Aqueous immune complexes in immunogenic uveitis

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A primary immunogenic uveitis was produced in rabbits by the intravitreal injection of bovine plasma albumin or ovalbumin. A secondary or recurrent uveitis was induced by intravenous injection of the specific soluble antigen several months after the cessation of primary inflammation. Aqueous antigen-antibody complexes were studied at times of maximal clinical response in both primary and recurrent forms during resolution of primary immunogenic uveitis, and in a nonspecific protein extravasation induced by aqueous paracentesis. Immune complexes could be demonstrated only during times of clinically evident inflammation in immunogenic uveitis. The results indicate the importance of antigen-antibody complex formation in the pathogenesis of this form of experimental ocular inflammatory disease. (INVEST OPHTHALMOL VIS SCI 23:715-718, 1982.)

Key words: immunology, uveitis, immune complexes, animal model

Circulating immune complexes have been observed in the serum and aqueous of patients with many forms of uveitis.1-3 The nature and importance of these immune complexes is unclear. Some experimental models of presumed immune complex-mediated ocular inflammatory disease have been studied. Reactions similar to Arthus reactions can be induced in the eye.4 In experimental serum sickness, circulating immune complexes deposit within the uveal tract in parallel with their deposition in the choroid plexus.5

The uveitis produced by the direct intravitreal injection of soluble protein is a well-studied reaction with both cell-mediated and humoral components.6,7 A secondary or recurrent uveitis can be produced in this model by the intravenous challenge of antigen months after the cessation of inflammation.6 The current study was undertaken to determine whether immune complexes could be demonstrated in the aqueous in primary and in secondary immunogenic uveitis.

Materials and methods

New Zealand white rabbits weighing 1.8 to 2.2 kg were used in the study. Ovalbumin, 1.5 mg (Sigma Chemical Co., St. Louis, Mo.), and bovine plasma albumin, 7.5 mg (Reheis Chemical Co., Chicago, Ill.), were passaged through an 0.22 micra filter (Nalge Sybron Corp., Rochester, N.Y.), suspended in 50 μl of normal saline, and injected into the right vitreous of each animal with a 27-gauge needle. In no case was the lens capsule damaged during the injection. Saline (50 μl) was injected into the left eye. Recurrent uveitis was produced 2 to 3 months after the initial immunization by the intravenous infusion of 20 mg of ovalbumin and 50 mg of bovine plasma albumin in 2.0 ml of saline. A different group of rabbits was used for the recurrent studies.

After the primary immunization, animals were examined by slit-lamp biomicroscopy every 2 days...
Table I. Immune complexes in rabbit aqueous (measured by Raji cell assay)

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<thead>
<tr>
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<th>Average ± S.D. (range)</th>
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<tr>
<td><strong>Primary vitreous immunization (right eye)</strong></td>
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<tr>
<td>Ovalbumin (1.5 mg, 9 days, 6 rabbits)</td>
<td>241 ± 96 (103 to 361)</td>
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<tr>
<td>Bovine plasma albumin (7.5 mg, 14 days, 5 rabbits)</td>
<td>81 ± 88 (~1 to 232)</td>
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<tr>
<td>Ovalbumin (1.5 mg, 20 days, 5 rabbits)</td>
<td>19 ± 8 (12 to 28)</td>
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<td><strong>Recurrent immune uveitis (i.v. challenge 2 to 4 months; aqueous samples taken 4 hr after challenge)</strong></td>
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<tr>
<td>Ovalbumin (20 mg i.v., 6 rabbits)</td>
<td>560 ± 485 (80 to 1442)</td>
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<tr>
<td>Bovine plasma albumin</td>
<td></td>
</tr>
<tr>
<td>Antibody titer &lt;1:128 (5 rabbits), no clinical response</td>
<td>-7 ± 9 (~16 to 3)</td>
</tr>
<tr>
<td>Antibody titer &gt;1:256 (5 rabbits), clinical response</td>
<td>267 ± 345 (17 to 838)</td>
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<td><strong>Nonspecific aqueous protein extravasation</strong></td>
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<tr>
<td>Aqueous samples 2 hr after aqueous paracentesis</td>
<td>27 ± 21 (~6 to 72)</td>
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- Results are expressed as percent difference between right and left eyes (\(\frac{\text{RE} - \text{LE}}{\text{LE}} \times 100\)).
- Average 101% (range 42% to 232%), discounting one rabbit not manifesting a clinical response.
- Tanned erythrocyte hemagglutination technique.

for 3 weeks, and severity of the inflammation was recorded in a semiquantitative manner. The animals were then examined every 1 to 2 weeks.

Blood samples were obtained from the ear prior to immunization and at various times during the course of the experiment. Hemagglutination titers were measured on sera by means of a conventional tanned red-cell technique.

Aqueous samples were obtained from animals topically anesthetized with Ophthaine drops; 0.12 to 0.15 ml of aqueous was drawn via a 27-gauge needle into a tuberculin syringe containing one drop of 3.8% sodium citrate. Aqueous samples were obtained from both the right and left anterior chamber, and protein determinations were performed on aliquots using the Lowry technique. A nonimmunogenic aqueous protein extravasation was produced as a control to delineate immune complex vs. nonspecific protein binding in the Raji cell radioimmunoassay. In these control rabbits aqueous was removed by the above technique, and 2 hr later it was again withdrawn. Samples of plasmoid aqueous were adjusted to have a protein concentration similar to that of the immune uveitis animals by the addition of either normal aqueous or saline.

Immune complex determinations were performed with a modification of the Raji radioimmunoassay described in detail by Theofilopoulos et al. Twenty-five microliters of aqueous were diluted 1:2 with normal saline and incubated with \(2 \times 10^6\) Raji cells that were at the 72 hr stage of propagation. After 45 min of incubation at 37° C the Raji cells were washed three times in minimal essential medium and a standardized amount of radioactively labeled iodine (\(^{125}\)I) mouse anti-rabbit immunoglobulin and 1% human serum albumin was added. An additional period of incubation with gentle agitation was carried out at 4° C for 30 min. The Raji cells were then repeatedly washed and the radioactivity of the cell pellet was determined. In initial studies, the concentration of immune complexes in the aqueous samples was extrapolated from a standard curve to which various known amounts of aggregated rabbit gammaglobulin and 1% human serum albumin was added. An additional period of incubation with gentle agitation was carried out at 4° C for 30 min. The Raji cells were then repeatedly washed and the radioactivity of the cell pellet was determined. In initial studies, the concentration of immune complexes in the aqueous samples was extrapolated from a standard curve to which various known amounts of aggregated rabbit gammaglobulin and complement were incubated with Raji cells and \(^{125}\)I-labeled anti-rabbit immunoglobulin. The amount of immune complexes was expressed as microgram equivalents of aggregated rabbit gammaglobulin per milliliter of aqueous. In all studies, the relative immune complex measurements from the control and treated eyes were compared and expressed as percent differences of immune complexes in the right vs. the left aqueous.

Results

Slit-lamp examinations demonstrated a maximal clinical response 9 days after ovalbumin and 14 days after bovine plasma albumin injection. A typical response consisted of iris injection and pupillary constriction, 2+ to 3+ cells and flare, and 2+ to 4+ fibrin. The extent of these changes in a single animal usually correlated with the immune complex measurement. Two to 3 weeks after ovalbu-
min and 3 to 4 weeks after bovine plasma albumin these changes were no longer present. Aqueous immune complexes were measured at the time of maximal response after antigen injection and also at the time where the inflammation appeared to be clinically resolving after injection with ovalbumin. The immune complex levels are expressed as a percent difference between the right (test) eye compared with the left (control) eye in Table I. The average percent difference for six rabbits injected with ovalbumin at 9 days was 241% (range 103% to 361%), and the average value for five rabbits injected with bovine plasma albumin and tested at 14 days was 81% (range 1% to 232%). A single rabbit in the latter group showed no clinical response, although an antibody titer was 1/2048. If this latter animal is excluded, the average percent difference was 101% (range 42% to 232%) in the bovine plasma albumin group. Twenty days after ovalbumin injection, at a time when clinical ocular inflammation had almost disappeared, the average percent difference in the two eyes was 19% (range 13% to 28%); hemagglutination titers were >1/512 at this time (Table I).

Two to four months after primary immunization, the eyes appeared normal. Four hours after repeat intravenous challenge, aqueous cells and flare were evident in all ovalbumin and half the bovine plasma albumin animals. Compared with primary immunization, little fibrin was present. In six rabbits given ovalbumin, aqueous immune complex determination showed a difference of 560% in the right vs. the left eye (range 80% to 1442%). Rabbits immunized and challenged with bovine plasma albumin fell into two groups. Five rabbits had no clinical response upon intravenous challenge (titer <1/128). The difference in Raji cell binding was −7% (range −16% to 3%). Five rabbits had a recurrent inflammatory response (titers >1/256). In this latter group the average difference was 267% (17% to 839%) (Table I).

The effect of a nonimmunogenic aqueous protein extravasation on the Raji cell assay was assessed by testing secondary aqueous after repeat anterior chamber paracentesis. Two hours after paracentesis, aqueous binding was 27% greater in the aspirated eye as compared with the normal eye (range −6% to 72%).

**Discussion**

This study illustrates that immune complexes can be measured in the aqueous of rabbits with experimental uveitis by means of a modified Raji cell radioimmunoassay. In primary immunogenic uveitis produced by the intravitreal injection of ovalbumin, higher aqueous levels of immune complexes were observed than in the ocular inflammatory model produced by injection with bovine plasma albumin. In the ovalbumin model, elevated levels of immune complexes could not be observed after the cessation of clinical inflammation. In secondary, or recurrent, immunogenic uveitis, complexes again were observed in both models.

Immune complexes have not been directly measured in the aqueous in immunogenic uveitis prior to the current study, but their presence has been inferred. Antigen retention in the vitreous can be identified for several weeks,7, 11, 12 and antibody and antibody-secreting cells have been demonstrated as early as 7 days after antigen injection.7, 13, 14 The presence of antigen-antibody complexes during the time of maximum clinical response suggests a major role in the pathogenesis of this form of uveal inflammation.

The binding of aqueous immune complexes at the time of clinically evident recurrent inflammation indicates their importance at this stage as well. Why immune complex levels should be higher in the recurrent uveitis than in the primary disease is not completely understood. Certainly the clinical duration and severity of the response is greater during primary immunogenic uveitis. But in the primary form, nonspecific protein and cellular exudates probably play a greater part, whereas in the secondary form, a selective immunogenic stimulus probably leads to the production of higher levels of antigen-antibody complexes. It has been amply demon-
strated that the eye functions as an accessory lymph node during such a secondary response. 6, 7 Previous injury to uveal blood vessels may also lead to greater protein extravasation in recurrent inflammation.

Although circulating immune complexes have recently been demonstrated in a number of clinical uveitides, their relationship to pathogenesis remains unknown. Pathologic changes produced in the eye by the intravenous infusion of immune complexes in rabbits is minimal. 14 In various forms of experimental serum sickness, immune complexes have been demonstrated in the uveal tract, but the ocular disease is of very low grade. 3 Recent studies in glomerulonephritis have in fact raised questions of whether circulating immune complexes are as important as previously supposed in renal disease. 10, 16 The formation of immune complexes in situ rather than deposition from the systemic circulation appears to be of greater importance in the pathophysiology of many diseases. This may also be the case in ocular inflammatory processes.

Concentrations of immune complexes in aqueous were relatively small. These small quantities plus a considerable variation in individual responses in rabbits make additional qualitative studies of immune complexes difficult. Our preliminary studies in an attempt to define the antigenic component of these aqueous immune complexes by either immunoassays or the injection of radioactively labeled antigen in the recurrent uveitis model have not been successful to date.

We are indebted to Virginia K. Cruse, Michael Hoffman, and Neelam Rand for their technical assistance.

REFERENCES