Chemoattractant and Neutrophil Degranulation Activities Related to Interleukin-8 in Vitreous Fluid in Uveitis and Vitreoretinal Disorders

Joke H. de Boer,* C. Erik Hack,† Arthur J. Verhoeven,‡ G. Seerp Baarsma,‡ Paulus T. V. M. de Jong,§ A. Jos J. M. Rademakers,‖ Willemien A. E. J. de Vries-Knoppert,¶ Aniki Rothova,# and Aize Kijlstra*

Purpose. To investigate whether the cytokine interleukin-8 (IL-8), a strong chemoattractant and activator for neutrophils, is responsible for neutrophil infiltration and degranulation in the eye in uveitis.

Methods. IL-8 and elastase were measured with specific enzyme-linked immunoassays in vitreous fluid samples obtained from 69 patients with various uveitis entities. Vitreous fluid of nonuveitis patients and eye bank eyes served as controls. The chemotactic activity of vitreous fluid was tested with the Boyden chamber technique.

Results. IL-8 was detected in 45% of the vitreous fluid samples from uveitis patients and in 26% of vitreous fluid samples from nonuveitis patients. Vitreous fluid samples with IL-8 levels exceeding 100 pg/ml were chemotactic for neutrophils. This chemotactic activity could be blocked by 41% to 79% with a monoclonal anti-IL-8 antibody. Elastase levels in vitreous fluid of uveitis patients with detectable IL-8 were significantly higher than those in vitreous fluid samples with no detectable IL-8.

Conclusion. These results indicate that IL-8 participates in the inflammatory processes in the eye by attracting and degranulating neutrophils. It is suggested that these processes contribute to the pathogenesis of tissue destruction in uveitis. Invest Ophthalmol Vis Sci. 1993;34: 3376–3385.

Uveitis or intraocular inflammation often results in impairment of vision. Although T cells are generally considered to be the key mediators in the pathogenesis of uveitis, it remains unknown which cells mediate the tissue damage in this disease. In various noninfectious inflammatory disorders such as autoimmune diseases and ischemic injuries, neutrophils have been implicated as mediators of tissue destruction. Neutrophils are able to release an array of agents such as the serine proteinase elastase and the two metallopro-

From *The Netherlands Ophthalmic Research Institute, Amsterdam, the Central Laboratory of the Netherlands Red Cross Bloodtransfusion Service, Amsterdam, †Eye Hospital, Rotterdam, ‡Department of Ophthalmology, Erasmus University, Rotterdam, §Department of Ophthalmology, Catholic University, Nijmegen, ¶Department of Ophthalmology, Free University, Amsterdam, #Department of Ophthalmology, University of Amsterdam, The Netherlands. Submitted for publication January 21, 1992; accepted April 27, 1993.

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measured the chemotactic activity of these VF samples and assessed to what proportion IL-8 contributed to this activity. Our results are consistent with the hypothesis that IL-8 participates in the inflammatory process of clinical uveitis as a chemoattractant and activator for neutrophils in VF.

METHODS

Patients

A diagnosis of uveitis was made according to the criteria of the International Uveitis Study Group.12 Paired VF samples (n = 69) and serum samples (n = 66) from uveitis patients were collected. From three uveitis patients only a VF sample was available. The samples were obtained during a therapeutic or diagnostic vitrectomy. The indications for a therapeutic vitrectomy were visual deterioration caused by vitreous opacities or progressive inflammation despite medical treatment. A diagnostic vitrectomy was performed in a few patients suspected of having an ocular lymphoma. Paired VF and serum samples from patients without uveitis undergoing a therapeutic vitrectomy (n = 23) and VF from eye bank eyes (n = 28; mean postmortem time 17 hours) were studied as controls. Undiluted VF samples were collected during a pars plana vitrectomy before the infusion line was opened. Blood samples were collected the same day the vitrectomy was performed, allowed to clot for 1 hour and centrifuged for 10 minutes at 1500g to obtain the serum. VF and serum samples were stored at −20°C until use.

The diagnosis of the nonuveitis patients included proliferative diabetic retinopathy (PDR, n = 9), vitreous hemorrhage of unknown cause (n = 4), proliferative vitreoretinopathy (PVR, n = 5), macular hole (n = 1), occlusion of the retinal vein (n = 1), complicated cataract surgery (n = 2), vitreal degeneration (n = 1). The exclusion criteria for VF from eye bank eyes were eyes from donors seropositive for human immunodeficiency virus, hepatitis C, neuroviral diseases, and sepsis.

IL-8 Assay

IL-8 was assessed with an enzyme-linked immunoassay (ELISA) using two monoclonal anti-human IL-8 antibodies. The preparation of these monoclonal antibodies and the development of the IL-8 ELISA has been described elsewhere.13 Briefly, ELISA plates (Nunc, Roskilde, Denmark) were coated overnight with monoclonal antibody anti-IL-8-1 (Central Laboratory of the Netherlands Red Cross blood transfusion service (CLB, Amsterdam). After washing, 100 µl of the sample dilutions together with 10 µl of biotinylated monoclonal anti-IL-8-4 (CLB; diluted in buffer containing 10% [v/v] of a 1:1 mixture of normal bovine and sheep serum, to avoid false-positive reactions) were added in each well and incubated for 2 hours. Then the plates were developed with streptavidin-horseradish peroxidase (Amersham Buckinghamshire, UK) followed by a signal-amplification step with catalyzed reporter deposition14 and, finally 3,5,3',5'-tetramethylbenzidine (Merck, Darmstadt, Germany) as a substrate.

Briefly, purified human recombinant IL-8 (rIL-8) obtained by transfecting Escherichia coli DH5 with the plasmid pMBL11 (British Biotechnology Ltd, Oxford, UK) was used as a standard and results were expressed in picograms per milliliter.15 All samples were tested in serial dilutions, starting at a 1:2 dilution in phosphate buffered saline, pH 7.4, supplemented with 0.02% Tween (w/v) and 0.2% gelatin (w/v). The concentration of IL-8 in samples was calculated by comparing the absorption of samples with that of serial dilutions of human rIL-8. The detection limit of the assay was 10 pg/ml. To exclude if TF interfered with the ELISA, two different concentrations of rIL-8 (125 pg/ml and 31.5 pg/ml) were added to different dilutions of VF samples (starting 1:2) of uveitis patients (n = 2) with IL-8 levels <10 pg/ml and raised elastase levels (181 ng/ml and 111 ng/ml), and VF of eye bank eyes (n = 2). The IL-8 ELISA was not inhibited by the VF dilutions tested.

Chemotaxis

The chemotactic activity of VF for neutrophils was measured with a modified Boyden chamber technique.15 The two compartments of the chamber were separated by two filters. The upper filter has a pore width of 8 µm (Sartorius AG, Germany) and the lower filter has a pore width of 0.45 µm (Millipore, Ireland). Samples were diluted in complete HEPES medium (containing 132 mM NaCl, 6 mM KCl, 1.2 mM CaCl2, 1 mM MgSO4, 1 mM CaCl2, 5 mM glucose, supplemented with 5% [w/v] human serum albumin and heparin 10 IU/ml). Serial sample dilutions, starting undiluted or 1:2, were placed in the lower compartment of the chamber. Neutrophils 105, 95% to 99% pure, isolated by Percoll (density 1.076, Pharmacia, Uppsala, Sweden) density centrifugation from a healthy donor,15 were placed in the upper compartment. The Boyden chamber was then incubated for 90 minutes at 37°C in a moist chamber. Thereafter the filters were removed and the 8-µm filters were fixed in butanol/ethanol (20/80, v/v) for 10 minutes and stained with Weigert solution (1% hematoxylin (v/v) in 95% ethanol (v/v) and an acidic FeCl3 solution (70 mM) in a volume ratio of 1:1). The filters were dehydrated with ethanol and made transparent in xylene. The filters were fixed upside down in malinol. The number of cells per field that passed the filter were determined by light microscopy. Human rIL-8 (10−8 M and 10−9 M) was used as a positive control. Controls included ran-
dom neutrophil migration in the presence of medium alone and VF samples of eye bank eyes (n = 2). VF samples of uveitis patients with detectable IL-8 (n = 9) and with IL-8 levels less than 10 pg/ml (n = 6) were tested in duplicate at different dilutions starting undiluted or 1:2 in medium. The same diluted experimental samples were also incubated with anti-IL-8-1 (CLB) antibody at a concentration of 2 μg/ml immunoglobulin G, a concentration that inhibits 80% of the chemotactic activity of 10^{-8} M human rIL-8.

**Elastase Assay**

Elastase in VF was measured with a capture ELISA using polyclonal rabbit anti-human elastase antibodies. These antibodies were prepared by immunization with purified human neutrophil elastase (Elastin Products Co. Inc., Pacific, MO). ELISA plates were coated overnight with 100 μl of anti-elastase antibodies at a concentration of 1.5 μg/ml in phosphate-buffered saline. One hundred microliters of serial dilutions of samples in 0.02% Tween (v/v) and 0.2% gelatin (w/v) were added to the wells and incubated for one hour at 37 °C. After washing, biotinylated polyclonal rabbit anti-human elastase antibodies were added at the optimal concentration in the presence of 1% (v/v) of a 9:1 mixture of normal rat and rabbit serum (to prevent nonspecific binding) and incubated for 1 hour. After washing, streptavidin-horseradish peroxidase was added diluted 1:1000 in 0.02% Tween (w/v) and 0.2% gelatin (w/v). 3,5,3',5'-tetramethylbenzidin was used as a substrate and the reaction was stopped with 100 μl of 2 M H_{2}SO_{4}. The absorbency of the reaction product was measured in a Titertek Multiskan Plus (Biomedical, Costa Mesa, CA) at 450 nm. Elastase levels were calculated by comparison with a standard curve of purified human neutrophil elastase (Fig. 1). Controls included coating with nonspecific rabbit immunoglobulin G. The detection limit of this assay was 2.5 ng/ml elastase.

**IL-6 Measurements**

IL-6 in VF was measured using an ELISA performed as described by Helle et al. A monoclonal anti-human IL-6 antibody (mAB CLB IL-6/16, donated by Dr. L.A. Aarden from the Central Laboratory of the Netherlands Red Cross blood transfusion Service) was used as a coating capture antibody. Polyclonal biotinylated sheep anti-human IL-6 immunoglobulin G (CLB) was used as a detection antibody. The detection limit of this assay was 10 pg/ml. Results with experimental samples were related to a standard curve of recombinant IL-6, and expressed in picograms per milliliter.

**Miscellaneous**

Albumin levels in serum and VF were measured by radial immunodiffusion (LP-Partigen-Albumin, Behring [Marburg, Germany]). The concentrations were calculated from a standard curve using a human reference serum (HOO-05, CLB). For calculation of the

![Figure 1](attachment:image.png)

*Figure 1.* Dose-response curve of human neutrophilic elastase in the ELISA with polyclonal rabbit anti-human elastase antibodies. Data show the mean and SD (bar) of eight experiments.
relative concentration ratio (RCR) the following equation was used:

\[
\text{vitreous IL-8 : serum IL-8} \div \text{vitreous albumin : serum albumin} \quad (1) \text{RCR}
\]

**Statistical Analysis**

Statistical analysis of the data was performed by using the SPSS statistical software package (SPSS Inc., Chicago, IL.). The differences among multiple patient groups were assessed with the nonparametric Kruskal-Wallis test. Differences between two patients groups were estimated with the nonparametric Mann-Whitney test or Chi-squared test using the Bonferroni method to correct for multiple comparison. Correlation coefficients were determined with the nonparametric Spearman test on log transformed data.

**RESULTS**

**IL-8 Levels**

IL-8 levels (10 pg/ml) were detected in 31 of 69 (45%) VF samples of uveitis patients (median of 31 samples = 33 pg/ml; range 10 to 1830 pg/ml, Fig. 2). In VF samples of patients with noninflammatory eye diseases, IL-8 levels were elevated in six of 23 samples (26%; median of 6 samples = 25 pg/ml; range 10 to 116 pg/ml). Only one of 28 (4%) VF fluid samples obtained from eye bank eyes had an increased IL-8 value (20 pg/ml). IL-8 in VF fluid from uveitis patients was significantly higher than that in VF fluid of eye bank eyes (\(P < 0.0001\)) and tended to be higher than that in VF fluid from patients with vitreoretinal disorders although this latter difference was not significant (Fig. 2). Circulating IL-8 was raised in 20 of 66 serum samples of uveitis patients (30%; median of 20 samples = 42 pg/ml; range 10 to 1329 pg/ml) and in one of 23 samples of patients with noninflammatory eye diseases (4%; 17 pg/ml) (\(P < 0.01\)).

To investigate VF IL-8 levels among various uveitis entities, the patients were subdivided into subgroups according to the localization of inflammation in the eye and to cause (Table 1). Raised IL-8 levels were observed in various groups. There was no relationship between localization of the inflammation and the presence of IL-8 in VF, but there were significantly more patients with IL-8 in serum in the total group with anterior or intermediate uveitis compared to posterior uveitis (\(P < 0.006\); \(P < 0.006\), respectively) or panuveitis (\(P < 0.0024\); \(P < 0.012\), respectively). In six of ten patients with acute retinal necrosis syndrome, IL-8 was detectable in the VF but could not be detected in the serum. Serum IL-8 was detected in 11 of 20 samples from patients with intermediate uveitis (\(P < 0.015\) versus patients with acute retinal necrosis). IL-8 could be detected in sera of all three patients positive for HLA B27, but only one of these patients had increased levels in VF. IL-8 levels were raised both in serum and VF in eight of the uveitis cases (Table 2). For these patients samples, the RCR was calculated\(^{18}\) using paired albumin levels as a reference. The RCR values ranged between 3 and 216. In two patients with severe albumin diffusion in the eye (10% and 50% of the serum value, respectively) and raised IL-8 levels in

![Figure 2](image-url) **FIGURE 2.** IL-8 levels in VF and sera from uveitis patients and nonuveitis patients. The broken line represents the detection limit of the assay.
TABLE 1. IL-8 Levels in Vitreous Fluid and Serum in Different Uveitis Entities

<table>
<thead>
<tr>
<th>Anatomical Diagnosis</th>
<th>Specific Diagnosis</th>
<th>Vitreous Fluid Samples With Increased IL-8</th>
<th>Serum Samples With Increased IL-8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. of Total</td>
<td>Median (pg/ml)</td>
</tr>
<tr>
<td>Anterior uveitis</td>
<td>HLA B27 related*</td>
<td>1/3</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>Anterior uveitis†</td>
<td>0/2</td>
<td>—</td>
</tr>
<tr>
<td>Intermediate uveitis</td>
<td>Intermediate uveitis†</td>
<td>7/21</td>
<td>21</td>
</tr>
<tr>
<td>Posterior uveitis</td>
<td>Toxoplasmosis</td>
<td>4/5</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Cytomegalovirus retinitis</td>
<td>0/2</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Toxocariasis</td>
<td>1/2</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>Sarcoidosis</td>
<td>0/1</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Posterior uveitis†</td>
<td>2/3</td>
<td>173</td>
</tr>
<tr>
<td></td>
<td>Masquerade syndrome§</td>
<td>3/4</td>
<td>22</td>
</tr>
<tr>
<td>Panuveitis</td>
<td>Acute retinal necrosis (ARN)</td>
<td>6/10</td>
<td>115</td>
</tr>
<tr>
<td></td>
<td>Fuchs' heterochromic cyclitis</td>
<td>0/2</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Mycotic endophthalmitis</td>
<td>1/1</td>
<td>272</td>
</tr>
<tr>
<td></td>
<td>Bechets disease</td>
<td>0/1</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Crohn's disease</td>
<td>1/1</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Panuveitis†</td>
<td>5/11</td>
<td>33</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>31/69</td>
<td>33</td>
</tr>
</tbody>
</table>

NP = not performed.
* One patient with Reiter's disease.
† Unknown origin.
‡ Difference in serum IL-8 levels between patients with intermediate uveitis and acute retinal necrosis is significant (P < 0.015).
§ Specific diagnosis: lymphoma (n = 3) and teratoid medullar epithelioma (n = 1).

serum (455 pg/ml and 944 pg/ml, respectively) no IL-8 was detected within the VF.

In the nonuveitis group, IL-8 was detected in the VF of three of nine PDR patients (median 13 pg/ml; range 10 to 76 pg/ml), in one of four patients with vitreous hemorrhage of unknown cause (23 pg/ml), in one patient with a retinal vein occlusion (27 pg/ml), and in one patient with PVR after perforating ocular trauma (116 pg/ml). IL-8 was slightly raised in one

serum (17 pg/ml) from a patient with PDR with no raised IL-8 in the VF.

No relationship was observed between IL-8 in VF or serum and age or sex of the patients. There was no evidence that IL-8 was related to duration of the disease or medical treatment. Most of the patients underwent surgery during a chronic stage of the disease except seven patients with acute retinal necrosis who underwent surgery within 4 months of disease onset. Six

TABLE 2. Albumin, IL-8 Levels, and Relative Concentration Ratio (RCR) in VF and Serum of Patients With Detectable IL-8 in Both Fluids

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>VF IL-8 (pg/ml)</th>
<th>Serum IL-8 (pg/ml)</th>
<th>VF Albumin (mg/ml)</th>
<th>Serum Albumin (mg/ml)</th>
<th>RCR*</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA B27 associated uveitis, Reiter's disease</td>
<td>44</td>
<td>48</td>
<td>6.90</td>
<td>48.2</td>
<td>6</td>
</tr>
<tr>
<td>Intermediate uveitis†</td>
<td>170</td>
<td>110</td>
<td>2.00</td>
<td>47.8</td>
<td>37</td>
</tr>
<tr>
<td>Intermediate uveitis†</td>
<td>37</td>
<td>75</td>
<td>2.16</td>
<td>46.0</td>
<td>11</td>
</tr>
<tr>
<td>Intermediate uveitis†</td>
<td>21</td>
<td>10</td>
<td>0.36</td>
<td>37.1</td>
<td>216</td>
</tr>
<tr>
<td>Intermediate uveitis†</td>
<td>15</td>
<td>172</td>
<td>1.00</td>
<td>39.4</td>
<td>3</td>
</tr>
<tr>
<td>Intermediate uveitis†</td>
<td>12</td>
<td>273</td>
<td>0.62</td>
<td>52.4</td>
<td>4</td>
</tr>
<tr>
<td>Posterior uveitis†</td>
<td>21</td>
<td>10</td>
<td>3.90</td>
<td>41.6</td>
<td>22</td>
</tr>
<tr>
<td>Panuveitis†</td>
<td>58</td>
<td>56</td>
<td>7.00</td>
<td>47.0</td>
<td>7</td>
</tr>
</tbody>
</table>

* The RCR value was calculated as follows: (vitreous IL-8/vitreous IL-8):(vitreous albumin/serum albumin).
† Unknown origin.
of them had IL-8 in VF. Fourteen patients were treated with systemic steroids at the time of vitrectomy and two of them had IL-8 in serum (455 pg/ml and 944 pg/ml). Half of the patients with raised IL-8 in VF were treated with corticosteroids systemically (n = 7), subconjunctivally (n = 3), or in eyedrop form (n = 6) at the time of vitrectomy.

Chemotactic Activity of VF Samples
To verify that the immunochemically detected IL-8 in VF was also biologically active, chemotactic activity of VF for neutrophils was investigated using the Boyden chamber technique. The mean random neutrophil migration induced by HEPES medium was nine cells per field (range 5 to 13 cells per field). The chemotactic activity of the two VF samples of eye bank eyes was 11 and 14 cells per field. In Figure 3 chemotactic activity of different dilutions of one VF sample with an IL-8 concentration of 325 pg/ml is shown. The dilution factor with the optimal chemotactic activity of VF samples of 15 patients is shown in Figure 4. The chemotactic activity of two VF samples increased after tenfold dilution of the samples. VF samples with IL-8 levels exceeding 100 pg/ml (n = 5) were all chemotactic for neutrophils whereas VF samples with IL-8 levels less than 100 pg/ml (n = 4) and VF samples with IL-8 levels less than 10 pg/ml (n = 6) did not attract neutrophils. Neutralization of the chemotactic activity of VF for neutrophil by incubating them with a monoclonal anti-IL-8 antibody varied between the five VF samples and ranked from 41% to 79%.

ELASTASE
Instead of studying the cytology of VF during intraocular inflammation, a neutrophil degranulation product, elastase, was measured as a marker for neutrophil activation (Table 3). An elastase level of 22 ng/ml (mean + 2 SD of elastase levels in VF of eye bank eyes) was used as the upper normal level for VF. Elastase levels were elevated in 28 of 66 VF samples from uveitis patients (42%) and in 7 of 21 VF samples of nonuveitis patients (33%). Elastase levels were significantly higher in VF of uveitis patients and nonuveitis patients than in that of eye bank eyes (P < 0.0005; P < 0.0015, respectively). Raised elastase levels in VF samples with detectable IL-8 were observed in 18 of 28 VF samples of uveitis patients (64%), in 3 of 6 VF samples of the nonuveitis group, and one VF sample of eye bank eyes (Fig. 5). Elastase levels in VF of uveitis patients with detectable IL-8 were significantly higher compared to elastase levels in VF samples without detectable IL-8 (P < 0.001).

IL-8 Versus IL-6 Levels
To investigate the possible interaction between different cytokines in VF, the intraocular IL-8 levels were compared with IL-6 levels. Both cytokine levels were measured in an immunochemical assay in 61 VF samples (Fig. 6). In the VF of uveitis patients, raised IL-8 levels were always accompanied by raised IL-6 levels but not vice versa. IL-6 levels were more frequently raised as compared to IL-8.

DISCUSSION
This study shows that IL-8 levels in VF are increased in 45% of the patients with uveitis and in 26% of the patients with noninflammatory eye diseases. To confirm that the immunochemically detected IL-8 in VF was biologically active, we performed chemotactic assays. VF samples of uveitis patients with an IL-8 level exceeding 100 pg/ml were chemotactic for neutrophils, an activity that could be blocked by a monoclonal anti-human IL-8 antibody. Only 10% of the uveitis VF samples and one VF sample from a patient with PVR had IL-8 levels in this range. Experimental animal models in the rat have shown that intravitreal injection of 100 pg to 10^3 ng of human rIL-8 induced cell infiltration into the eye, with an optimal effect seen at 10 ng.\(^{11}\) However, the concentration of IL-8 that affects the human eye might be different from that inducing ocular inflammation in the rat model. Furthermore, it is possible that the active in vivo level of IL-8 is significantly less than that required for a positive reaction in the Boyden chamber technique.
Low IL-8 levels in VF might participate in processes within the eye other than neutrophil recruitment, because it is known that the various biologic effects of IL-8 levels are concentration dependent, whereby low levels induce T cell chemotaxis, whereas higher levels are needed for neutrophil attraction.9

Different explanations are possible for the presence of IL-8 in VF in vitreoretinal disorders. PVR seems to be an exaggerated wound healing response after retinal detachment surgery or perforating trauma and neutrophil recruitment can participate in the elimination of necrotic tissue.19 Other inflammatory mediators than IL-8 and growth factors have already been demonstrated in VF of patients with PVR and PDR.19-21 It was also recently reported that IL-8 can induce corneal neovascularization in rabbits.22 We found IL-8 in the VF of three patients with PDR and in one patient with vitreous hemorrhage. Whether the angiogenic properties of IL-8 might play a role in retinal neovascularization deserves further investigation.

Unfortunately, no information was available about the number of infiltrating cells in VF, clinically or counted at vitrectomy. Instead of studying the cytology of the VF during intraocular inflammation, we measured a neutrophil degranulation product, elastase, as a marker for neutrophil activation in vivo.

### TABLE 3. Vitreous Fluid Elastase Levels in Uveitis, Noninflammatory Eye Diseases, and Eye Bank Eyes

<table>
<thead>
<tr>
<th>Samples</th>
<th>Median elastase (ng/ml)</th>
<th>Range (ng/ml)</th>
<th>Elastase &gt;22 ng/ml per No. of Samples Investigated (%)</th>
<th>IL-8 &gt;10 pg/ml per No. of Samples With Elastase &gt;22 ng/ml (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uveitis</td>
<td>15.5*</td>
<td>&lt;2.5–10,124</td>
<td>28/66 (42%)</td>
<td>18/28 (64%)</td>
</tr>
<tr>
<td>Nonuveitis</td>
<td>14.0*</td>
<td>&lt;2.5–86</td>
<td>7/21 (33%)</td>
<td>3/7 (43%)</td>
</tr>
<tr>
<td>Eye bank eyes</td>
<td>4.0*</td>
<td>&lt;2.5–31</td>
<td>2/28 (7%)</td>
<td>1/2</td>
</tr>
</tbody>
</table>

* Difference in elastase levels in VF of uveitis and nonuveitis patients compared with VF from eye bank eyes is significant (P < 0.0005 and P < 0.0015, respectively).
Elastase is a proteolytic enzyme considered to play a role as a mediator of tissue destruction. Comparable elastase levels were observed in VF of patients with uveitis and vitreoretinal disorders, which indicates that neutrophils participate in both eye diseases. The observation that elastase levels in VF with detectable IL-8 were significantly higher than in VF samples without IL-8 suggested that IL-8 is a chemoattractant and activator for neutrophils in VF. In 10 of the 28 uveitis samples (36%) and in 4 of the 7 nonuveitis samples with raised elastase levels no IL-8 was detectable. One explanation for this might be that IL-8 may have already been cleared from VF. Conversely, this raised elastase might be due to the presence of other neutrophil chemotactic factors, such as C5a or leukotriene B4, known to be released into the eye.\textsuperscript{23,24} Other elastase containing cells, for instance, monocytes and macrophages, that infiltrate in the eye during uveitis can theoretically be a source of elastase in VF. Monocytes only contain 6% of the amount of elastase of neutrophils and the proteolytic activity of macrophages is largely due to cysteine proteases.\textsuperscript{25,26} Eosinophils also contain elastase but these cells are not commonly involved in uveitis.\textsuperscript{27–29} Therefore, neutrophils may be regarded as the main source of elastase in VF. In the majority of patients with detectable IL-8 in VF, serum levels of IL-8 were normal, suggesting that IL-8 in VF of these patients reflected local production. Our data do not allow conclusions regarding the source of IL-8. The cytokine may have originated from resident ocular cells, such as retinal pigment epithelial cells, which have been shown to produce IL-8 in vitro after stimulation with IL-1 or TNF.\textsuperscript{30} It is also possible that IL-8 was produced by infiltrating cells. Eight patients had detectable IL-8 both in the eye and in the circulation (Table 2). For these patients we calculated the RCR to correct for changes in permeability of the blood vitreous barrier. The detection limit of the IL-8 assay was 10 pg/ml, which prevented us from calculating normal RCR values for IL-8 in VF and serum. It is therefore difficult to accurately estimate which RCR indicates intraocular production of IL-8. Furthermore, it is not clear whether IL-8 behaves in the same way as albumin in its diffusion behavior across the blood–retinal barrier. Two patients with an increased permeability of the blood–retinal barrier had raised IL-8 in serum but no detectable IL-8 in their eye. More studies concerning intraocular kinetics of cytokines, are warranted to clarify this issue.

Previous studies have already demonstrated that cytokines participate in the process of ocular inflammation.\textsuperscript{31} The cytokines TNF-\(\alpha\), IL-1\(\alpha/\beta\), IL-6, IL-8, and Interferon-\(\gamma\) have uveitogenic properties when injected intravitreally.\textsuperscript{11,32–35} Furthermore, the presence of the cytokines IL-2, IL-6, and IFN-\(\gamma\) has been demonstrated in ocular material obtained during clinical uveitis.\textsuperscript{36,22,37} In vitro studies have demonstrated that human retinal pigment epithelial cells produce IL-8 as well as IL-6 on stimulation with IL-1 or TNF-\(\alpha\).\textsuperscript{30,38,39} To investigate if a similar mechanism was operating in the in vivo situation, we compared IL-6 and IL-8 levels in human VF. Raised IL-8 levels were always accompanied by raised IL-6 levels but not vice versa (Fig. 5) and IL-6 levels were more frequently raised than IL-8 levels. This latter observation might be caused by differences in the regulatory processes of production and elimination of these cytokines during inflammation.

Raised IL-8 levels in VF and sera were detected in
different intraocular diseases, which suggest that IL-8 is a common inflammatory mediator within the eye (Table 2). In 60% of patients with acute retinal necrosis, IL-8 was detectable in VF but not in serum. Acute retinal necrosis is characterized by a severe necrotizing inflammation of the retina. It is caused by reactivation of latent neurotropic viruses of the herpes group and often leads to blindness. The observation that IL-8 is not systemically detectable in these patients supports the opinion that acute retinal necrosis is a local inflammatory process. IL-8 was detectable in 55% of the serum samples of patients with intermediate uveitis of unknown origin. Other investigators have detected high levels of a new circulating protein in these patients. These observations may suggest a systemic involvement in this type of inflammatory eye disease. Because some of the patients with intermediate uveitis are known to develop a systemic disease later on, it would be interesting to investigate whether this is related to the occurrence of circulating cytokines. Circulating IL-8 has been detected in various systemic diseases. The range of serum IL-8 levels is very wide between different diseases but also within one disease. Compared to serum IL-8 levels in uveitis, low serum IL-8 levels were observed in patients with Crohn’s disease (maximum 21 pg/ml), comparable levels were observed for patients with acute pancreatitis (maximum 1388 pg/ml), and, as suspected, high levels were detected in patients with septic shock (maximum 66,000 pg/ml).

This study shows that IL-8 participates in the pathogenesis of clinical uveitis and that neutrophil attraction and activation may be involved in the process of tissue destruction in ocular inflammation. Cytologic and histologic studies have repeatedly demonstrated the presence of neutrophils in the eye during uveitis. For example, Hogan observed that next to lymphocytes and monocytes, neutrophils were often present in the aqueous humor of uveitis patients. In the retina of patients with Behçets disease, neutrophils infiltrate the area around obstructed blood vessels. In other diseases such as rheumatoid arthritis, the presence of IL-8 in synovial fluid has been reported and it is known that neutrophils play a role in tissue destruction in this disease. Studies concerning the interaction between different chemoattractants and infiltrating cells may yield important information about the inflammatory processes within the eye and may offer new prospects for the treatment of clinical uveitis.

Key Words
interleukin-8, elastase, chemotaxis, uveitis, vitreous fluid

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References
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