Inflammatory response of guinea pig to injected limbal tissue. Joel Corwin and Bernard Schwartz.

This study examined the possibility that homologous limbal tissue, including trabecular meshwork, serving as an antigen, could elicit inflammatory cells responsible for both aqueous obstruction and inflammation, the possible role of this response in ocular disease was also studied. Four sets of guinea pigs were sensitized with complete Freund’s adjuvant. One set received only adjuvant and saline, one set received adjuvant plus uvea, and two sets received adjuvant plus limbal tissue. The animals injected with only adjuvant and saline all showed normal ocular histology. Sensitizing guinea pigs with limbus and adjuvant produced mononuclear infiltration of the uvea, trabeculum, and episclera. Lymphocytes and plasma cells predominated. The response induced by the limbus seemed to consist of two components: a uveal and a specific limbal component. The lymphoid infiltration of the uvea was identical to the response induced by uveal antigen in our uvea control group. The limbal component was characterized by clusters of lymphocytes and plasma cells in the trabecular and episcleral regions. This study showed that limbal tissue antigen induces an inflammatory response in both uvea and limbus, and that the inflammatory response in the trabeculum is greater with limbal than uveal antigen.

The role of homologous uveal and retinal antigens in the stimulation of experimental uveitis in many species of animals has been previously described. Initially, Collins’s immunized guinea pigs and monkeys with homologous uveal tissue antigen and complete Freund’s adjuvant and produced a uveitis. The concept was advanced by Aronson et al.2, 3 and Wacker et al.,4–6 who used homologous uveal and retinal antigens in both guinea pigs and rabbits. Meyers’7 then induced uveitis in the guinea pig with purified retinal rod outer segments or pigment epithelium as antigen.

In this paper, the possibility was examined that homologous tissue from another site, the trabecular meshwork-limbal area, could serve as antigen to induce inflammation in this area. An inflammatory process of the trabecular meshwork would be of interest as a model for obstruction to aqueous outflow by inflammatory mechanisms, such as might occur in some types of glaucoma.6

Materials and methods

Animals. Forty female Albino Hartley guinea pigs, weighing between 400 and 500 gm, were used. The animals were divided into four groups of 10, with two control groups and two experimental groups. Because of illness, two animals from the positive control group and one animal from the experimental group were eliminated from the experiment.

Antigens. Fresh ocular tissue was obtained from normal Hartley guinea pigs killed with ether. The eyes were stored at −20°C. Retina and uvea were removed from the posterior half of the eye while still frozen. Toothed forceps were used to totally remove the aqueous, lens, and iris from the anterior chamber so that only sclera, limbus, and cornea remained. Excess sclera was removed by cutting around the cornea until a white ring, approximately 1 mm in width, remained. The limbus was dissected away from the cornea using curved corneal scissors under ×4 magnification. The resulting ring of limbal tissue including trabeculum was frozen and stored at −20°C until adequate tissue for the experiment had been collected.

Immunizations. The tissues were thawed and ground in a motor-driven Teflon and glass homogenizer with the addition of 1 ml of physiologic saline to each group to make the desired concentration of tissue homogenate. The homogenate was mixed with an equal volume of complete Freund’s adjuvant (Difco Laboratories, Detroit, Mich.) and utilized the Mycobacterium butyricum strain.

Table I. Inflammatory responses in guinea pigs following immunization with retino-uveal antigen (uvea) and limbal antigen (LB I and LB II)

<table>
<thead>
<tr>
<th>Region</th>
<th>Antigen</th>
<th>Uvea</th>
<th>LB I</th>
<th>LB II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1+</td>
<td>2+</td>
<td>3+</td>
</tr>
<tr>
<td>Ciliary body</td>
<td>8</td>
<td>7</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Trabeculum</td>
<td>15</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Episclera</td>
<td>4</td>
<td>11</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

* LB II vs. Uvea significant at 0.05 > p > 0.02.
1+ = Mild inflammation
2+ = Moderate inflammation
3+ = Heavy inflammation
Measure is of cell density (number of eyes).

0146-0404/78/0817-081400.40/0 © 1978 Assoc. for Res. in Vis. and Ophthal., Inc.
Fig. 1. Normal iris and trabecular meshwork, negative control, injected with saline and complete Freund's adjuvant. (×210.)

The animals in the positive control group, referred to as the uvea group, received 20 mgm (wet weight) of retino-uvea weekly for a period of 5 weeks. The experimental groups, referred to as LB I and LB II groups, were immunized with 20 and 30 mgm (wet weights) of limbal tissue antigen, respectively, for a period of 5 weeks. The negative control group received saline and adjuvant. All animals were injected with 0.2 ml of their respective solutions intramuscularly in the right hindleg weekly.

Histology. Animals were killed after 5 weeks, 1 week after their fifth injection. Samples were fixed in 10% formalin. After paraffin embedding, 25 sagittal sections for each eye, 6 μm in thickness, were mounted and stained with hematoxylin and eosin.

Method of analysis. The 25 sections were then graded in a masked manner for occurrence of cells (+ or −) and cell density (0, 1+, 2+, 3+) (Table I) in the iris, ciliary body, choroid, episclera, and trabeculum. Every nucleated cell was counted in the trabecular region, from Schwalbe's line to the scleral spur and an equivalent area of iris processes, under ×40 magnification (Table II). Previous experiments showed that this area was the area of heaviest involvement. Approximately 15 to 20 nucleated cells per area were considered 1+ inflammation, 21 to 30 nucleated cells per area were considered 2+ inflammation, and more than 30 nucleated cells per area were considered 3+ inflammation.

Each eye was analyzed separately, as some of the animals developed unilateral inflammation. Statistical analyses were performed on the occurrence of cells and cell density data using a Chi-square test with Yates correction factor for de-
termining differences between two independent samples. A comparison of frequency distributions, using a Mann-Whitney U Test, was made on the trabecular area cell counts. Two-tailed tests were used, and a p level of less than 0.05 was chosen to indicate significance.

**Results**

**Ocular histopathology**

**NEGATIVE CONTROLS.** The controls had an average of 10 nucleated cells per trabecular field. This count served as a baseline for the experimental animals. Occasional lymphocytes were seen in the trabecular area, but there were no dense collections of inflammatory cells (Fig. 1).

**UVEA GROUP.** The most common lesions involved the corona of the ciliary body and the episclera. Lymphocytes and plasma cells were the predominant cells seen, and polymorphonuclear leukocytes were rare. Occasional choroidal involvement appeared to occur, apparently by direct extension from the ciliary body. No cells were seen in the retina.

**TRABECULAR GROUPS.** As in the uvea group, a mononuclear infiltration involving the ciliary body and episclera was seen. Once again, clusters of lymphocytes and plasma cells predominated, with polymorphonuclear leukocytes rarely seen. There was, however, an increase in the mononuclear response to limbal antigen in the trabecular and episcleral regions when compared with the retino-ueva antigen (Table I and Fig. 2). Occasional cells in the choroid were seen. No cells were seen in the retina.

**Data analysis.** Statistical analysis of the occurrence of cells showed that the increased inflammatory response in the episcleral region,
using high-dose limbal antigen in the LB II group (30 mg/week), when compared to retina-uveal antigen, although suggestive, was not statistically significant (Table I). On the other hand, the number of eyes with 1+ cells in the trabecular region was significantly higher (0.05 > p > 0.02) (Table I). Using the Mann-Whitney U Test, it was found that the increased number of mononuclear cells with either dose of limbal antigen was significant when compared to retina-uveal antigen (0.02 > p > 0.05 for uvea vs. LB I and 0.002 > p > 0.01 for uvea vs. LB II) (Table II). The difference in number of cells between the two doses of limbal antigen was not, however, statistically significant.

Discussion. This study showed that limbal tissue, antigen, which included the trabecular meshwork, induced a mononuclear infiltrate of the ciliary body similar to the one invoked by uveal antigen. In addition to the response of the uvea, there was also a significant increase in the number of mononuclear cells in the trabeculum. Others have mentioned the presence of cells in the trabecular meshwork and episcleral tissue as part of a generalized inflammation induced by retina-uveal antigen. This study showed that limbal tissue was superior in producing more inflammation in trabeculum as compared to uveal tissue, whether it was the trabecular or scleral component of the limbal antigen that elicited the inflammatory response in the trabeculum was not determined in this experiment, since the trabecular meshwork could not be dissected out for use as a separate antigen.

The results of this study imply that there is an antigen which induces lymphocytes and plasma cell migration into the trabecular and episcleral regions. Inflammatory cells could cause obstruction at either the trabecular or episcleral levels or perhaps both and cause increased intraocular pressure.

We thank Mathea Allansmith and Roberta Meyers for reviewing the manuscript.

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Key words: limbus, trabecular meshwork, episclera, antigen, aqueous obstruction, lymphocytes, plasma cells

REFERENCES


Elster content of the scleral spur, trabecular mesh, and sclera. ROBERT A. MOSES, WALTER J. Grodzki, Jr., BARRY C. STARCHER, and MICHAEL J. GALIONE.

The scleral spur and trabecular mesh of the human eye contain approximately 3% elastic tissue. Elastic tissue forms less than 2% of the sclera.

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