Lymphocyte-stimulating activity of ocular tissues in sympathetic ophthalmia

George E. Marak, Jr., Ramon L. Font, Merrill C. Johnson, and F. Paul Alepa

Blastogenic activity of ocular tissue extracts was elicited in lymphocyte cultures from peripheral blood of two of four patients with sympathetic ophthalmia. Retinal pigment epithelium, retina, and lens were mitogenic, while choroidal antigen was usually inactive. There was no evidence of cellular hypersensitivity to ocular tissue in several other forms of uveitis. The problems in interpreting these observations are discussed.

Key words: Autoimmune uveitis, cellular hypersensitivity, lymphocyte transformation, sympathetic ophthalmia, uveitis

Sympathetic ophthalmia is a bilateral uveitis of uncertain etiology. The sensitizing limb of the pathogenetic mechanism is generally believed to involve autoimmunity to uveal pigment.1 Circulating antibodies to ocular tissues are found irregularly in this disease,2-4 therefore it is difficult to support the idea of an inflammation mediated by circulating antibody. Cellular mechanisms of immunopathology have never been adequately evaluated. Skin testing with suspensions of uveal tissue formerly employed in the diagnosis of sympathetic ophthalmia does not clearly distinguish between autoimmunity and histoincompatibility.

Mindful of the problems that histocompatibility antigens may play in the in vitro evaluation of cellular hypersensitivity to tissue antigens, the demonstration of in vitro reactions to soluble antigens in a number of recent studies of suspected autoallergic disease5-7 encouraged us to believe that the sensitizing antigen in sympathetic ophthalmia might be soluble and therefore could be evaluated by the blastogenic effect of ocular tissues on the lymphocytes of patients with sympathetic ophthalmia.

Material and methods

Four recent cases of sympathetic ophthalmia were observed. All cases were clinically well documented. Two cases were histopathologically confirmed and the other two demonstrated a

From the Divisions of Ophthalmology and Rheumatic Diseases, Georgetown University Hospital, Ophthalmic Pathology Branch, Armed Forces Institute of Pathology, and Division of Nuclear Medicine, Walter Reed Army Institute of Research, Washington, D. C.

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Reprint requests to: Dr. Marak, Division of Ophthalmology, Georgetown University Hospital, Washington, D. C. 20007.
**Table I. Blastogenic activity of ocular tissues in sympathetic ophthalmia**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Number of patients</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sympathetic ophthalmia</td>
<td>4</td>
<td>Two greater than 2.6× control</td>
</tr>
<tr>
<td>Reiter's syndrome</td>
<td>2</td>
<td>None greater than 2.6× control</td>
</tr>
<tr>
<td>Sarcoid</td>
<td>2</td>
<td>None greater than 2.6× control</td>
</tr>
<tr>
<td>Toxoplasmosis</td>
<td>1</td>
<td>None greater than 2.6× control</td>
</tr>
<tr>
<td>&quot;Histoplasmosis&quot;</td>
<td>1</td>
<td>None greater than 2.6× control</td>
</tr>
<tr>
<td>Normal subjects</td>
<td>11</td>
<td>None greater than 2.6× control</td>
</tr>
</tbody>
</table>

**Table II. Data on Patient S. S.**

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Admission blood (c.p.m., average of three)</th>
<th>Remission, no steroids (c.p.m., average of three)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal subject</td>
<td>Patient</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PHA</td>
<td>1.5M</td>
<td>1.2M</td>
</tr>
<tr>
<td>Control</td>
<td>1,857</td>
<td>1,123</td>
</tr>
<tr>
<td>Iris epithelium</td>
<td>2,025</td>
<td>4,718 (4.2×)</td>
</tr>
<tr>
<td>Iris stroma</td>
<td>1,774</td>
<td>1,851</td>
</tr>
<tr>
<td>Lens</td>
<td>2,811</td>
<td>6,737 (6×)</td>
</tr>
<tr>
<td>Retina</td>
<td>1,614</td>
<td>3,818 (3.4×)</td>
</tr>
<tr>
<td>Optic nerve</td>
<td>1,706</td>
<td>2,919 (2.6×)</td>
</tr>
<tr>
<td>Choroid</td>
<td>2,904</td>
<td>1,485</td>
</tr>
<tr>
<td>Retinal pigment epithelium</td>
<td>1,923</td>
<td>6,401 (5.7×)</td>
</tr>
</tbody>
</table>

Results

Soluble ocular antigens have little stimulating effect on the lymphocytes of normal patients. There is usually no activity, but occasionally normal cultures demonstrated as high as 2.6 times the activity found in control cultures lacking antigen. It appeared that there was more stimulation with donor eyes having only mild autolytic changes. Two of the four patients with sympathetic ophthalmia, as well as all of the uveitis patients, had no evidence of lymphocyte transformation above control values (Table I).

The sedimented antigens were inactive in most normal and uveitis cultures. Occasionally the sediments of ocular tissue depressed the thymidine uptake below the values found in control cultures without added antigen.

Two of the four patients with sympathetic ophthalmia had lymphocytes (in blood
samples taken prior to steroid therapy) that were transformed in the presence of soluble ocular antigens. Ocular antigens were also blastogenic for one patient's lymphocytes obtained 8 months later, after the patient had been off steroid medication for one month. During the time this patient was receiving large doses of steroids (20 mg. of prednisone every other day), six cultures at monthly intervals were not stimulated by ocular antigens.

In the first patient, antigens from the lens, retina, and retinal pigment epithelium stimulated blastogenic transformation of the patient's lymphocytes. The soluble choroidal antigens as well as the sedimented antigens did not produce blastogenic activity (Table II).

The second patient showing a positive lymphocyte culture responded only to retinal pigment epithelial antigen. None of the other ocular antigens cultured was mitogenic for this patient's lymphocytes (Table III).

**Discussion**

There are many variables that may influence the blastogenic activity of ocular tissues on the lymphocytes of patients with sympathetic ophthalmia. Because the importance of these factors is not known, positive results must be interpreted cautiously.

Antigen concentration is well known to affect mitogenic activity. We selected 0.1 mg. of protein per culture because this gave adequate stimulation of lymphocytes and was consistent with protein concentrations of antigen giving good activity in other systems. In a strict sense the activity of ocular antigens cannot be directly compared until dose-response curves are evaluated for each of the antigens. Although we obtained no activity with sedimented antigens, insoluble antigens may have been suspended during preparation. Detailed analysis of sedimented antigen by sonication, enzymatic hydrolysis, etc., could demonstrate that the major portion of the active antigen is not soluble.

Histocompatibility considerations have generally been ignored in ocular immunology. Skin testing with tissue antigens (as has been done both clinically and experimentally in order to demonstrate cellular hypersensitivity) simply does not clearly distinguish between autoimmunity and histoincompatibility, even though graft rejection may occur more slowly than the tuberculin type of delayed skin test. Fortunately, histoincompatibility does not appear to be a major factor in lymphocyte transformation with ocular tissues. Perhaps transplantation antigens are weakly expressed in ocular tissues or have been altered by the autolytic changes occurring in donor eyes. While this problem is unsettled, it may be better to use mixed antigens from two or more donors rather than antigens from a single donor.

Enhancing antibody may block the blastogenic response of a sensitized lymphocyte to its specific antigen. In one of the many cultures we found that substitution of normal serum for patient's serum unmasked a blastogenic response to soluble choroidal antigen. Enhancing antibody must be evaluated before a specific antigen can be implicated in the pathogenesis of sympathetic ophthalmia.

The inflammatory activity at the time of culture may influence the results, although we found positive cultures during active disease as well as in remission. We have not had an opportunity to study a recurrence of the uveitis. The severity or dura-
tion of the disease may be related to in vitro indices of cellular hypersensitivity in sympathetic ophthalmia as it is in Guillain-Barré syndrome. It is important to know whether patients with more severe or persistent inflammation are more likely to demonstrate cellular hypersensitivity to ocular tissues.

In our two positive cases, the patient with the least severe clinical inflammation demonstrated hypersensitivity only to retinal pigment epithelial antigen. The other positive patient who had much more severe inflammatory signs had lymphocytes that were stimulated by several ocular tissues. Since the clinical picture in sympathetic ophthalmia varies from a mild anterior uveitis with a few cells in the vitreous to a violent inflammation with papillitis and exudative retinal detachment, the specificity of the presumed hypersensitive response may have some bearing on the clinical picture. This variable mitogenic activity may also be a secondary phenomenon simply reflecting a situation with more extensive inflammation, as discussed below.

The blastogenic activity of lens in one of our two positive cases is interesting because of the association of lens-induced uveitis with sympathetic ophthalmia. While the lens may have sympathogenic properties, the association could also be fortuitous, since the lens partially shares antigens with all other ocular tissues. In many normal cultures the lens demonstrated the great nonspecific mitogenic activity so that some nonspecific lymphocyte-stimulating property of the lens antigens could also be involved.

While several forms of uveitis demonstrate no lymphocytes sensitized to ocular tissues, in none of these diseases does the inflammatory reaction involve the ocular structures so extensively as in patients with sympathetic ophthalmia. The demonstration of migration inhibition factor in the presence of CNS basic protein in patients after cerebrovascular accidents illustrates the difficulty in interpreting whether cellular autoimmunity is a primary pathogenic factor or is merely a secondary result of inflammation. This problem is especially important in evaluating late cases of sympathetic ophthalmia and makes it imperative repeated cultures be obtained throughout the course of disease.

Steroids appeared to interfere with the blastogenic activity of ocular tissues. We were unable to obtain any positive cultures when our patients were on immunosuppressive doses of steroids.

Soluble antigens from lens, retina, optic nerve, and retinal pigment epithelium induced lymphocyte transformation in patients with sympathetic ophthalmia. Neither soluble nor sedimented choroidal antigen was mitogenic except on one occasion when a patient’s cells were incubated with normal serum.

Two positive cases cannot lead to any definitive conclusions, however, these observations suggest that a new hypothesis may be added to the list of the many speculations about the pathogenesis of sympathetic ophthalmia. Antigens outside the choroid cannot be excluded from participation in the immunopathogenesis of sympathetic ophthalmia. The avascular tissue between the middle limiting membrane of the retina and Bruch’s membrane at first glance is structurally suitable to be a privileged site. The paradoxical observation that autosensitization with retinal tissue usually elicits a uveitis is consistent with such a hypothesis.

These studies are encouraging in that it appears histoincompatibility may not be a major barrier to in vitro evaluation of ocular autoimmunity. Wong has recently confirmed the blastogenic activity of ocular tissues in seven of eight patients with sympathetic ophthalmia.

We wish to thank Drs. David Nemser Cohen and A. Raymond Pilkerton for referring patients for this study.

REFERENCES


