Conn. 06510. This work was supported in part by Public Health Service Grants USPHS 5 PO6 RR 00393 and USPHS 1 RO1 RR 00700, and 1 RO1 EY01769-01. Submitted for publication Dec. 23, 1975. Reprint requests: Dr. Yin-Lok Lai, Section of Comparative Medicine, Yale University School of Medicine, 375 Congress Ave., New Haven, Conn. 06510.

Key words: keratoconjunctivitis, sialodacryoadenitis, virus, rat, enzootic megaloglobus, cataract, retinal degeneration.

REFERENCES

The effect of splenectomy on corneal graft rejection. WILLIAM M. BOURNE, BRYAN M. GEBHARDT, ALAN SUGAR, ROGER F. MEYER, AND HERBERT E. KAUFMAN.

Recent studies have suggested that the spleen may be essential for the "immunologic privilege" enjoyed by corneal grafts. 1, 2 In one of these studies, enhancement of subsequent skin allograft survival was elicited in rats by preinjecting donor lymphoid cells into the anterior chamber of the eye or the vein of the animal. This prolongation of skin graft survival did not occur when splenectomized recipient rats were used, so that the spleen is somehow necessary for this enhancement-like response. 3 The low incidence of corneal graft rejection may be due in part to the intravascular presentation of antigen via the lymphatic anterior chamber and the subsequent spleen-dependent response. Antigen from grafts in many other organs presents first in the draining lymph nodes and therefore may be handled differently. Since sensitizing antigen from penetrating corneal transplants probably first enters the recipient via the anterior chamber, it may lead to enhancement just as did the lymphoid cells and, therefore, may play a role in prolonging corneal graft survival. Prior splenectomy might then be expected to lead to a greater percentage of rejected corneal grafts and/or earlier graft rejection, just as it eliminated the prolonged skin graft survival described above. This possibility seemed to deserve further investigation. Furthermore, it would provide a convenient means of attaining higher rejection rates of clear penetrating corneal grafts in experimental animals.

We examined this experimental model in rabbits.

Materials and methods. Central 7 mm. penetrating keratoplasties were exchanged between the right eyes of 19 normal adult outbred albino rabbits and 19 similar rabbits that had undergone splenectomy 2 weeks previously. The entire spleen plus any accessory splenic tissue was removed. The animals were anesthetized with sodium pentobarbital (30 mg. per kilogram) for both the splenectomy and keratoplasty procedures. The operating microscope was used for the keratoplasty. Heparin solution (10,000 U. per milliliter) was applied topically after the anterior chamber had been entered. Interrupted 10-0 nylon sutures were used and removed on the tenth postoperative day. One per cent atropine ointment was applied to each eye daily for 1 week following keratoplasty. Technical failures, grafts not clear on the seventh postoperative day, and rabbits that died during the 10 week period of study were all eliminated from the project. No steroids were administered. The rabbits were examined daily for the first month and then every few days until grafts. 1, 2

<table>
<thead>
<tr>
<th>No Splenectomy</th>
<th>Splenectomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number rejected</td>
<td>6/14 (43%)</td>
</tr>
<tr>
<td>Mean survival time (days)</td>
<td>30.5</td>
</tr>
</tbody>
</table>

Table I. Rejection rates of penetrating corneal transplants in rabbits followed for 10 weeks

Studies published recently have indicated that the spleen may play an essential role in the "immunologic privilege" enjoyed by corneal grafts.
the study was terminated 10 weeks following keratoplasty. Sera from the recipient rabbits were examined intermittently for hemagglutinating activity directed at donor antigens, but no satisfactory test for hemagglutination in rabbits could be obtained. Grafts were considered rejected when they became too hazy for iris detail to be seen. One ocular infection occurred, and this rabbit was eliminated from the study. Vessels eventually reached the graft in all eyes except one.

Results. Eight of the 38 rabbits were eliminated from the study according to the above criteria. The results in the remaining 30 rabbits are presented in Table I. No statistically significant difference in rejection rate or mean survival time was found between the two groups. When the paired samples were considered (13 of the original 19 pairs of rabbits remained intact), again no significant difference was found.

Discussion. Our results do not in any way negate those of the previous studies showing the effects of splenectomy in inbred rats, but demonstrate that quite different results can be obtained when different experimental animals are used. In addition, our animals were outbred rather than inbred, and the histocompatibility differences are not uniform. The studies in rats are applicable to penetrating corneal grafts only if a significant amount of the graft antigen first presents itself to the immune system of the recipient via the anterior chamber and intravenous route, bypassing the regional lymphatics. Antigen injected into the corneal stroma apparently does not travel by this pathway, although that from penetrating grafts may. In any event, we have shown that prior splenectomy has no effect upon the rejection rate of penetrating corneal transplants in rabbits. The significance of the contrast between our findings in rabbits and those of Kaplan and Streilein in rats requires further investigation.


Key words: Corneal allograft, splenectomy, rabbit penetrating keratoplasty, corneal graft rejection, keratoplasty enhancement.

REFERENCES


Corneal glycogen synthesis. I. Evidence for a gluconeogenic pathway in beef cornea. J. STEVENS ANDREWS.

Beef eye anterior chambers were perfused with media containing radiolabeled glycogen precursors. Incorporation of 14C from l-alanine-U-14C into corneal epithelium glycogen suggested the presence of a gluconeogenic pathway in the eye. Failure to isolate radioactive glucose from l-alanine-U-14C-containing perfusate after passage through the anterior chamber strongly suggests a corneal site for this pathway.

Glycogen is an important metabolic energy reserve in those tissues which synthesize and store it. The observations that glycogen is present in corneal epithelium and metabolically active prompted an investigation of the pathways of synthesis in beef cornea.

Since most, if not all, glucose utilized by the corneal epithelium comes from the aqueous humor of the anterior chamber, a method of perfusing beef eye anterior chambers with chemically defined media was devised. Incorporation of 14C-labeled glycogen precursors in the perfusion medium permitted a preliminary assessment of the pathways of glycogen synthesis.

Methods. Chemicals used in this investigation were the purest grade commercially available and were used without further purification. Radio-labeled substrates were purchased from New England Nuclear, Inc., Boston, Mass. Beef eyes were obtained at a local abbatoir.

Perfusion media were prepared according to Dikstein, using the basal salt solution with glutathione (BSSG).

The medium was placed in a single reservoir and a mixture of water-saturated 5 per cent CO2:20 per cent O2:75 per cent N2 bubbled into the reservoir. After division of the single perfusion stream into eight channels, the medium was pumped by an eight-channel pump through beef eye anterior chambers at a rate of either 170 or 60 al per minute. In certain experiments, two reservoirs were used consecutively and a two-way Teflon stopcock was used to switch from the first to the second reservoir.