The complement system is involved not only in host defense against infection but also in autoimmune tissue damage. Using a radioimmunoassay, we sought to measure levels of C3a, C4a and C5a (activated complement fragments with anaphylatoxin functions) in human vitreous humor from eyes with and without vitreal inflammation. Vitreous from 11 patients with clinical evidence of vitritis (Group 2) had significantly higher levels of protein, C3a and C4a and significantly higher ratios of these anaphylatoxins to protein than vitreous from 19 patients without clinical evidence of vitritis (Group 1). The finding that not only the absolute levels of anaphylatoxins but also the ratios of anaphylatoxins to protein were significantly higher in vitreous from Group 2 than vitreous from Group 1 or normal plasma suggested that complement activation was taking place in inflamed vitreous. Because of irreversible binding to leukocytes, C5a is difficult to measure and correlate with complement activation, and we were unable to detect it in any vitreous sample. Invest Ophthalmol Vis Sci 29:1195-1198, 1988

The complement system is an important mediator of inflammation. C3a, C4a and C5a are activated complement fragments with anaphylatoxin functions that include smooth muscle contraction, increased vascular permeability, and release of histamine from mast cells and lysosomal proteases from neutrophils. C5a is not only an anaphylatoxin but also a potent chemotactic agent for neutrophils.

The complement system has been studied in human aqueous humor. Normal human aqueous humor was found to contain functional C1, C4, C2, C3, C5, C6 and C7, and the mean values of all these complement components were increased in inflamed aqueous humor. Studies of anaphylatoxin levels showed that normal human aqueous humor does not have measurable levels of C3a, C4a or C5a, but these anaphylatoxins were measured in aqueous humor from eyes with anterior uveitis. The higher ratios of anaphylatoxin to protein levels in inflamed aqueous humor when compared to normal plasma or noninflamed aqueous humor suggested that complement was being activated by either the classical or alternative pathways.

There are no studies of the complement system in human vitreous humor. In the present study, we sought to measure levels of C3a, C4a and C5a in human vitreous from eyes with and without inflammation.

Materials and Methods. Plasma Collection: Venous blood samples were collected from eight normal human adult volunteers after informed consent. A volume of 5 ml of blood was drawn using sterile vacutainers (Becton-Dickinson, Rutherford, NJ) containing disodium EDTA. Each sample was centrifuged at 2000 rpm at 4°C for 10 min. The resulting plasma was aspirated from each sample tube, separated into 1 ml portions and stored at -70°C until needed.

Vitreous Humor Collection: Samples of human vitreous humor were obtained from 30 patients with the approval of the UCLA Human Subjects Protection Committee. The patients who provided vitreous were divided into two groups. Group 1 consisted of 14 patients who underwent vitrectomy for repair of retinal detachment or treatment of epiretinal membranes and five patients who underwent vitrectomy to clear vitreous hemorrhages. The patients in this group did not have clinical evidence of vitreal inflammation or inflammatory lesions of the retina or choroid. Some of these patients had debris, red blood cells or pigment cells in their vitreous. Group 2 consisted of 11 patients with clinical evidence of vitreal inflammation with or without inflammatory lesions of the retina or choroid (Table 1). All these patients had white blood cells and flare in their vitreous which were graded from 1-4+ using standard criteria described by Schlaegel.

Vitreous was collected at the time of diagnostic vitreous tap or at the time of diagnostic or therapeutic vitrectomy. In eyes undergoing vitrectomy, approximately 0.2 ml of vitreous was cut and aspirated undiluted into the vitrectomy line from which it was withdrawn into a 1 ml tuberculin syringe. In eyes undergoing a diagnostic vitreous tap, a 1 ml tuberculin syringe with a 30-gauge needle was used to withdraw approximately 0.2 ml of liquid vitreous from a site 3.5-4 mm posterior to the superotemporal limbus. When appropriate, vitreous was submitted for viral, fungal or bacterial cultures and viral titers. The syringe with the remaining vitreous was placed in ice immediately and picked up by a technician. Preliminary studies showed that this was the optimal method of collecting vitreous that adding diso-
A fixed amount of antibody (to C3a, C4a, or C5a) to bind its radioactively labeled antigen (125I-labeled C3a, C4a, or C5a) in competition with unlabeled antigen. Both the standard curve and test samples were run simultaneously. Radioimmunoassay is an extremely sensitive technique that permits quantitation of anaphylatoxins in nanograms/milliliter. All laboratory assays of vitreous were performed by a technician without knowledge of the patient's clinical diagnosis. Statistical comparisons were made using the two-tailed student t-test.

Results. Vitreous from patients in Group 1 without clinical evidence of vitreal inflammation had measurable levels of C3a in 18/19 patients and C4a in 19/19 patients. All patients in Group 2 with clinical evidence of vitreal inflammation had measurable levels of C3a and C4a in their vitreous (Table 1). The two patients with microbial endophthalmitis and severe vitreal inflammation (4+ cells and flare) had higher degrees of vitreal inflammation (1+ cells and flare). Substantial levels of anaphylatoxins were also found in six patients with the acquired immunodeficiency syndrome and an associated retinitis. We were unable to detect C5a in any vitreous sample.

### Table 2. Mean anaphylatoxin and protein levels in vitreous and plasma ± SEM*

<table>
<thead>
<tr>
<th>Patient sample</th>
<th>Protein (mg/ml)</th>
<th>C3a (ng/ml)</th>
<th>C3a/protein</th>
<th>C4a (ng/ml)</th>
<th>C4a/protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1: Vitreous from eyes without vitritis (n = 19)</td>
<td>3.18 ± 0.49°</td>
<td>98.0 ± 14.1°</td>
<td>40.36 ± 6.79°</td>
<td>76.5 ± 18.9°</td>
<td>29.55 ± 8.47°</td>
</tr>
<tr>
<td>Group 2: Vitreous from eyes with vitritis (n = 11)</td>
<td>6.16 ± 0.80°</td>
<td>679.8 ± 217.7°</td>
<td>99.65 ± 27.87°</td>
<td>428.5 ± 93.8°</td>
<td>67.49 ± 12.88°</td>
</tr>
<tr>
<td>Normal plasma (n = 8)</td>
<td>114.94 ± 1.26°</td>
<td>84.1 ± 6.4°</td>
<td>0.73 ± 0.06°</td>
<td>189.3 ± 40.3°</td>
<td>1.67 ± 0.38°</td>
</tr>
</tbody>
</table>

* Values in a column without common superscript symbols are significantly different (P < 0.05).
Table 2 summarizes the mean levels of protein, C3a and C4a as well as the mean ratios of these anaphylatoxins to protein in vitreous from patients in Groups 1 and 2 and in plasma from normal subjects. Vitreous from patients in Group 2 with clinical evidence of vitritis had significantly higher levels of protein, C3a and C4a and significantly higher ratios of these anaphylatoxins to protein than vitreous from patients in Group 1 without clinical evidence of vitritis. Although plasma had a significantly higher concentration of protein than vitreous, vitreous from patients in Group 2 had significantly higher levels of C3a and C4a and significantly higher ratios of these anaphylatoxins to protein than plasma. Although the absolute levels of C3a and C4a were similar in vitreous from patients in Group 1 and normal plasma, the ratios of C3a or C4a to protein were significantly higher in vitreous than plasma.

**Discussion.** We found that vitreous from eyes in Group 1 with retinal detachments or vitreous hemorrhages had measurable levels of C3a and in some cases C4a. Although these levels were similar to background levels found in plasma, the ratios of anaphylatoxins to protein were significantly higher in vitreous than plasma because vitreous has much less protein. Disruption of the blood-retinal barrier in these eyes could be associated with elevated levels of complement in the vitreous. Generation of C3a or C4a in some of these eyes could result from activation of either the classical or alternative pathways. For example, in the case of vitreous hemorrhages, activated enzymes of the coagulation cascade which activate complement via the classical pathway could generate anaphylatoxins in the vitreous. Another possibility, however, is that complement in the relatively closed environment of the vitreous cavity may undergo spontaneous breakdown as it does in plasma with release of activated complement fragments that accumulate and reach detectable levels in the vitreous, which does not circulate as freely as plasma or aqueous. A study of radioactively labeled serum proteins that were injected into the vitreous of rabbits showed a slow rate of loss from the eye. In a previous study of aqueous humor from eyes with a history of prior surgery or inflammation, we measured levels of protein in aqueous that were similar to those found in vitreous from Group 1 in the present study. However, we generally were unable to measure aqueous levels of anaphylatoxins, perhaps because they were unable to accumulate and reach detectable levels in the anterior chamber due to rapid turnover of aqueous.

Regardless of how they were generated, low levels of C3a and C4a are present in the vitreous of patients from Group 1. The role that these inflammatory mediators play in these eyes is not known. With time, these anaphylatoxins may be converted to their less active forms by removal of C-terminal arginine by serum carboxypeptidase. The radioimmunoassay that we used does not distinguish between anaphylatoxins and their physiologically less active forms (C3a des Arg and C4a des Arg).

All vitreous samples from eyes in Group 2 with vitritis contained C3a and C4a. The finding that not only the absolute levels of anaphylatoxins but also the ratios of anaphylatoxins to protein were significantly higher in vitreous from Group 2 than vitreous from Group 1 or plasma suggested that complement activation was taking place. In patients with inflammation of the retina or choroid, anaphylatoxins in the vitreous may represent a spillover from inflammation within the retinal or choroidal tissues. On the other hand, microbial endophthalmitis may be associated with the generation of anaphylatoxins in the vitreous itself.

We were unable to measure C5a in any vitreous sample in this study. C5a is not only an anaphylatoxin but also a potent chemotactic agent for neutrophils. C5a is much more difficult to evaluate than C3a because it binds irreversibly to neutrophils, so that it is more difficult to measure and correlate with complement activation. Leukocytes in the relatively closed environment of the inflamed vitreous may bind C5a so that it could not be detected.

Two patients with microbial endophthalmitis had the highest levels of C3a and C4a as well as some of the highest ratios of these anaphylatoxins to protein in the vitreous. Studies of aqueous humor also demonstrated that patients with bacterial endophthalmitis had the highest levels of C3a and C4a as well as some of the highest ratios of these anaphylatoxins to protein. In a previous study, we suggested that the complement system was an important early defense in bacterial endophthalmitis in guinea pigs. Our findings in human vitreous and aqueous humor are consistent with our laboratory studies by showing substantial activation of complement in response to bacterial endophthalmitis.

**Key words:** complement, anaphylatoxins, C3a, C4a, C5a, uveitis

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**References**

1. Hugli TE and Chenoweth DE: Biologically active peptides of complement: Techniques and significance of C3a and C5a


