The ocular immune response

III. Effect of antilymphocyte serum on the development of immunogenic uveitis

Ronald M. Burde and Stephen Waltman

Rabbits receiving an intravitreal injection of egg albumin developed immunogenic uveitis spontaneously in approximately one week. Pretreatment with systemic horse antirabbit lymphocyte serum (HARLS) was highly effective in preventing the development of the uveitis. Pretreatment with normal horse serum had no effect on the uveitis. Pretreatment with HARLS also prevented sensitization of the animal to the egg albumin as the recurrent uveitis which accompanies a later intravenous challenge of the prestimulated animal did not occur. Treatment with HARLS could not prevent the secondary response after the animal had been previously sensitized.

Key words: Antilymphocyte serum, second set responses, immunogenic uveitis, allergic uveitis, immune reaction suppression, horse serum, intramuscular injections, intravenous injections, hypersensitivity, albumin, intravitreal injection, histopathology, rabbits.

Rabbits receiving an intravitreal injection of foreign protein develop uveitis spontaneously in approximately one week. Previous investigations have shown that this experimental ocular inflammation is an immunologic phenomenon and have suggested that at least part of the inflammatory response may be due to a delayed hypersensitivity reaction.1 The primary immune response involves sensitization of extraocular lymphoid tissue. Immunosuppressive agents such as whole-body irradiation will prevent this occurrence.2

Following initial sensitization the animal develops a state of local ocular hypersensitivity. At this time challenging the animal with the same antigen systemically, i.e., extraocular route, will cause a rapid recurrence of the uveitis. This will occur even if the original antigen is no longer present in the eye.2

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Antilymphocyte serum (ALS) is a potent immunosuppressive agent that is currently in use both clinically and experimentally. It has previously been used to modify the ocular immune response in corneal homotransplantation. The present paper reports the effect of ALS on the development of immunogenic uveitis induced by intravitreal egg albumin.

Methods and materials

Horse antirabbit lymphocyte serum (HARLS) was prepared as previously described. It was stored at −20°C with 1:10,000 merthiolate added as a preservative. After absorption with red blood cells, it had a lymphagglutinating titer of 1:4,000 and a hemagglutinating titer of 1:16.

Crystalline egg albumin was dissolved in normal saline solution to a final concentration of 20 mg per milliliter. This solution was sterilized by membrane filtration and was used immediately or after storage for 24 hours at −20°C.

Albino rabbits weighing 3 to 5 pounds were used in this set of experiments. Under local anesthesia 0.10 ml of the albumin solution (2 mg) was injected into the vitreous of the right eye by means of a tuberculin syringe with a No. 27 gauge needle, after the globe had been rotated inferiorly by superior rectus muscle fixation with a forceps. The puncture site was compressed with a cotton spud and there was no evidence of leakage of the injected material on cessation of pressure.

Two treatment schedules were followed during this experiment. The schedules were very similar. In the first test situation 11 rabbits received HARLS intramuscularly starting 3 days before the intravitreal injection. They received 0.5 ml of HARLS each day for 6 days and then 0.5 ml every other day for 10 days. Simultaneously, ten animals received injections of normal horse serum (NHS) and 7 animals received injections of normal saline intramuscularly.

In the second part of the experiment 18 animals received HARLS starting 2 days prior to intravitreal albumin injection. They received 0.5 ml of HARLS intramuscularly each day for 6 days followed by 0.5 ml every third day for 15 days. Fourteen animals received injections of NHS and 5 animals injections of normal saline on a similar dosage and time schedule. The total dose of HARLS received by the animals was 5.5 ml in both groups.

The rabbits were examined daily with the use of a hand light or slitlamp by an examiner who was unaware of which animals were being treated.

Blood was drawn from several animals in each group for antibody studies. The serum was frozen at −20°C for approximately 2 months prior to assay.

Passive cutaneous anaphylaxis was performed according to the method of Ovary. Precipitating antibodies were looked for by mixing various serial dilutions of serum and antigen at room temperature. The mixtures were left overnight at 4°C and read. Histologic sections of the involved eyes were interpreted by an observer who was unaware of the prior treatment.

Results

The first signs of uveitis were recognizable four days after intravitreal injection. A circumcorneal limbal flush appeared along with a concomitant engorgement of the iris vessels. The pupil became miotic and an anterior chamber haze appeared. Cells and flare were noted in the vitreous between 24 and 72 hours. Macroscopically visible hazy vitreous exudates were apparent. Since it is difficult to evaluate the presence of cells and flare in the anterior portion of the albino rabbit eye, an animal was classified as having uveitis only if it had definite limbal and iris vascular engorgement and vitreous exudates.

The various clinical signs were graded on a scale of 0 to 4. This was useful only in observing the development of the uveitis. All animals that developed reactions demonstrated a marked response. In the first part of the experiment 28 animals were treated starting 3 days before ocular inoculation. They were treated for 15 days. All 7 of the saline-treated animals developed uveitis as did all 10 of the animals treated with NHS. During the period of active treatment, none of the 11 animals treated with HARLS developed uveitis. Two animals were put to death because of broken legs on day 12. Five of the remaining 9 animals developed uveitis be-
 tween days 13 through 39. The animal that developed uveitis on day 13 had a typical reaction. In all of the other rabbits vitreous exudates appeared suddenly without prior or subsequent limbal, iris, or anterior chamber involvement. These exudates gradually cleared over the next three weeks. The 4 other HARLS-treated animals remained free of signs of uveitis for 52 to 72 days. The initial and late results in this group are shown in Tables I and II.

The difference between the HARLS-treated group and either one of the control groups is statistically significant (p < 0.05 for the least favorable results).

The second treatment schedule was more efficacious in preventing immunogenic uveitis. Eighteen animals were treated with HARLS until day 18. None had developed uveitis by day 53, at which time the experiment was terminated. Five animals were killed on day 14 for pathologic study. All of the control animals developed uveitis. The results are summarized in Table III.

The difference between either control group and the HARLS group is highly significant (p < 0.001).

Table IV summarizes the combined long-term results of both groups. All animals were observed for at least 50 days after intravitreal injection.

To determine if HARLS had prevented ocular sensitization or merely suppressed the inflammatory response, the following experiment was performed. Ten weeks following the intravitreal injections, 5 animals that had been treated with HARLS were given 20 mg. of egg albumin intravenously. Similarly, 6 NHS- and 6 saline-treated animals were also given albumin intravenously. All 12 control animals developed reactivation of the uveitis in a total of 12 hours whereas none of the HARLS-treated animals showed any signs of a reaction.

Six animals that had had uveitis 10 weeks previously were given 1 c.c. of HARLS intramuscularly for 4 days and then challenged with 20 mg. of albumin intravenously. All animals had reactivation

### Table I. Development of immunogenic uveitis. Results at day 12, treatment schedule 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of animals</th>
<th>Uveitis</th>
<th>No uveitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>HARLS</td>
<td>11</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>NHS</td>
<td>10</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Saline</td>
<td>7</td>
<td>7</td>
<td>0</td>
</tr>
</tbody>
</table>

### Table II. Development of immunogenic uveitis. Late results with treatment schedule 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of animals</th>
<th>Uveitis</th>
<th>No uveitis</th>
<th>Average time of onset of uveitis (range) (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HARLS</td>
<td>9</td>
<td>5</td>
<td>4</td>
<td>26 (13-39)*</td>
</tr>
<tr>
<td>NHS</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>6.5 (5-9)</td>
</tr>
<tr>
<td>Saline</td>
<td>7</td>
<td>7</td>
<td>0</td>
<td>5.4 (5-6)</td>
</tr>
</tbody>
</table>

*Numbers in parentheses show range.

### Table III. Development of immunogenic uveitis. Late results with treatment schedule 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of animals</th>
<th>Uveitis</th>
<th>No uveitis</th>
<th>Average time of onset of uveitis (range) (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HARLS</td>
<td>13</td>
<td>0</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>NHS</td>
<td>14</td>
<td>14</td>
<td>0</td>
<td>6.0 (4-7)</td>
</tr>
<tr>
<td>Saline</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>6.0 (5-8)</td>
</tr>
</tbody>
</table>
Table IV. Combined results from Tables II and III

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of animals</th>
<th>Uveitis</th>
<th>No. uveitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>HARLS</td>
<td>22</td>
<td>5</td>
<td>17</td>
</tr>
<tr>
<td>NHS</td>
<td>24</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>Saline</td>
<td>12</td>
<td>12</td>
<td>0</td>
</tr>
</tbody>
</table>

of their uveitis. The reactivation was comparable to that seen in 6 animals treated with NHS for 4 days.

Control eyes enucleated one week after onset of uveitis showed a dense mononuclear infiltrate of the iris and ciliary body. Plasma cells and lymphocytes were predominant. Around the vitreous exudates mononuclear cells were also present in small numbers. The choroid of several animals showed a few patchy areas of minimal round cell infiltrates. Histopathologic examination of the HARLS-treated eyes confirmed the clinical findings of the absence of uveitis. One HARLS-treated animal that developed late atypical uveitis was examined histopathologically. The cellular response around the vitreous exudate was more marked than in the other eye. The iris and ciliary body were also infiltrated with mononuclear cells. This occurred despite the lack of clinical evidence of iritis.

Blood drawn at day 14 from 15 animals (5 in each group) was tested for evidence of serum antibody by passive cutaneous anaphylaxis. The sera of two rabbits treated with NHS demonstrated the presence of antibodies to egg albumin by this method. All others were negative. No precipitating antibodies were found in the serum of treated or control animals. The blood had been drawn 2 and 5 weeks after intravitreal injection.

Discussion

ALS has proven to be a potent immunosuppressive agent in preventing the development of immunogenic uveitis. It prevented development of the primary immune response which is presumably due to sensitization of lymphoid tissue. It also prevented the development of local ocular hypersensitivity. When a HARLS-treated animal was given egg albumin intravenously it did not develop recurrent uveitis as did the control animals. It is not clear if local ocular hypersensitivity is due to immunologically competent cells arising in the eye or merely to the migration of such cells to the eye. Whatever the mechanism, ALS was able to prevent its development.

Cell-mediated immunity and antibody production may both be important in immunogenic uveitis. ALS is more efficient in blocking cell-mediated immune responses. It is capable, however, of preventing systemic antibody production. It may also prevent local ocular antibody production. It is still not clear which type of immune response is predominant in the development of immunogenic uveitis.

Previous investigations have shown that antigens introduced into the eye often give rise to detectable serum antibodies. This response is variable and depends on the antigen used. Furthermore, the previous investigations have used antigen loads 5 to 15 times that employed in the present study. This could be a possible explanation for the finding of serum and antibodies in only two of our animals.

With the dosage levels employed, ALS was not capable of preventing the recurrent uveitis that follows intravenous challenge of a previously sensitized animal. This agrees with previous work which has shown that ALS is more effective in preventing primary than secondary responses.

The usefulness of the current uveitis model is limited because the uveitis is short lived. It subsides spontaneously 2 to 3 weeks after onset. This makes it difficult to assess the therapeutic efficacy of a drug. Autoimmune uveitis has been produced in the rabbit but the model is not ideal. Uveitis occurs in less than half of the animals and often is demonstrable only by microscopic section. Should a better
uveitis model become available ALS may prove to be a useful agent in preventing and reversing the disease as it has proved valuable in both aspects of experimental allergic encephalomyelitis.\textsuperscript{11} If this occurs it may be worthwhile to use ALS or ALG in those cases of uveitis in man that are unresponsive to corticosteroids and other immunosuppressive therapies.

REFERENCES