Increased CD4⁺ Expression and Decreased IL-10 in the Anterior Chamber in Idiopathic Uveitis

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Purpose. To compare cell types and cytokines in aqueous humor from patients with uveitis either occurring in association with a systemic disease or apparently isolated and not associated with a systemic disease.

Methods. Cells were collected by centrifugation of fresh aqueous humor from uveitis and controls, and immunofluorescence techniques were performed with markers for T cells, B cells, and monocytes. Cytokines were measured in the aqueous supernatants, and serum samples were assayed for soluble interleukin-2 receptors.

Results. When aqueous samples from idiopathic uveitis were compared with those from uveitis associated with a systemic disease, there were increases in CD3⁺, CD4⁺ (p = 0.001), and activated CD4⁺ T cells (p = 0.02) and a decrease in B cells (p = 0.0013). This was not reflected in the peripheral blood where there were no differences in the cell types or in soluble interleukin-2 receptor levels. No cells were obtainable from control aqueous. Interleukins-10 and -12, interferon-γ, and transforming growth factor-β2 were detected in aqueous supernatants. Interleukin-10 was reduced (p = 0.024) in uveitis in comparison with controls.

Conclusions. The results suggest a selective recruitment of CD4⁺ T cells within aqueous humor but only in idiopathic uveitis. In both disease groups there was a decrease in the immunoregulatory cytokine interleukin-10, which might enable an immune response to occur in an otherwise highly immunosuppressive microenvironment. Increases in activated CD4⁺ T cells combined with depressed interleukin-10 levels could partially explain why, for example, in acute anterior uveitis, the inflammatory disease is often more severe.

Uveitis is an intraocular inflammatory disease, leading to blindness in many cases that can occur either as an idiopathic disease (i.e., there are no systemic features on questioning or on investigation) or in association with a systemic disease such as sarcoidosis or Behcet’s disease. Although posterior uveitis (affecting the posterior segment) describes a range of different clinical entities, all forms are similar immunohistologically, characterized by an infiltration of mainly CD4⁺ T cells. Cyclosporin A (CsA) can be effective in arresting the disease progression in many cases, highlighting the importance of T cells. The ability to adoptively transfer disease using activated retinal antigen-specific CD4⁺ T cells in an experimental model is further evidence for CD4⁺ T cell-mediated processes inducing the irreversible destruction of the photoreceptor cells of the retina. Posterior uveitis in humans is therefore considered to be a T cell-mediated autoimmune disease, although the immunopathogenic mechanisms remain unclear. Anterior uveitis (affecting the anterior segment), on the other hand, can be associated with HLA-B27 where it is thought to have an infectious etiology. However, the role of T cells in non-HLA-B27 anterior uveitis is less well understood.

It is not known whether the immune processes in idiopathic uveitis are identical to those in which a systemic immune disease is also occurring, because where there is an associated systemic disease, the ocular inflammatory activity does not necessarily parallel systemic inflammation. A previous study reported a positive correlation between lymphocyte activation, as measured by interleukin-2 receptor (IL-2R) expression, and clinical uveitis activity, and it was suggested that in some cases of idiopathic uveitis, there was abnormal systemic immune activation. In another study comparing T cells from idiopathic posterior uveitis with those from posterior uveitis associated with a systemic disease, a significant increase in IL-2Ra chain expression was demonstrated on CD4⁺ T cells in the blood in uveitis groups in comparison with healthy controls (p < 0.05). In contrast, only those uveitis patients with a systemic disease showed significant increases in IL-2R expression on the CD8⁺ T cells compared with controls.

The aim of the present study, therefore, was to find out whether there are intrinsic differences in the immune cell types, cytokines, or both within the aqueous humor, the fluid from the anterior segment of the eye, comparing idiopathic uveitis with uveitis associated with a systemic disease and with controls. The cell subsets and cytokines within the aqueous humor were investigated and soluble IL-2R (sIL-2R) levels in serum samples were measured as a marker of peripheral immune activation.
METHODS

Patients

Patients were recruited from the uveitis clinic at Moorfields Eye Hospital and aqueous humor (AH), and blood samples were only obtained after informed consent and Ethics Committee approval. Nineteen patients (mean age, 43.2 ± 3.5 years) with idiopathic uveitis were diagnosed as either having panuveitis (n = 5) or idiopathic acute anterior uveitis (AAU, n = 14) after detailed questioning and appropriate investigations were all negative. Eleven of these patients were not receiving therapy at the time of sampling, 6 received topical steroids, and another 2 were receiving prednisolone. In addition, 13 uveitis patients (mean age, 40.9 ± 3.5 years) with an associated systemic disease comprised of 7 AAU (4 ankylosing spondylitis, 1 multiple sclerosis, 1 Wegener’s Granulomatosis, 1 Reiter’s disease), 1 panuveitis, 4 sarcoidosis, and 1 Behcet’s disease. Of these, 5 patients were receiving medication at the time of sampling. For the sIL-2R study, 370 patients’ serum samples were collected. Within this group 248 had uveitis without systemic disease, and the remaining 122 had uveitis associated with systemic disease. One hundred of these patients were on combined therapy of CsA and steroids at the time of sampling. Serum samples were also collected from 26 healthy donors.

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All the studies involving human subjects were conducted according to the tenets of the Declaration of Helsinki.

AH Sampling Procedure

The anterior chamber paracentesis was carried out using a 30-gauge needle, on a 1-ml insulin syringe, via the temporal limbal approach and a rolling technique. AH (100–200 μl) was aspirated and immediately aliquoted into 3 microfuge tubes precoated with EDTA to prevent cell clumping.

The AH samples were centrifuged at 400 g for 5 minutes at 4°C. Supernatants were stored at −70°C until assayed for cytokines by enzyme-linked immunosorbent assay (ELISA).

Immunofluorescence

The cell pellets were washed twice in phosphate-buffered saline (PBS) before being resuspended in 15 to 20 μl PBS. In parallel, 100 μl of anticoagulated peripheral blood (PB) was added to 2 ml Lysis buffer (Becton Dickinson, Oxford, UK) for 10 to 20 minutes at room temperature to remove red blood cells. PB cells were then washed at least twice before staining. Three color staining was performed using directly conjugated monoclonal antibodies (mAbs), in three combinations: Tube A contained isotype-matched control mAbs, B contained CD3 (Pan T)/CD14 (monocytes)/CD19 (B cells), and C contained CD4/CD8/CD25 (IL-2R; all from Becton Dickinson). Briefly, cells were incubated with mAbs for 45 to 60 minutes in the dark on ice. Cells were then washed twice with PBS and fixed in 0.4% paraformaldehyde and stored at 4°C in the dark until acquired for flow cytometric analysis. Triple color immunofluorescence was analyzed using a FACScan flow cytometer (Becton Dickinson) equipped with a 15-mW argon laser and filter settings for fluorescein isothiocyanate (530 nm), PE (phycoerythrin; 585 nm), and PerCP (peridinin chlorophyll protein; >650 nm). Compensation settings were obtained from single, double, and triple color-staining controls. For every sample, 5000 events were acquired, resulting in 1500 to 2000 events gated within the lymphocyte region (R1).

Cytokine Determination

Cytokines in the AH were measured using commercially available sandwich ELISA kits specific for each human cytokine (Quantikine, R&D Systems, Abingdon, UK). To reduce the effect of other proteins in the AH which might be inhibitory, every sample was diluted 5 to 20 fold, depending on the cytokine. The minimum detectable concentrations of each cytokine were as follows: IL-4, IL-12, interferon-γ (IFN-γ), 3.0 pg/ml; IL-10, 1.5 pg/ml; tumor necrosis factor-α (TNF-α), 4.4 pg/ml; and transforming growth factor-β (TGF-β2), 7 pg/ml. For detecting TGF-β2 in the AH, acidification was carried out. In all assays, OD_{550nm} values obtained from diluent negative controls were subtracted from all readings (samples and standards), before making standard curves from which to calculate sample concentrations. For determining the sIL-2R levels in the serum samples, a commercially available ELISA was used (T Cell Sciences, Cambridge, MA) to detect 78.1 to 5000 pg/ml with a minimum detection level of 6 pg/ml.

Statistics

Statistical analysis of the results was performed with nonparametric methods (Mann–Whitney U test and Pearson correlation coefficient). Results for the sIL-2R levels are expressed as median value ± 95% confidence interval (CI).

RESULTS

Cell Types in AH

No lymphocytes were detectable in any of the AH samples from the noninflammatory control group (n = 10). The AH and PB from 19 patients with idiopathic disease and from 13 uveitis patients with an associated systemic disease were examined for the percentages of B cells (CD19), T cells (CD3), and CD4 and CD8 T-cell subsets and activated T cells (CD25) within the lymphocyte region (R1; Fig. 1). In addition, the percentages of monocytes (CD14) were compared within the total gated population of all cells. Significant increases in the expression of CD3 (p = 0.0001), CD4 (p = 0.0001), and activated CD4 (p = 0.02), but not CD8 or activated CD8, were observed in the AH compared with PB in idiopathic uveitis (Fig. 2). There was a decrease in CD19 (B cells) expression in AH from the idiopathic uveitis group compared with PB (p = 0.0013), but no differences in expression of CD14 were detected. In contrast, when the AH was compared with PB in the uveitis group associated with a systemic disease, there were no significant differences in the expression of CD3, CD4, and CD8. Among the AH sample groups, the only significant difference was an increase in activated CD4^{+} T cells (CD25-expressing) in AH from idiopathic uveitis compared with those in uveitis associated with systemic disease (p = 0.02), although all levels were low (<10%). In PB there were no differences in expression of any cell-surface markers when the uveitis groups were compared with controls (n = 8).
Cytokines in AH

The AH samples from 10 controls, from 12 uveitis patients with an associated systemic disease, and from 8 idiopathic uveitis patients were examined for IL-2, IL-4, IL-10, IL-12, IFN-γ, TNF-α, and TGF-β2. Of these, only IL-10, IL-12, IFN-γ, and TGF-β2 were present at detectable levels (Fig. 3). In comparison with control AH, there were no differences in levels of IL-12, IFN-γ, and TGF-β2. However, the levels of IL-10 were significantly decreased for uveitis associated with a systemic disease ($p < 0.049$) and idiopathic uveitis ($p < 0.024$), compared with noninflammatory controls. There was a positive correlation between IFN-γ and IL-12 in idiopathic uveitis ($r = 0.03$) and in uveitis associated with systemic disease ($r = 0.12$). A positive correlation was also observed between IL-10 and TGF-β2 in idiopathic uveitis ($r = 0.78$) but not in uveitis associated with systemic disease ($r = -0.58$; Table 1). In contrast, negative correlations were found for IFN-γ and TGF-β2 in idiopathic uveitis and in uveitis associated with systemic disease ($r = -0.07$ and $-0.08$, respectively). Interestingly, there was a positive correlation between IFN-γ and IL-10 in idiopathic uveitis ($r = 0.08$) but not in uveitis associated with systemic disease ($r = -0.08$).

When T-cell subsets were compared with cytokine levels, a positive correlation was found for CD8 expression and IFN-γ ($r = 0.21$) in idiopathic disease, whereas the correlation was negative in uveitis associated with systemic disease ($r = -0.66$). There was also a positive correlation between the level of IL-10 and CD8 expression in uveitis associated with systemic disease ($r = 0.83$), which was not observed in idiopathic uveitis.

sIL-2R Levels in Serum

When idiopathic uveitis (95% CI, 390–450; median, 405) was compared with uveitis associated with a systemic disease (95% CI, 410–500; median, 450) and with controls (95% CI, 340–603; median, 365), no significant differences in the levels of sIL-2R were observed in the serum. Omitting those patients receiving steroids and CsA from the study did not affect the results.

DISCUSSION

In this study only patients with active disease were investigated, and the cell types within the AH are significantly differ-
ent when comparing uveitis and controls. In contrast, the PB cell types do not differ between the groups. One explanation for this is that the group of uveitis patients with an associated systemic disease, comprising both anterior and posterior forms of uveitis, is too diverse to permit detection of any minor alterations in cell phenotypes within the blood. Alternatively, the immune processes occurring within the eye are too localized to be reflected in the PB population. The sIL-2R levels in serum obtained from the disease groups did not differ, suggesting that serum is not the ideal site for studying immune abnormalities in intraocular inflammatory diseases because it does not reflect the immune processes occurring within the eye. We have previously reported significant increases in activated CD4<sup>+</sup> and CD8<sup>+</sup> T cells in PB in posterior uveitis compared with controls. However, that patient population comprised 70 cases of posterior uveitis and was therefore different from the present study, which might explain the conflicting results.

The cellular phenotypes in the AH were recently compared in two distinct forms of idiopathic anterior uveitis: the more benign form (Fuchs' heterochromic cyclitis) and the more aggressive idiopathic anterior uveitis. It was found that CD4<sup>+</sup> T cells were the main T-cell subset in the AH of patients with idiopathic anterior uveitis in contrast to Fuchs', in which CD8<sup>+</sup> T cells predominated. This suggested that CD4<sup>+</sup> T cells are upregulated within the anterior chamber during severe forms of anterior uveitis, whereas CD8<sup>+</sup> T cells are more evident in benign disease, perhaps in downregulating immune responsiveness. In the present study there was a selective increase in CD4<sup>+</sup> T cells in the AH during idiopathic disease, which again supports the hypothesis that CD4<sup>+</sup> T cells are involved in more aggressive forms of disease, because inflammation in idiopathic uveitis is often clinically more severe than in uveitis associated with a systemic disease. In addition, the percentage of B cells (expressing CD19) is

![Figure 2. Histogram of mean percentage expression ± SEM of T-cell subsets (CD3, CD4, CD4/CD25, CD8), B cells (CD19), and monocytes (CD14), as determined by flow cytometry. Aqueous humor and PB were compared from uveitis associated with a systemic disease (AHs; PBs; n = 13), idiopathic uveitis (AHi; PBi; n = 19), and noninflammatory controls (AHc; PBc; n = 10).]
lower in the AH in idiopathic uveitis, agreeing with previous immunohistochemical studies. In uveitis associated with a systemic disease, it is unclear whether the T cells enter the eye as part of a peripheral immune response, whether there is a specific mechanism attracting T cells to the eye, or whether there is a passive extravasation of cells into the eye during a breakdown of the blood-retinal barrier. To address some of these questions in another multisystem immune disorder, sarcoidosis, research has focused on comparing immunologic abnormalities within the different immune compartments (bronchoalveolar lavage fluid, AH, PB). In one study, T-cell clones were established from bronchoalveolar lavage fluid, PB, and transbronchial biopsies, and their T-cell receptor (TCR) repertoire compared. It was found that different TCR V beta genes were used by the T cells in each compartment, suggesting that they were independent of each other. However, others have failed to detect restricted TCR usage in lung or PB from sarcoid, and it is therefore still unclear whether antigen-driven T-cell activation occurs independently in each immune compartment.

There were significant decreases in IL-10 in the AH samples in both uveitis groups compared with controls. There was a positive correlation between IL-10 and TGF-β2, suggesting that within the anterior segment of the eye, these cytokines are...
cooperating. It is important to note that this cytokine association was only found in idiopathic uveitis and not in uveitis associated with systemic disease. There was also a positive correlation between CD8 and IFN-γ in idiopathic disease and between CD8 and IL-10 in uveitis associated with a systemic disease, suggesting that different immune processes are occurring in the two disease groups. Cytokines such as IL-10 and TGF-β2 are thought to play important roles in the immunoregulation of disease processes due to their inhibitory effects on cell functions (T cells, monocytes) and on proinflammatory cytokines such as TNF-α. Thus, the effect of IL-10 and other cytokines on the T cells within the AH needs to be thoroughly investigated if cytokine immunotherapy is to be applied in the future.

### Acknowledgments

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### References


### Table 1.

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Values given as picograms per milliliter. Pearson test for correlation resulted in a positive correlation for IL-10 and TGF-β2 (r = 0.78).