Serum Levels of Tumor Necrosis Factor-Alpha and Interleukin-6 in Ocular Cicatricial Pemphigoid

Soon Jin Lee, Zhengzhi Li, Brian Sherman, and C. Stephen Foster

Purpose. These studies examined regulation of the cytokines interleukin-6 and tumor necrosis factor-alpha in ocular cicatricial pemphigoid (OCP), a systemic autoimmune disease.

Methods. Serum levels of interleukin-6 and tumor necrosis factor-alpha in sera collected from 35 patients with OCP, 29 normal persons and 17 patients with ocular inflammatory diseases were determined using an enzyme-linked immunosorbent assay.

Results. Levels of interleukin-6 were significantly decreased in sera of patients with OCP (median, 28.9; range, 7.5 to 136.7 pg/ml, \( P < 0.001 \)) compared with sera from normal subjects (median, 65.2; range, 21.1 to 303.9 pg/ml). Sera from patients with non-OCP, extraocular inflammatory diseases and uveitis, showed no such decrease. In contrast, tumor necrosis factor-alpha levels were significantly elevated in OCP patients (median, 22.5; range, 8.3 to 44.4 pg/ml, \( P < 0.001 \)), whereas no such increase was observed in sera from patients with extraocular inflammatory disease or uveitis, compared to normal sera controls (median, 17.4; range, 5 to 27.2 pg/ml).

Conclusions. These results suggest that elevated serum tumor necrosis factor-alpha levels and decreased serum interleukin-6 levels can be added to the increasing list of systemic immunologic correlates of active OCP, again emphasizing that OCP is a systemic disease whose primary manifestation is ocular. Invest Ophthalmol Vis Sci. 1993;34:3522-3525.

The term cytokine refers to a class of proteins, produced by a variety of cells, that play critical roles in many biologic processes including inflammation and immunity. Cytokines play important roles in many physiological responses and are involved in the pathophysiology of a wide range of diseases. Because of their regulatory nature, their potential as therapeutic agents has been explored. The clinical application of pure recombinant cytokines or their inhibitors in cancer, infectious disease, and autoimmune disease has recently met with some success.

Interleukin-6 (IL-6) is a wide variety of activities on numerous cell types. IL-6 is expressed by tumor cells, mitogen-stimulated lymphocytes and cytokine-stimulated fibroblasts. IL-6 enhances immunoglobulin secretion by B lymphocytes and serves as a second signal in murine T cell activation. Increased serum levels of IL-6 have been reported during renal transplant rejection episodes, and IL-6 has been associated with certain autoimmune diseases.

Tumor necrosis factor-alpha (TNF-\( \alpha \)) also is produced by many different cell types, including macrophages, T cells, and NK cells; it has a variety of physiological properties, including stimulation of fibroblast growth and neutrophil function. TNF-\( \alpha \) enhances both IL-2 receptor and human leukocyte antigen-II expression on T cells and is an autocrine T cell growth factor. Elevated TNF-\( \alpha \) gene expression has been observed in grafted versus host disease, and TNF-\( \alpha \) has been associated with rheumatoid arthritis.

As a first step in understanding the abnormal regulation of cytokine networks in autoimmune eye diseases, we examined IL-6 and TNF-\( \alpha \) levels in the sera of patients with ocular cicatricial pemphigoid (OCP), a systemic autoimmune disease, as well as in sera from patients with other ocular inflammatory eye diseases, and in sera from normal subjects. We found decreased levels of IL-6 and increased levels of TNF-\( \alpha \) in OCP sera compared to sera from normal groups. These re-
results support the notion that OCP is an autoimmune disease associated with an abnormal cytokine network.

MATERIALS AND METHODS

Sample Collection
Before initiating treatment, informed consent about the nature and possible consequences of this study was obtained from all participants. Sera were collected from patients with OCP (n = 35), uveitis (n = 8), other extraocular inflammatory diseases (n = 9), and normal (control) individuals (n = 29) and stored at -70°C until assayed. The diagnosis of OCP was confirmed by immunohistochemical demonstration of immunoreactants at the epithelial basement membrane zone. The protocols used in this study followed the tenets of the Declaration of Helsinki and were approved by the Massachusetts Eye and Ear Infirmary Human Experimentation Committee.

Determination of Tumor Necrosis Factor-Alpha and Interleukin-6 Levels
Serum TNF-a and IL-6 levels were measured by a two-site sandwich enzyme-linked immunosorbent assay, using a commercial kit (T-cell Sciences, Inc., Cambridge MA). Briefly, 96-well plates coated with either an anti-TNF-a or an anti-IL-6 monoclonal antibody were incubated with sera samples from each group. After washing the plates to remove unreacted sera components, enzyme-conjugated monoclonal antibodies directed against either TNF-a or IL-6 were added and incubated. Unbound enzyme-conjugated antibodies were removed by washing and the substrate, O-phenylenediamine, was added. The reaction was terminated and absorbance at 490 nm was measured. All samples were tested in duplicate and mean values were determined. The values for TNF-a and IL-6 were calculated using a standard curve determined using purified TNF-a and IL-6 provided with these kits.

Statistical Method
The Wilcoxon test was used to compare the mean serum levels of TNF-a and IL-6 between the different groups of patients and control subjects. The level of significance chosen was P < 0.05.

RESULTS
Patients included in this study varied in age from 20 to 60 years. No significant differences were observed between normal subjects grouped on the basis of age or sex, so normal subjects were not divided into groups for comparison with patients with ocular inflammation. Extraocular inflammatory disease patients included seven diagnosed with scleritis, one with sarcoidosis-associated chronic conjunctivitis, and one with episcleritis.

First, we studied TNF-a in the sera of normal subjects grouped by either age or sex. No significant differences were observed between subjects older or younger than 50 years (P = 0.429) or between male and female subjects (P = 0.154; Fig. 1). For this reason, samples from patients or control subjects were not divided into groups before comparison of serum TNF-a levels.

TNF-a levels were significantly increased in the sera of patients with OCP (median, 22.5; range, 8.3 to 44.4 pg/ml, P < 0.001) compared to serum levels in normal subjects (median, 17.4; range, 5 to 27.2 pg/ml; Table 1). Statistically significant increases in serum TNF-a levels were not observed in patients with uveitis (median, 22.5; range, 8.9 to 30.1 pg/ml; P = 0.257) or extraocular inflammatory diseases (median, 19.5; range, 14.2 to 22.5 pg/ml; P = 0.057) compared to normal subjects (Table 1).

Serum IL-6 levels also did not significantly differ in normal subjects grouped by either age or sex (Fig. 2). Serum IL-6 levels were significantly lower in OCP patients (median, 28.9; range, 7.5 to 136.7 pg/ml; P < 0.001) compared to normal subjects (median, 65.2; range, 21.1 to 308.9 pg/ml; Table 1). There was no significant difference in the mean serum IL-6 levels of patients with extraocular inflammatory diseases (median, 45.8; range, 0 to 450 pg/ml; P = 0.476) or with patients.
uveitis (median, 79.6; range, 0 to 435.6 pg/ml; \( P = 0.200 \)) compared to normal subjects (Table 1). Because the difference in age of the normal group for IL-6 had borderline significance, we reanalyzed the data with age-matched group for comparison of serum IL-6 levels from patients with OCP, extraocular inflammatory disease, and uveitis and those from normal subjects. The new \( P \) values were OCP = 0.01 (n = 9), uveitis = 0.163 (n = 8) and extraocular inflammatory diseases = 0.199 (n = 7), all similar to the values obtained without age matching.

**DISCUSSION**

Elevated serum levels of IL-6 and TNF-\( \alpha \) have been previously reported in autoimmune diseases such as rheumatoid arthritis, suggesting abnormal regulation of these two cytokines in the peripheral immune system.\(^4\)\(^,\)\(^5\) Our studies examined the serum levels of these two cytokines in patients with OCP, an extraocular inflammatory problem that we and others have contended represents local expression of a systemic autoimmune disease.\(^7\)\(^,\)\(^8\) Serum TNF-\( \alpha \) levels were substantially elevated in OCP patients, suggesting a potential role for this cytokine in the increased activation of the peripheral immune system, as judged by elevated serum levels of soluble IL-2 receptor and soluble CD8 (unpublished observation). TNF-\( \alpha \) has been shown to potentiate both CD8 T cell function in vivo and in vitro and the release of soluble CD8 from synovial fluid mononuclear cells in vitro.\(^9\) The observed increase of serum-soluble CD8 levels in OCP patients may result from elevated levels of TNF-\( \alpha \), which has been shown to trigger CD8 T cell activation.\(^9\) The elevated serum TNF-\( \alpha \) levels observed in OCP patients in this study suggest an association between expression of this cytokine and OCP, a finding reported for other autoimmune diseases, such as rheumatoid arthritis.\(^5\)

Increased IL-6 expression has been implicated in polyclonal B cell activation, which is observed in autoimmune diseases such as rheumatoid arthritis.\(^3\) However, IL-6 levels in the sera of our OCP patients were decreased, suggesting that a different mechanism may be involved in the pathogenesis of OCP. Although these studies comprised a relatively small number of subjects and a large variation in mean values was noted, the findings obtained from this study warrant further investigation with a larger study population. Reduced IL-6 serum levels were reported in cyclosporin-induced nephrotoxicity in human renal allotransplant recipients.\(^10\) Decreased serum levels of IL-6 observed in this study may have resulted from abnormal function of cells secreting IL-6 or from secondary effects of other cytokines involved in the cytokine regulatory network. More studies are needed to fully establish the potential relationship between serum levels of IL-6 and TNF-\( \alpha \) and their relationship to conjunctival inflammation.

**Key Words**

tumor necrosis factor, interleukin-6, cicatricial pemphigoid, ocular immunology, cytokines

**Acknowledgments**

The authors thank Dr. George T. Cicila for critically reviewing the manuscript.
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References