Elevated Interleukin-10 Levels Correlate With Disease Activity in Systemic Lupus Erythematosus

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Background. We analyzed the relationship between serum interleukin (IL)-10 levels and disease activity in patients with systemic lupus erythematosus (SLE).

Methods. The study enrolled 50 SLE patients and 30 healthy adults who were used as the control group. The IL-10 levels in the serum were determined and compared according to disease activities, which were measured using the SLE Disease Activity Index (SLEDAI) and laboratory parameters such as C3, C4, anti-dsDNA antibodies, IgG, IgM and IgA.

Results. The IL-10 levels of the SLE patients were significantly higher than those of the control group (34.3 ± 7.2 vs 5.8 ± 1.2 pg/ml, p < 0.05). Serum IL-10 titers in SLE patients positively correlated with the SLEDAI (r = 0.42, p < 0.01) and anti-dsDNA antibodies (r = 0.3, p < 0.05), but negatively correlated with complement C3 levels (r = −0.45, p < 0.01). However, there was no significant association between IL-10 and C4 levels.

Conclusions. Our study demonstrated that serum levels of IL-10 were elevated in SLE patients and increased IL-10 correlated with SLE disease activity. (Mid Taiwan J Med 2000;5:37-42)

Key words
disease activity, interleukin-10, systemic lupus erythematosus

INTRODUCTION

Interleukin (IL)-10 is a major immunoregulatory cytokine and has a number of immunomodulating effects on the immune system. IL-10 is a potent in vitro inducer for B lymphocyte differentiation [1] as well as an inhibitor of T-helper lymphocytes [2] and antigen-presenting cell function [3]. IL-10 was initially identified as a type 2 T-helper cell cytokine which was produced by many cell types, including T lymphocytes, B lymphocytes, monocytes, macrophages, and mast cells [4]. IL-10 suppresses type 1 T helper lymphocyte by decreasing IL-2 and interferon (IFN)-γ production [5,6]. It has an autoregulatory negative feedback function, since IL-10 is secreted by the human mononuclear cell which can suppress its own IL-10 m-RNA production [7]. Human IL-10 and viral IL-10 stimulate the DNA replication of B lymphocytes through an IL-10 antigen receptor or a CD40 surface antigen of the B lymphocytes, thereby producing large quantities of IgG, IgM, and IgA through B lymphocyte proliferation and activation [1].

Systemic lupus erythematosus (SLE) is an autoimmune disease that its exact cause is still unknown. It is characterized by an increased amount of B lymphocytes, mainly reflected by the production of autoantibodies [8] and decreased cellular immune responses
The latter defect is related to a dysfunction of both T-helper lymphocytes [11] and antigen-presenting cells [12]. Furthermore, continuous administration of neutralizing anti-IL-10 antibodies delayed the onset of autoimmunity in F1 (New Zealand black x New Zealand white) hybrid mice, which is a murine model of SLE [13]. These findings indicate that IL-10 is an important candidate factor in the pathogenesis of SLE, and may play a role by reducing T cell responses and inducing B cell hyperactivity.

In a previous study [14], IL-10 titers were elevated and correlated with disease activity in SLE patients. However, different results were demonstrated by Llorente et al [15]. Moreover, the study of IL-10 in SLE patients has never been done in Taiwan. We decided to focus on the role of IL-10 in SLE patients characterized by increased B lymphocyte hyperactivity. This study was designed to determine the serum IL-10 levels in SLE patients and to assess their relationship with disease activity.

### MATERIALS AND METHODS

#### Subjects

From July 1998 through July 1999, 50 patients (four males and 46 females; mean age, 26.5 ± 53 years; range, 16 to 60 years) with SLE, who visited the Division of Immunology and Rheumatology at China Medical College Hospital, were enrolled in this study. Diagnosis of SLE was classified according to the American College of Rheumatology criteria [16]. Thirty sex- and age-matched healthy individuals were used as the control group (two males and 28 females; mean age, 26.8 ± 4.5 years; range, 18 to 52 years). Lupus disease activity was assayed using the SLE Disease Activity Index (SLEDAI) [17]. Out of the 50 patients, 35 were on steroids and 23 were also receiving immunosuppressive drugs at the time of sampling (21 on hydroxychloroquine, seven on azathioprine, two on intravenous cyclophosphamide and one on weekly oral methotrexate). The mean daily dose of prednisolone administered was 20.5 ± 8.7 mg; range, 5 to 80 mg (Table 1).

#### Serum IL-10 Level

The serum IL-10 level was determined using a commercial human IL-10 enzyme-linked immunoadsorbent assay (ELISA) kit (Biosource, San Diego, California). The ELISA was performed according to the instructions by the manufacturer. All samples were done in duplicate and the interassay variation was < 6%. The assay range of this IL-10 ELISA was 0–500 pg/ml. The correlation between absorbency values and IL-10 concentrations were perfectly linear ($r = 0.99$).

#### Serologic Tests

Anti-dsDNA antibodies were measured using a commercial indirect immunofluorescence test (MBL Co., Nagoya, Japan). The complements C3, C4, immunoglobulin IgG, IgM and IgA (Roche Diagnostics, Basel, Swiss) were determined using Nephelometry.

#### Statistical Analysis

The comparison of IL-10 levels between
the SLE patients and control subjects was performed using an unpaired Student's *t*-test. The correlation between the serum IL-10 levels and the disease activity indices in SLE patients, including SLEDAI, C3, C4, anti-dsDNA, IgG, IgM and IgA, was analyzed using Spearman's rank correlation test. A *p* value less than 0.05 was considered significant. All the statistical calculations were performed using the SPSS program (version 6.0 for Windows 95).

### RESULTS

The mean serum IL-10 level of the SLE patients was significantly higher than that of the control subjects (34.3 ± 7.2 vs 5.8 ± 1.2 pg/ml, *p* < 0.05, Table 1). There was no significant difference of IL-10 titers between SLE patients with or without steroid therapy (34.8 ± 6.5 and 30.7 ± 4.5 pg/ml respectively). However, serum IL-10 titers did not correlate with the total IgG, IgM, or IgA levels. No specific organ involvement was associated with the serum IL-10 concentrations (data not shown). Serum IL-10 titers in the SLE patients positively correlated with the SLEDAI (*r* = 0.42, *p* < 0.01) and with anti-dsDNA antibodies (*r* = 0.30, *p* < 0.05). A negative correlation was found between the IL-10 and C3 (*r* = −0.45, *p* < 0.01). However, there was no significant association between IL-10 and C4 levels (*r* = 0.24, *p* = 0.7, Table 2).

### DISCUSSION

In this study, we demonstrated that serum IL-10 levels were significantly higher in SLE patients than in healthy individuals, which is the same result as in a previous report [18]. This suggests that IL-10 may play an important role in the pathogenesis of SLE. At the same time there were significant associations between IL-10 and disease activity in SLE patients, which are similar with the results in a report by Houssiau et al [14]. They showed that serum IL-10 titers in SLE patients positively correlated with the SLEDAI and with anti-dsDNA, but negatively correlated with C3 levels. However, C4 titers were not detected in the study by Houssiau et al. Moreover, serum IL-10 concentrations were lower in their study than in ours. IL-10 was detected in the serum of 27 of 72 patients with SLE (37.5%) and in only one of the 30 control subjects (3%). In contrast, Llorente et al [15] did not find any associations. A possible explanation for this discrepancy is that our study was performed on the serum whereas Llorente et al tested IL-10 expression *ex vivo* (at the RNA level, a less quantitative measurement) and after a 24-hour culture of peripheral blood mononuclear cell *in vitro*.

The role of IL-10 in SLE was recently suggested due to these findings: (1) that continuous administration of anti-IL-10 antibodies delays onset of autoimmunity in NZB/W F1 lupus prone mice [13]; (2) that peripheral blood mononuclear cells from SLE patients spontaneously produce high amounts of IL-10 *in vitro* [15]; and (3) that anti-IL-10 antibodies decrease immunoglobulin and anti-DNA antibody production by SLE lymphocytes *in vitro* and *in vivo* [19].

Our data, by indicating an absence of correlation between serum IL-10 and total immunoglobulin levels, show evidence against the role of IL-10 as a polyclonal B lymphocyte activator *in vivo*. Several possible explanations for this discrepancy have been proposed. IL-10 does not seem to directly affect humoral immunity in SLE. The disease severity in lupus patients was reported to be associated with an

### Table 2. Correlations between serum IL-10 levels and parameters of disease activity in 50 SLE patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th><em>r</em></th>
<th><em>p</em> value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLEDAI</td>
<td>+ 0.42</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>C3</td>
<td>− 0.45</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>C4</td>
<td>+ 0.24</td>
<td>NS</td>
</tr>
<tr>
<td>Anti-DNA Ab</td>
<td>+ 0.30</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>IgG</td>
<td>+ 0.15</td>
<td>NS</td>
</tr>
<tr>
<td>IgM</td>
<td>+ 0.13</td>
<td>NS</td>
</tr>
<tr>
<td>IgA</td>
<td>− 0.12</td>
<td>NS</td>
</tr>
</tbody>
</table>

IL-10 = interleukin-10; SLE = systemic lupus erythematosus; *r* = Spearman's rank correlation coefficient; SLEDAI = SLE Disease Activity Index; NS = not significant.
increased ratio of cells secreting IL-10; on the other hand, IFN-γ and the number of cells secreting IFN-γ diminished with IL-10 administration in vivo [20]. These data showed that the expression of these cytokines might be coordinately regulated. Another explanation for this discrepancy is that serum IL-10 levels may serve as an anti-inflammatory response to suppress pro-inflammatory cytokines during the active stage of SLE. It has been reported that TNF-α and IL-6 are elevated in the active stage of SLE [21], and IL-10 has been shown to inhibit the synthesis of such cytokines [22]. Considering these anti-inflammatory properties of IL-10 in several autoimmune diseases, we surmised that IL-10 would increase as a counteraction against active inflammation in SLE patients. In addition, data obtained by Llorente et al in severe combined immuno-deficiency syndrome (SCID) mice injected with human SLE peripheral blood mononuclear cells add further support to this intriguing hypothesis as in vivo administration of anti-IL-10 antibodies preferentially down-regulates the anti-dsDNA response rather than the total immunoglobulin levels [19].

Until now there have been many hypotheses about the role of IL-10 in the pathogenesis of SLE and more studies on this subject will likely be performed. Our study showed that serum IL-10 levels were elevated in SLE patients and that IL-10 correlated well with the disease activity of SLE.

ACKNOWLEDGMENT

This study was supported by a grant (DMR-88-006) from the China Medical College Hospital.

REFERENCES

全身性紅斑性狼瘡介白質-10之升高和其疾病活動度呈相關性

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背景  偵測全身性紅斑性狼瘡患者血清介白質-10之濃度及分析其和疾病活動度之相關性。

方法  本研究對象為 50 位全身性紅斑性狼瘡患者，並以 30 位正常人為對照組。使用方法是以介白質-10和全身性紅斑性狼瘡疾病活動度指標 (SLEDAI) 及實驗室數據如補體 (C3 和 C4)、抗 DNA 抗体、免疫球蛋白 G、免疫球蛋白 M 和免疫球蛋白 A 加以比較。

結果  全身性紅斑性狼瘡患者介白質-10 濃度在統計學上顯著地高於正常人 (34.3 ± 7.2 比 5.8 ± 1.2 pg/ml, p < 0.05)。介白質-10 和 SLEDAI (r = 0.42, p < 0.01) 及抗 DNA 抗体 (r = 0.1, p < 0.05) 呈現正相關性。而與 C3 卻為負性相關性 (r = -0.45, p < 0.01)。然而，介白質-10 與 C4 在統計學上並無相關性。

結論  本研究顯示在全身性紅斑性狼瘡患者介白質-10會有較高之濃度，且介白質-10 和患者之疾病活動度具有相關性。 (中華醫誌 2000;5:37-42)

關鍵詞
疾病活動度，介白質-10，全身性紅斑性狼瘡

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收文日期：10/22/1999  修改日期：1/13/2000
接受日期：1/18/2000