The fate of experimental corneal grafts in herpetic keratitis and keratouveitis

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Corneal graft opacification may occur in the course of ocular inflammation and graft rejection may develop concomitantly or stimulated by the inflammation. The incidence of immune graft rejections during episodes of iritis or external infections is difficult to document, due to the fact that it is not possible at the present time to separate clinically one process from the other. In our experience the incidence of graft rejection in penetrating keratoplasties for herpetic keratitis is not greater than that found in other cases of keratoplasty with a similarly altered anatomic status (corneal pathology, vascularization, etc.). Other authors, however, feel that the rate of rejections in patients with herpetic keratitis is unquestionably very high. Whereas it is possible that the two processes may appear simultaneously or the immune reaction follow the herpetic reactivation, we have no laboratory test to determine the first. It seemed to us that an experiment could furnish some information on this problem because the parameters of graft reaction in the experimental animal have been well established and the histologic alterations studied in detail. The purpose of these experiments was, therefore, to determine the fate of penetrating grafts during episodes of corneal epithelial Herpes simplex virus (HSV) infection and after HSV uveitis.

Materials and methods

Forty-five adult albino or pigmented rabbits (3 kilograms) were used throughout this experiment. Penetrating keratoplasties 6 mm in diameter were made between pairs of animals. In one series, grafts were done between albino rabbits (allografts, homografts) and in another, grafts were exchanged between albino and pigmented rabbits. It has been reported that there is a high incidence of spontaneous graft rejection between these two varieties. In addition, four rotational autografts were performed. In all cases, 7-0 silk sutures were used and were removed 7 to 10 days later. No steroids were used postoperatively.

Experiment 1, HSV infection of graft epithelium. Herpes simplex virus of the “McKrae strain,” was used for all infections. Undiluted infected tissue culture stock (20× mid) was used for the epithelial infection. The corneal epithelium was lightly scarified with a small needle, and a drop of virus suspension stock was instilled in the conjunctival cul-de-sac. The lid was then closed and gently rubbed over the cornea.

The four autografts at the time of infection were four weeks old. The 15 homografts were all albinos, and the postoperative ages of the graft at the time of epithelial infection were: 2 weeks, 4 weeks, 2 months, 3 months, and 6 months. No...
Table I. The fate of isografts and allografts with HSV epithelial keratitis

<table>
<thead>
<tr>
<th>Isografts (autografts):</th>
<th>Age of graft</th>
<th>Postinfection (6-8 days)</th>
<th>Postinfection (2 months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>4 to 6 weeks</td>
<td>Hazy; vascular (III)</td>
<td>Mild haze (4/6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(6/6)</td>
<td>Scar (2/6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total cloudy</td>
<td>2/6</td>
</tr>
<tr>
<td>Allografts (homografts):</td>
<td>2</td>
<td>Opaque; vascular (2/15)</td>
<td>Opaque (2/15)</td>
</tr>
<tr>
<td></td>
<td>7-10 days</td>
<td>Hazy; vascular (1/15)</td>
<td>Scar (1/15)</td>
</tr>
<tr>
<td></td>
<td>3 weeks</td>
<td>Hazy; vascular (6/15)</td>
<td>Clear-hazy (6/15)</td>
</tr>
<tr>
<td></td>
<td>4 weeks</td>
<td>Hazy; vascular (3/15)</td>
<td>Scar (1/15)</td>
</tr>
<tr>
<td></td>
<td>5 weeks</td>
<td>Hazy; vascular (1/15)</td>
<td>Clear (1/15)</td>
</tr>
<tr>
<td></td>
<td>9 weeks</td>
<td>Hazy; vascular (2/15)</td>
<td>Scar (1/15)</td>
</tr>
<tr>
<td></td>
<td>12 weeks</td>
<td>Total cloudy</td>
<td>5/15</td>
</tr>
</tbody>
</table>

Note: All grafts were infected four to six weeks after surgery. Surviving rabbits were followed for two months after healing. Stromal opacities with various degrees of vascularization are defined as scars.

From then on, the graft was examined with the slit lamp to determine the appearance of the graft, vessels in the cornea, iritis, exudates, and hypopyon. The intraocular pressure was measured in some of the rabbits with the MacKay-Marg applanation tonometer.

Criteria to evaluate corneal allograft rejection.
I. Clinical.
   A. Host vessels invading scar and graft stroma.
   B. Graft haziness starts near area of vascularization and advances centrally two to four weeks postoperatively.
   C. Endothelial or stromal rejection line.
   D. Permanent opacification.

II. Histological.
   A. Midstromal and deep-stromal vascularization associated with round cell infiltration.
   B. Round cells are typically lymphocytic and plasmatic in lesser degree.
   C. Round cell infiltration of endothelium with destruction of endothelial cells.

In our experience, first and second set rejections often show gradual, localized (1, A, above) cloudiness in contrast to severe, diffuse, and fairly rapid cloudiness with vascularization which occurs infrequently in homografts, but commonly seen in heterografts.

Criteria to evaluate graft pathology.
Stage I. Mild haziness = discrete corneal edema.
Stage II. Hazy = blurred iris details due to edema; fine vessels in stroma.
Stage III. Severe haziness and vascularization = thick graft, iris details not seen due to edema and cellular infiltration.
Histology study. Six representative eyes from each group were enucleated, fixed in 10 per cent formalin and processed for light microscopic examination.

Results

The fate of homografts and autografts with HSV epithelial keratitis. Defective wound healing was observed in two-week-old allografts with epithelial herpetic infection. They eventually developed stromal edema and gaping of the wound.

The clinical picture and the final results of all the remaining transplants were similar between allografts and autografts (Table I). Two days after the eye was infected, the corneal graft and the adjacent host cornea showed a punctate, linear, or diffuse staining (Fig. 1) and, frequently, dendritic figures. Four days later, the lesions became more numerous and coalesced to form a larger epithelial defect. This was associated with a severe conjunctivitis and vascularization of the host cornea which was very prominent in all eyes at the eight-day period. The most frequent site for deeply staining lesions to develop was at the host-graft junction, from which vessels grew into the opaque graft (Stage III). After 10 days, the inflammatory reaction started to subside, corneal edema and epithelial disease decreased and became localized, vessels regressed or became less prominent. Two animals which developed a severe keratitis with a geographic corneal ulcer died of encephalitis. Three

grafts showed stromal scars, but no typical graft rejection occurred in any of the 15 homografts (Table I). Five of the 15 grafts showed scars and deep vessels two months after infection (Fig. 2).

The fate of grafts in eyes with HSV uveitis.

Albino-albino grafts (15 grafts, 4 to 6 weeks old). In previous studies, the incidence of spontaneous rejections between these animals has been less than 2 per cent. The intracameral injection of stock virus caused a moderate to severe keratoiritis with a peak 6 to 8 days after injection. The ocular inflammation was initially characterized by conjunctival inflammation and vascularization of the host cornea with small vessels advancing into the graft.
Fig. 4. Peripheral area of a cloudy graft showing polymorphonuclear leukocytes, plasma cells, and some lymphocytes in the anterior third of the stroma. (Hematoxylin-eosin, ×100.)

Table II. Fate of corneal allografts in HSV kerato-iritis

<table>
<thead>
<tr>
<th>Rabbits</th>
<th>Postinfection (6-8 days)</th>
<th>Postinfection (17-20 days)</th>
<th>Typical rejection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albino-albino</td>
<td>Hazy; vascular (8)</td>
<td>Clear graft (0)</td>
<td>None</td>
</tr>
<tr>
<td>(15)</td>
<td>Iris (8)</td>
<td>Hazy (2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glaucoma (2)</td>
<td>Opaque (6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mortality (7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albino-pigmented</td>
<td>Hazy; vascular (7)</td>
<td>Clear graft (0)</td>
<td>One (1) before HSV infection</td>
</tr>
<tr>
<td>(15)</td>
<td>Iris (7)</td>
<td>Hazy (7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mortality (7)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: All grafts were infected four to six weeks after surgery.

Iritis varied from 2+ to 3+. Graft haziness varied from moderate to severe haziness and vascularization. Two of the animals showed an intraocular pressure of over 30 mm. Hg (MacKay-Marg); and seven animals died at this period of time. Seventeen to twenty days later, corneal vessels started to regress with clearing of the graft and improvement of the iritis. Three weeks after infection, two of the eight remaining grafts had a moderate haze (scarring) and peripheral vascularization. The grafts cleared further in four rabbits which survived over one month.

Grafts between albino and pigmented rabbits (14 six-week old grafts). This series was performed because of the reported high incidence of spontaneous graft rejection between these two groups. In the six-week period which preceded the ocular infection, only one albino rabbit developed a typical homograft reaction (10.5 per cent). Seven of the 14 remaining rabbits died during the period of maximum ocular inflammation. The remaining seven rabbits (five albino and two pigmented) were followed for three months after inoculation with HSV, and they showed slight haze with fine vessels (Fig. 3). The pattern of ocular inflammation and decreasing graft haziness was similar to that described above for the transplants between albino rabbits. In none of the remaining seven rabbits did a typical homograft reaction develop (Table II).

Histological study. Eyes with active in-
Infection of graft epithelium showed areas of epithelial destruction and infiltration of the superficial stroma by polymorphonuclear leukocytes. Leukocytic infiltration was also present in the limbal area. Eyes with areas of scarring and vascularization showed polymorphonuclear (PMNL) leukocytes and round cells at the limbus as well as in the stroma around vessels, but mostly in the anterior half of the cornea (Fig. 4). Endothelial cells were present (Fig. 5).

Cloudy grafts with active uveitis showed a profuse number of polymorphonuclear leukocytes in the anterior chamber and endothelium with a few round cells. Similar types of cells were seen at the limbus and ciliary body. Vessels in the graft were mid-stromal and were surrounded by polymorphonuclear leukocytes (PMNL’s).

Comment

These experiments show that as a result of epithelial herpetic keratitis or herpetic uveitis, corneal autografts and allografts become cloudy. In the ensuing weeks, the majority of these transplants will clear noticeably, while others may remain hazy or with localized areas of scarring. This, in our previous studies and in the criteria established to determine the presence of an immunologic graft reaction, we could see that the behavior of our experimental grafts do not fit well within the parameters of graft rejection. Whereas, it is possible that rejected-opaque grafts in the rabbit may clear considerably in many months, this has not occurred in a period of six to eight weeks in our experience. The fact that autografts opacify and some cleared like the allografts, it is also contrary to the concept of autograft rejection.

Also, in contradiction to this concept of graft rejection, is the lack of similarity to the typical clinical and histologic picture of rejection. One exception could be the presence of lymphocytes and plasma cells in the stroma around new vessels (Experiment I), which is also found in graft reactions; however, the distribution of these cells was also atypical for graft rejection.

Recent concepts suggest HSV infection of corneal grafts may change the antigenic characteristics of the tissue in such a way that the graft may be destroyed as a xenograft; however, an Auer reaction can also produce severe keratitis, or the immune reaction can co-exist with the inflammatory herpetic process. We had expected that animals sensitized to corneal allografts should reject their transplants when an inflammatory process developed in the eye, since this is a common observation in humans. However, graft rejection did not occur as it was observed in a previous study in which immunogenic uveitis was developed in eyes bearing clear transplants. Since grafts between rabbits are well-tolerated and usually require a second set to be rejected, we introduced several variants into the experiments to facilitate the rejection; such as, younger grafts (unhealed scar), the use of silk material for sutures, and the use of animals susceptible to spontaneous rejection (albino-pigmented). The low incidence of graft reactions between
rabbits of one species might be due to their inbreeding, rather than to the lack of antigens in the transplanted cornea. This weak tissue sensitization may account for the absence of typical and full rejection of corneal allografts in these experiments and in those reported previously with non-specific intraocular inflammation. Localization of PMNL, round cells, and vessels in the graft was mostly in the anterior part of the stroma, which is consistent with histologic observations in corneas with epithelial herpetic keratitis. The profuse leukocytic exudate into the anterior chamber following intraocular herpetic infections should theoretically bring enough sensitized lymphocytes to the endothelium to cause a rejection of this layer, but this did not occur. The majority of cells in the anterior chamber during the acute inflammatory process were polymorphonuclear cells which do not participate in the immune reaction. It is possible also that an inhibition of a host immune response to the graft, insufficient sensitization, or a shift of the immune response to herpes virus antigens may explain the absence of a corneal graft rejection.

REFERENCES