Penetrating Corneal Transplantation in the Inbred Rat: A New Model

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A model of orthotopic penetrating keratoplasty has been developed in the inbred rat using both avascular and prevascularized recipient beds. The surgical procedure is conventional and can be achieved with standard instrumentation. Isografts into avascular recipient beds (Fisher 344 into Fisher 344 strain combination) were successful and survived indefinitely with excellent corneal function judged either visually by clarity and lack of oedema, or histologically at autopsy. Allografts into avascular beds (DA into Fisher 344 strain combination) became cloudy and oedematous at a median of day 12 postgraft; 43% spontaneously recovered clarity while the remaining 57% remained opaque or became scarred. Penetrating grafts also were performed in eyes prevascularized by the placement of sutures approximately 3 weeks prior to transplantation. Most isografts into prevascularized and inflamed beds underwent a transient episode of oedema, which quickly resolved and was felt to result from postsurgical inflammation. All allografts into prevascularized beds became oedematous and cloudy; 76% went on to fail completely, while 24% cleared without treatment. End-point histology showed normal graft morphology in the isografts; failed allografts showed a picture consistent with immunologic rejection. The model, which allows corneal transplantation to be performed against a constant histocompatibility barrier, may be useful in studies of rejection. Invest Ophthalmol Vis Sci 26:23-30, 1985

It has long been clear that the development of a model of orthotopic penetrating keratoplasty in inbred animal strains would be of value in studies of corneal graft rejection, in that transplantation could be performed against a well-defined histocompatibility barrier and factors associated with anatomic position could be considered.

The rabbit is the most commonly used animal in experimental models of penetrating corneal transplantation. Advantages of the rabbit include the relative ease of handling and caging of the species, the similarity in size and shape between the rabbit and human eye, which allows the use of conventional ophthalmic surgery techniques, and the vast published literature describing various aspects of this model.

From an immunologic point of view, however, there are a number of problems associated with the use of the rabbit, preeminent amongst which is the difficulty of studying immunologic mechanisms in an outbred strain where the degree of histocompatibility between donor and recipient animals cannot be controlled. Laboratory rabbit strains are generally bred in closed colonies (so that a limited degree of histocompatibility can be expected) but are not inbred. Imperfect knowledge of the rabbit major histocompatibility complex (MHC) antigen system and a dearth of available antibody reagents to either MHC antigens or leucocyte subsets compound the problem.

Models of heterotopic full-thickness corneal transplantation in the inbred mouse and orthotopic lamellar corneal transplantation in the inbred rat have been described. The major drawback to these approaches is that no appropriate continuing assessment of endothelial viability (and therefore corneal function) can be made. Additionally, corneal graft rejection is known to be influenced by both anatomic position and the degree of vascularization of the recipient bed, the “privilege” ascribed to corneal grafts breaking down with increased recipient vascularization and inflammation. An interesting approach was described by Lang and his colleagues, who inserted full-thickness corneal pieces into interlamellar corneal pockets in inbred rats and were able to follow the fate of the grafts in both avascular and vascularized eyes across histocompatibility barriers of varying strengths.

We have developed a model of orthotopic penetrating keratoplasty in the inbred rat. The procedure
can be performed by a skilled technician and success is achieved reliably using standard procedures and instrumentation. Because of the relationship between vascularization or inflammation and subsequent allograft rejection following corneal transplantation, appropriate models were developed in both avascular and prevascularized recipient eyes.

Materials and Methods

Experimental Animals

Virgin female rats of the Fisher 344 (RT1|) and DA (RT1a) inbred strains were used. Rats weighed 150-200 g at operation. The right eye only of recipient animals was operated upon, so that no animal was blinded.

Premedication and Anaesthesia

Recipient: Fifteen minutes before beginning the procedure, one drop each of 1% atropine sulphate (“Atropin”, Sigma), 1% N-ethyl-N(4-pyridyl methyl)tropamide (“Mydriacyl”, Alcon) and 10% viscous phenylephrine hydrochloride (“Neosynephrine”, Winthrop Laboratories) was instilled into the right eye of the animal. Periorbital hair and whiskers obscuring the field of view were trimmed with scissors. The left eye was taped closed to prevent corneal drying. Inhalation anaesthesia was induced using a halothane vaporizer (Fluotec 3) providing 3–3.5 ppm halothane in oxygen at a flow rate of 2 L per min oxygen. The halothane concentration was varied over the range 2.5–4 ppm during the procedure, at the discretion of the operator. Gases were delivered with a small nose cone made from a syringe barrel, and a simple scavenging system was employed. At the end of the operation, the animal was given oxygen for up to a minute before being returned to its cage.

Donor: The donor animal was killed by overdose of inhalation anaesthetic, once the recipient was anaesthetized.

Surgical Procedure

The operation was performed under a Zeiss Mark I operating microscope with ×12.5 eyepiece and ×16 objective lens magnification.

The recipient eye was propsected by inserting two loops of 4-0 silk under the superior and inferior rectus muscles and weighting the ends with artery forceps. The donor button then was prepared. The donor eye was propsected with forceps. A 3-mm diameter trephine was used to score the cornea and was twisted until the anterior chamber was just entered as evinced by appearance of anterior chamber fluid. Corneal scissors were used to complete the dissection. The button was left sitting on the donor eye, while the recipient was prepared. The same trephine was used to score a partial thickness 3-mm diameter disc on the recipient cornea, the dissection being completed with scissors as before.

The donor button then was transferred to the recipient eye and secured with four interrupted 10-0 monofilament nylon sutures (Alcon, 1921), one in each quadrant. One or two additional interrupted stitches then were placed in each quadrant, with care being taken not to enter the anterior chamber. Loose ends were cut as short as possible, but no attempt was made to bury the knots. The cornea was kept moist with a balanced salt solution designed for ophthalmic use (Alcon ophthalmic BSS) throughout the operation. Failure to keep the crystalline lens wet during the procedure invariably resulted in cataract formation. No attempt was made to reform the anterior chamber at the end of the procedure. Immediately following surgery, one drop each of 1% atropine sulphate and 0.5% chloramphenicol (“Chlor-
sig," Sigma) was applied to the grafted eye. Chloramphenicol ointment, 1%, was applied to the graft after the animal had recovered from the anaesthetic.

All instruments except the trephine were autoclaved before use. The trephine was soaked for 5 min in 70% alcohol. Care was taken to keep the operating field clean, but no attempt was made at strict asepsis, and the recipient animal was not draped. The whole procedure took, on average, 30–40 min with the graft taking 15–20 min to suture in place.

Postoperative Care and Inspection

Antibiotic ointment and atropine sulphate were instilled into the grafted eye routinely every second day for the first week postgraft. The sutures were not removed postoperatively. Each animal was inspected thrice weekly under the operating microscope and the graft scored for clarity, degree of oedema, inflammation, iris synechiae, correct siting of the graft, wound healing, extent of vascularization and cataract formation. A proportion of the later grafts also were examined at the slit lamp.

Prevascularization of the Rat Eye

In some experiments, recipient corneas were prevascularized before insertion of a graft. Two 10-0 nylon sutures were placed in the cornea and the
knobs left exposed. Two to three weeks later, when vessels had grown well in towards the sutures, the stitches were removed and penetrating keratoplasty performed as before.

**Histology**

Recipient animals were killed at intervals for endpoint histology. Enucleated globes were fixed in buffered formalin and embedded in JB-4 (Polysciences). Two-micron-thick sections were stained with hematoxylin and eosin (H and E) and duplicates with May-Grünwald-Giemsa.

**Results**

**Penetrating Grafts into Avascular Beds**

In a series of 21 Fisher 344 to Fisher 344 transplants, grafts were found to be clear and thin at 48 to 72 hours postgraft and remained so indefinitely (Fig. 1). Histologic sections of long-surviving grafts (>100 days) showed normal morphology (Fig. 2).

Conical allografts from DA to Fisher 344 strain animals (N = 28) also cleared in the same way as did isografts. However, all corneas thickened and clouded to a variable extent between days 4 and 28 (median, day 12) postgraft. Forty-three percent (N = 12) of these grafts showed minimal oedema and regained clarity spontaneously after 1 or 2 weeks. The remaining 57% (N = 16) became steadily more and more oedematous and opaque (Fig. 3) or shrunken and scarred. Histologic sections of these corneas showed a moderate-to-marked mononuclear cell infiltrate, a thin epithelium and frequently missing or damaged endothelium, features consistent with immunologic rejection (Fig. 4).

**Penetrating Grafts into Prevascularized Beds**

Isografts and allografts into prevascularized beds were clear and thin within 4 days (median, 1 day) postgraft. Of 13 isografts, all but three developed a transient degree of oedema between 5 and 10 days postgraft, which spontaneously resolved within a fur-
Fig. 4. B. Endothelium and posterior stroma. Note the mononuclear cell infiltrate.

ther 10 days. In a series of 21 allografts, all became thick and cloudy within 12 days postgraft (median day 6) and five (24%) regained clarity (at days 22, 23, 27, 32, and 38 postgraft). The eventual outcome of all grafts is summarized in Table 1. Histology of corneal isografts showed near normal architecture with a minimal stromal infiltrate of polymorphonuclear leucocytes in some sections, whereas the failed allografts appeared to have undergone rejection (Fig. 5), with sections showing endothelial and sometimes epithelial cell loss, a marked mononuclear cell infiltrate and stromal oedema. Examination of the five allografts that appeared to have cleared, showed evidence of some damage with thin epithelium and a slight mononuclear cell infiltrate.

**Discussion**

This study suggests that penetrating keratoplasty in the inbred rat will provide a useful model for the analysis of corneal graft rejection. The technique is simple, and the outcome of surgery predictable. It has a number of advantages over the traditional rabbit experimental model. The surgery is at least as successful, the operating time per animal is the same or less, and the animals are less expensive to purchase and maintain. In addition, the ready availability of inbred strains allows transplantation to be performed against a constant histocompatibility barrier, and monoclonal antibodies are available for the identification of cells involved in the allograft response. The authors are aware that an apparently similar technique has been used to investigate the healing-in process of

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<th>Strain combination</th>
<th>Recipient eye</th>
<th>Eventual graft outcome</th>
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<tr>
<td>Fi 344-Fi 344</td>
<td>Avascular</td>
<td>Clear 21, Oedematous 0</td>
</tr>
<tr>
<td>DA-Fi 344</td>
<td>Avascular</td>
<td>Clear 12, Oedematous 16</td>
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<td>Fi 344-Fi 344</td>
<td>Prevascularized</td>
<td>Clear 13, Oedematous 0</td>
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<td>DA-Fi 344</td>
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rat corneas stored in two different storage media, but in which no comparison of the immunologic response to isografts and allografts was made.

Several aspects of the technique of corneal transplantation in the rat require comment. The major difficulties encountered were related to the relatively large size of the crystalline lens and the very shallow anterior chamber. When cutting the cornea, it was found to be essential to lift it well away from the iris and the lens. No particular difficulty was encountered in trephining and cutting the recipient or donor buttons. The lens invariably became cataractous if allowed to dry, and repeated irrigation with a balanced salt solution throughout the procedure was necessary. The rat cornea was found to be relatively tough compared with the human or rabbit cornea, and use of a very sharp needle was essential. The Alcon sutures proved most satisfactory. Interrupted sutures were more effective in achieving a water-tight closure than was a running stitch. It was found to be safer to allow the anterior chamber to reform spontaneously after surgery, rather than attempting to reform it at the end of the procedure.

The rat eye proved to be easier to handle than the rabbit eye in a number of respects. Coagulation of the anterior chamber fluid (as occurs in rabbits) was not a problem and application of topical heparin was unnecessary. The epithelium rapidly covered exposed sutures so that knots left exposed generally were covered within 3-4 days. Infection was seldom a problem.

In view of the small size of the graft, and the inevitable damage to the endothelium that must occur during the procedure, we questioned how much endothelium actually was transferred with the donor button. Examination of donor buttons after excision showed endothelial cells present over the button, with loss at the cut edge only. The fact that grafts appeared clear and thin within 72 hr (and usually with 24 hr) of the procedure also suggested that donor endothelium was present and functioning. Furthermore, preliminary experiments have shown no evidence of rat

Fig. 5. A, Section of corneal isograft placed into prevascularized beds at 4 weeks postgraft (Magnification x1,920; hemotoxylin and eosin).
Isografts into normal eyes showed no evidence of rejection, remaining clear and thin indefinitely. The majority of isografts into prevascularized beds showed a transient degree of oedema, which resolved without treatment. Clearly this could not have involved a response to foreign antigens on the graft and was most probably a temporary inflammation, induced by the surgical procedure in an already inflamed eye, which subsided as the graft healed in. Histologic examination of several animals killed during this phase showed a polymorphonuclear cell infiltrate in the grafted corneas.

The majority of allografts into avascular eyes appeared to undergo an irreversible rejection. A lesser
number thickened briefly and then cleared again, although some of these grafts then developed a scarred appearance. We suggest that these animals may have undergone a rejection episode, which resolved spontaneously without treatment, leaving a degree of scarring in some cases. Corneal allografts into avascular human and rabbit eyes seldom reject; the reason for the higher percentage of rejected grafts in the rat model may result from the additional surgical trauma and inflammation that are inevitable in such small eyes.

Seventy-five percent of allografts into prevascularized eyes failed; end-point histology showed edematous grafts with the endothelium usually missing, the epithelium sometimes thin and damaged, and a moderate-to-marked mononuclear cell infiltrate in the stroma. The appearance was thus strongly indicative of rejection.

Animals that reached 100 days with clear, thin grafts were deemed to be long survivors and were killed. Histologic examination was thus possible for every graft. A review of all sections showed that occasional grafts had failed because of infection. Some allografts showed evidence of retro-endothelial membrane formation although the incidence of this complication declined as the operator became more experienced, probably because of better apposition of wound edges.

In summary, a model of orthotopic penetrating keratoplasty in the inbred rat has been developed, in which the majority of allografts placed into prevascularized beds appear to undergo a classical rejection phenomenon. The model may be useful in studying the mechanisms of corneal graft rejection.

**Key words:** penetrating keratoplasty, orthotopic transplantation, inbred rats, animal model

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**References**