Aqueous Humor Interleukin-6 Levels in Uveitis

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The level of Interleukin-6 (IL-6) in the aqueous humor of 24 patients with 2 types of uveitis was measured with a specific bioassay using the murine hybridoma cell line B9. Sixteen patients had Fuchs' heterochromic cyclitis (FHC) and 8 had toxoplasma uveitis (TU). Sixty-three percent of each of the FHC and TU groups had raised levels of IL-6 in their aqueous (mean: 543 and 19,228 units/ml respectively). Thirteen control aqueous samples, obtained at surgery for senile cataract, showed IL-6 levels of <10 units/ml. Serum obtained at the same time as each aqueous humor sample also showed IL-6 levels of <10 units/ml, indicating that the raised levels of IL-6 found in the aqueous of uveitis patients did not result from serum leakage, but from local production. This is the first report on intraocular IL-6 levels, and indicates that IL-6 may play a role as an inflammatory mediator in uveitis.


Although the etiology of most cases of uveitis is unknown, disturbances of the immune system have long been suspected of playing an important role in the pathogenesis or perpetuation of intraocular inflammation. Fuchs' heterochromic cyclitis (FHC) is probably the most puzzling of all of the uveitis syndromes, and an immunologic basis is strongly suspected.1 Many immunologic aberrations previously detected in this condition have been found in peripheral blood—eg, reduced T-suppressor cell function,1 raised levels of immune complexes,1 and humoral2 and cellular2 immunity to a corneal antigen. These abnormalities may, in part, be epiphenomena, since none of the patients exhibited evidence of systemic disease. To gain further insight into the immunopathogenesis of FHC and other types of uveitis, analysis of intraocular fluids is essential.

Interleukin-6 (IL-6), also known as B-cell stimulating factor 2 (BSF-2), B-cell differentiaion factor (BCDF), 26-kD protein, interferon-β2, plasmacytoma/hybridoma growth factor, and hepatocyte stimulating factor is a multi-functional cytokine which can be expressed by a wide variety of cell types, eg, T-cells, macrophages, monocytes, fibroblasts, and endothelial cells. IL-6 is a heterogeneous 19-30-kD phosphoglycoprotein eliciting a broad range of physiologic and immune responses.4,5 Apart from the stimulation of immunoglobulin synthesis by B-cells,6 IL-6 also augments T-lymphocyte responses7 and induces the synthesis of acute phase proteins.8-10 IL-6 can be induced by interleukin-1 (IL-1), tumor necrosis factors (TNF) α and β, and endotoxin.11,12 Serum levels of IL-6 are increased in patients with severe burns,13 acute bacterial infections,14 and Castleman’s disease,15 and in normal volunteers administered with endotoxin,16 in cancer patients treated with recombinant TNFα (rTNF) and in renal transplant recipients.17 Local IL-6 production has been detected in synovial fluid of patients with rheumatoid arthritis but not in those with active osteoarthritis.18

IL-6 may have a role to play as an inflammatory mediator in uveitis, since B-cell overstimulation is believed to occur in FHC,19 and since the endotoxin-induced uveitis seen in rats is associated with high levels of IL-6 in the aqueous of these animals (manuscript submitted). Currently, no reports on IL-6 in the eye exist; therefore, determination of aqueous IL-6 levels in uveitis was undertaken. It was decided to study two markedly different but well-defined groups of uveitis patients: FHC, with its chronic low-grade (mainly) anterior chamber activity, and active TU, with its acute (often recurrent) posterior/pan uveitis. The comparison of dissimilar uveitis entities may increase our knowledge of immunopathogenic mechanisms in these group of conditions.

Materials and Methods

Aqueous humor samples were obtained from 16 patients with FHC undergoing extracapsular cataract extraction or trabeculectomy (10 males and 6 females, aged 23-72 yr), and from 8 patients with TU (during active disease) for diagnostic purposes (6...
males and 2 females, aged 15–49 yr). Aqueous humor specimens were obtained also from 13 patients undergoing extracapsular cataract extraction for senile cataract (5 males and 8 females, aged 46–82 yr). All patients and controls were informed about these investigations, and their consent was obtained. Every precaution was taken at the time of each anterior chamber tap to ensure that the aqueous was not contaminated with blood and to avoid contact with corneal endothelium, iris, or lens. The aqueous samples were stored at −40°C until use. Cells were not removed from the aqueous prior to freezing. In each patient 10 ml serum was obtained by venepuncture at the same time the aqueous was aspirated.

**IL-6 Bioassay**

IL-6 levels were measured by a [3H]-thymidine ([3H]d-Thd) uptake assay using the murine hybridoma cell line B9, essentially as described by Aarden et al. Briefly, samples to be tested were titrated in 2-fold dilutions starting at 1:20 dilution, and 100 μl aliquots were placed in 96-well flat bottomed culture plates (Nunc, Roskilde, Denmark) in triplicate. All sera were heat-inactivated (56°C, 30 min) prior to testing. For aqueous humor samples this was found to be unnecessary. (Washed B9 cells were added per well 5×10^3) in 100 μl RPMI 1640 medium supplemented with 25 mM HEPES, 5% (v/v) fetal calf serum, 50 μM 2-mercaptoethanol, and penicillin/streptomycin. After 72 hr of culturing in a humidified incubator at 37°C and 5% CO₂, cells were labeled by a 4-hr pulse with 0.5 μCi [3H]d-Thd (Amersham, Amersham, UK); nuclei were harvested; and incorporated radioactivity was counted. In each experiment values were related to a diluted standard preparation of recombinant IL-6 (rIL-6); 1 U/ml is the rIL-6 concentration that leads to half-maximal ([3H]d-Thd incorporation in the assay.

Statistical analysis was performed using the Mann Whitney U test.

**Results**

These are summarized in Table 1 and Figure 1. Ten of 16 patients with FHC and 5 of 8 of the TU group had raised levels of IL-6 in the aqueous. The mean (±2 SD) of those samples with elevated levels were 543 (±1775) and 19,228 (±50,078) U/ml for the FHC and TU groups, respectively. IL-6 was not detected in the aqueous in any of the control group. The level of IL-6 in the serum of each patient and control was <10 U/ml. Statistical analysis showed a significant difference (P < 0.01) in the aqueous IL-6 levels in both the FHC and TU groups as compared to controls. To check the specificity of the IL-6-like activity in aqueous humor, samples containing IL-6 at concentrations higher than 10,000 U/ml were preincubated for 30 min at room temperature with an equal volume of heat-inactivated (30 min, 56°C) rabbit antiserum against human rIL-6, diluted 1:25 in phosphate buffered saline (PBS) pH 7.4 or with PBS alone, prior to the B9 assay. This procedure demonstrated that rabbit antibodies neutralized all IL-6-like activity in aqueous humor.

**Discussion**

To our knowledge this is the first report of IL-6 measurement in the eye. The finding of raised levels of IL-6 in the aqueous of uveitis patients adds further evidence to the theory of disturbed immune homeostasis in this group of conditions. The low levels of IL-6 (<10 units/ml) found in all serum samples indicate that the high aqueous IL-6 levels seen in this study have not resulted from breakdown of the blood–aqueous barrier and subsequent serum leakage. The mean aqueous IL-6 value in the TU group is much larger than that of the FHC group. This may be related to the difference in inflammatory activity between the two groups. Unfortunately, because of lack of clinical details, it was not possible to determine whether there was anterior chamber (AC) activity in two patients of the TU group. Of the other six patients, three had a posterior and three a panuveitis. The highest aqueous IL-6 levels (41,600 and 51,200 U/ml) were seen in two of the patients with panuveitis. The highest IL-6 level (3000 U/ml) in the FHC group was in a patient with uncontrollable glaucoma who had recently undergone unsuccessful drainage surgery and who, compared to the other FHC patients, had a moderate amount of AC activity. None of the other FHC patients with raised IL-6 levels had values higher than 750 U/ml. Although the numbers of patients in each group are very small (thereby not allowing statistical comparison), it appears that the degree of AC activity may be associated with the level of IL-6. No association between the age of the patient

**Table 1. IL-6 levels in uveitis**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Number (n = 37)</th>
<th>Raised aqueous IL-6 (%)</th>
<th>Raised serum IL-6 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fuchs' heterochromic cyclitis</td>
<td>16</td>
<td>63*</td>
<td>0</td>
</tr>
<tr>
<td>Toxoplasma uveitis</td>
<td>8</td>
<td>63*</td>
<td>0</td>
</tr>
<tr>
<td>Controls</td>
<td>13</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*P < 0.01.
and IL-6 level could be made in either group. None of the FHC patients was receiving systemic steroids, and only one was applying topical steroid (the patient mentioned above), but details of therapy in the TU group are not known.

Although we cannot exclude the possibility that some of the IL-6 measured in aqueous humor was released from intracellular pools of dead mononuclear cells, this is unlikely to be an important contributing source of IL-6. Others have shown the maximum number of mononuclear cells in aqueous of patients with active ocular toxoplasmosis to be 150,000/ml.\textsuperscript{21} The 750 or fewer cells present in the 5 μl of aqueous tested probably did not contribute significantly to the minimum concentration of IL-6 (10 pg/ml) required in the B9 assay.

Although the exact immunopathogenic mechanisms in uveitis are still understood only poorly, it is apparent that cytokines play a part. Recent studies\textsuperscript{22,23} have shown that Interleukin-2 and gamma interferon are present in the eye during inflammation, the latter cytokine being associated with the induction of class II antigens in the iris.

There is increasing evidence that TNFα, IL-1, and IL-6 interact in a complex network.\textsuperscript{4} Administration of endotoxin, a well-known inducer of TNFα in vitro, to volunteers leads to increased circulating TNFα as well as of IL-6.\textsuperscript{16,24} Furthermore, treatment of cancer patients with rTNF infusion also induces circulating IL-6.\textsuperscript{14} These results suggest the possibility of a "cascade" reaction in which TNF serves as an "intermediate" between the endotoxin-stimulus and the IL-6 response. Since IL-1 is also a potent inducer of IL-6 in vitro\textsuperscript{11,12} and since TNFα stimulates the production of IL-1 in monocytes, endothelial cells, and fibroblasts,\textsuperscript{25} one could speculate that IL-1 is also part of this cascade. Both IL-1 and TNFα have been shown to induce an acute ocular inflammatory response when injected into rabbit eyes.\textsuperscript{26-29} In view of the above, the possibility has to be considered that these cytokines induced the uveitis indirectly, i.e., by stimulating local production of endogenous IL-6. On the other hand, it is possible that the production of IL-6 is an end event of this cascade, and that the presence of IL-6 itself is not required for the induction of uveitis. Additional evidence as to the significance of IL-6 in intraocular inflammation has come from animal studies. We have recently shown that injection of endotoxin into the footpad of rats results in significant IL-6 levels in the circulation and even higher levels in the eye (manuscript submitted). Also, the inability of the eye to develop uveitis after repeated injections of endotoxin (tolerance) is associated with the selective absence of IL-6 in the eye although it is still detectable in the serum (manuscript submitted). Therefore, the ability or inability to develop uveitis appears to be related to the presence or absence of IL-6 in the eye.

The effect of IL-6 in the aqueous in FHC may be to stimulate specific B-cell clones in the eye. There is good evidence to support this theory; local production of IgG and raised immune complexes in the aqueous have previously been reported in this condition.\textsuperscript{30,31} We have found evidence of both IgG oligoclonal bands and a relative increase of IgG1 in the aqueous in FHC (manuscript submitted). Also, the presence of increased numbers of mast cells seen in iris biopsies in FHC\textsuperscript{32} may be related to IL-6 production: a recent report has shown the presence of TNF in mast cells.\textsuperscript{33} How IL-6 is involved with TU also is unclear. The relationship between the parasite and the production of IL-6 in the eye needs further investigation. The IL-6 response may be due directly to the
parasite, or through an immune response against the parasite.

Our results indicate that IL-6 may be a mediator in intraocular inflammation. Further studies on IL-6 levels in other uveitis syndromes are in progress, and this may provide additional information about how IL-6 contributes to the pathogenesis of uveitis.

**Key words:** interleukin-6, bioassay, aqueous humor, heterochromic cyclitis, toxoplasma uveitis

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**References**