg/mL，p < 0.001）。同時，其腫瘤壞死因子-α也明顯比正常人高（85.3 ± 42.1 pg/mL及34.3 ± 12.8 pg/mL，p = 0.02）。然而，在介白質-10於兩組則無明顯差異。另外，僵直性脊椎炎患者血中介白質-1β值和腫瘤壞死因子-α和僵直性脊椎炎疾病活動度巴斯量表（BASDAI）有正相關（p = 0.007，r = 0.32及p = 0.001，r = 0.41）。同時，ESR和腫瘤壞死因子-α亦有正相關（p = 0.01，r = 0.31）。

結論  僵直性脊椎炎疾病活動度巴斯量表（BASDAI）分數高之病人，較分數低之病人或正常人有較高血中介白質-1β和腫瘤壞死因子-α值，這表示發炎前細胞激素在活動性發炎期中扮演重要角色。（中華醫學 2009;14:10-5）

關鍵詞
介白質-1β，介白質-10，腫瘤壞死因子-α，僵直性脊椎炎

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