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Experimental Orthotopic Penetrating Keratoplasty

—A Rat Penetrating Keratoplasty Model—

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An orthotopic penetrating keratoplasty model was developed in the rat. An oversized(0.5mm) graft was used and 8 interrupted sutures were applied. These sutures were not removed. Eleven grafts out of 13 were rejected by the 3rd week in the disparate group(Brown Norway rat to Lewis rat transplantation group), which was characterized by edema, opacity, and neovascularization. All grafts remained clear in the syngeneic group(Lewis rat to Lewis rat transplantation group). Immunohistochemical examination was performed. This model seems to be a reliable and reproducible one to evaluate rejection reaction in corneal transplantation

Key Words : *Animal model, Penetrating keratoplasty, Rat, Orthotopic, Rejection reaction*

INTRODUCTION

Corneal transplantation is a very successful procedure to restore vision in patients with corneal opacification. However, 6-40% of patients with corneal transplants undergo allograft rejection reaction, and currently, rejection reaction is a leading cause of graft failure (Alldredge and Kramer, 1981 ; Arentsen, 1983 ; Chandler and Kaufman, 1974). Therefore, it is crucial to understand the mechanism of rejection reaction in order to prevent this phenomenon.

Recent studies began to demonstrate the efficacy of the rat penetrating keratoplasty model for studying allograft rejection reaction (Moran et al., 1988 ; Williams and Coster, 1985). It is an orthotopic transplantation model and also a histocompatability antigen controllable model. In the present study, we describe our own rat penetrating keratoplasty model which has the following different points from previously described rat model, 1) an oversized graft was performed, 2) the corneal sutures were not removed, and 3) immunohistochemical staining was done.

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MATERIALS AND METHODS

Rats

Female rats of the Lewis and Brown Norway inbred strains were used. The rats weighed 150-200 gm at operation. Lewis rats were used as recipients and donors in the syngeneic transplantation group and recipients in the disparate transplantation

group. Brown Norway rats were used as donors in the disparate transplantation group.

Penetrating keratoplasty procedure

The rats were anesthetized with intramuscular injection of ketamine (25 mg/kg) and xylazine(3 mg/kg). After anesthesia, 10% phenylnephrine and 2.5% mydriacyl were instilled in one eye of the recipient animal for maximal dilation of the pupil. Only one eye was operated on so that functional visual acuity was maintained. Some of the animals were killed by lethal injection of a barbiturate for preparation of the donor cornea. With an aid of an operating microscope a 3.5-mm diameter trephine was used to cut the central corneal button from the donor eye. After entering the anterior chamber, the excision was then completed with a corneal scissors. The button was left sitting on the donor eye with drops of a balanced salt solution while the recipient was being prepared. The recipient cornea was prepared by using a 3.0-mm trephine, the excision being completed with scissors as before. The donor button was then transferred and sutured to the recipient wound with 8 interrupted 10-0 nylon sutures (Fig. 1). The loose ends were cut as short as possible. The cornea and lens were moistened throughout the procedure with balanced salt solution. Subconjunctival and topical gentamycin were administered at the end of the procedure. No steroids were given. The sutures were not removed postoperatively. All animals were followed-up for 3 weeks.

Clinical evaluation

Each animal was inspected twice a week under the operating microscope. Quantitative evaluation was based on grades of 0-4 for each of the following categories : corneal opacity, edema, and vascularization (Table).

Histologic and immunohistochemical evaluation

All recipients animals were killed by a lethal dose of barbiturate 3 weeks after the operation. The corneas of the enucleated eyes were stained using hematoxylin-eosin, as well as using monoclonal antibodies against macrophage (OX-42), T helper/inducer cells (W3/25), and T suppressor/cytotoxic cells (OX-8). The avidin-biotin-peroxidase complex method (Hsu et al., 1981) was used for this immunohistochmical staining.

RESULTS

Syngeneic corneal transplantation group

Fifteen Lewis to Lewis syngeneic corneal transplantations were performed. Two rats were killed during observation because of wound disruption. In 13 rats, mild postoperative edema (grade 1-2) was seen on the all grafted cornea immediate postoperatively. The edema usually cleared within 5 days. Starting at the 2nd week, peripheral vascularization was seen surrounding the sutres in many of the grafts (grade 1-2), but the grafted corneas were relatively clear with no edema (grade 0-2). By the 3rd week, all of the grafts remained clear (grade 0-1) without any sign of rejection reaction so that the underlying iris was clearly visible (Fig. 2). There was a slight increase in vascularization but not extending to the center (grade 2-3). Hematoxylin-eosin staining at the 3rd week showed normal-looking corneal layer in the grafts and the few inflammatory cells, especially around the sutures (Fig. 3). Immunohistochemical evaluation revealed that these inflammatory cells were macrophages (OX-42) and occasional T helper/inducer cells (W3/25) (Fig. 4)

Disparate corneal transplantation group

Among the 17 Brown Norway to Lewis disparate transplantation group, 4 rats were excluded because of wound disruption (two grafts) and hyphema (two grafts). There was mild corneal edema (grade 1) just after transplantation, and this initial corneal edema was resolved within a week. In 11 rats, rejection reaction occured by the

Table. Items and Scales of Rejection Score

Grade	Opacity	Edema	Neovascularization
0	none	none	none
1	slight	slight	present but not to the suture
2	moderate (iris vessel obscured)	moderate	to the suture
3	marked (hardly visible iris)	marked	passed the suture
4	extreme (whitish opaque)	extreme	to the center

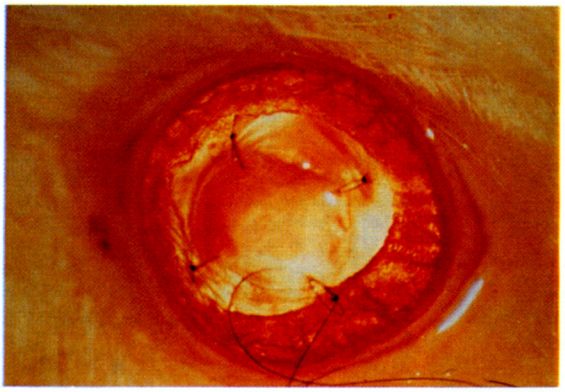

Fig. 1. Rat penetrating keratoplasty model. Good wound apposition between donor button and recipient cornea was obtained after 4 cardinal sutures.

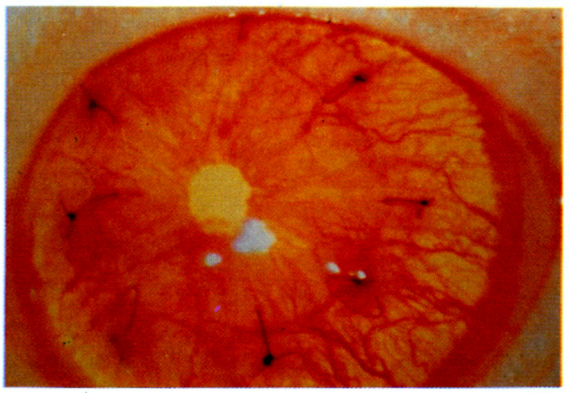

Fig. 2. Corneal graft in the syngeneic group, 3 weeks postoperatively. The cornea was clear without edema so that the underlying iris was clearly visible. Occasional neovascularization was found around the sutures.

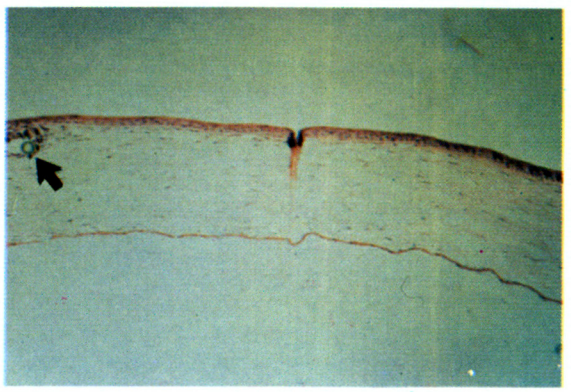

Fig. 3. Hematoxylin-eosin staining of grafted cornea in the syngeneic group, 3 weeks postoperatively. The grafted cornea showed normal looking corneal layers, and some inflammatory cells were seen around the sutures (arrow).

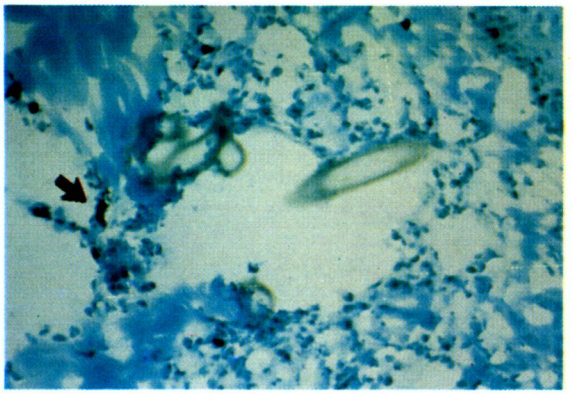

Fig. 4. Immunohistochemical staining of grafted cornea in the syngeneic group, 3 weeks postoperatively. Macrophages (arrow) were seen around the sutures.

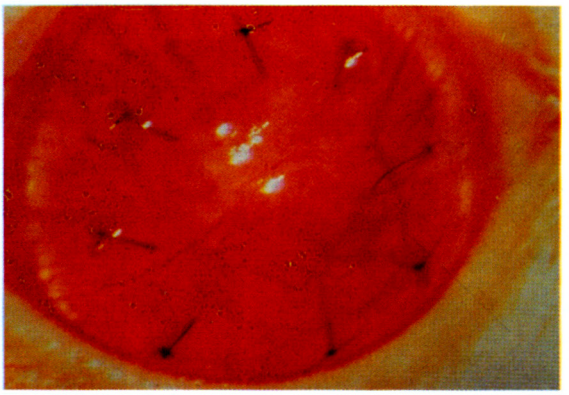

Fig. 5. Corneal graft in the disparate group, 3 weeks postoperatively. The iris vessels were hardly visible through the opaque cornea, and new vessels grew almost to the center.

2nd to 3rd week and was characterized by opacity (grade 2-3), increased edema (grade 2-3), and progressive vascularization (grade 3-4) in the corneas (Fig. 5). There was a heavy infiltration of inflammatory cells in the donor cornea by the 3rd week (Fig. 6), and the endothelial layer was almost destroyed. Immunohistochemical staining at the 3rd week revealed that these inflammatory cells were mainly T suppressor/cytotoxic cells (0×-8), occasional T helper/inducer cells (W3/25), and macropages (0×-42) (Fig. 7). Two rats had clear grafts without any signs of rejection by the 3rd week.

DISCUSSION

The rat keratoplasty model was developed rece-

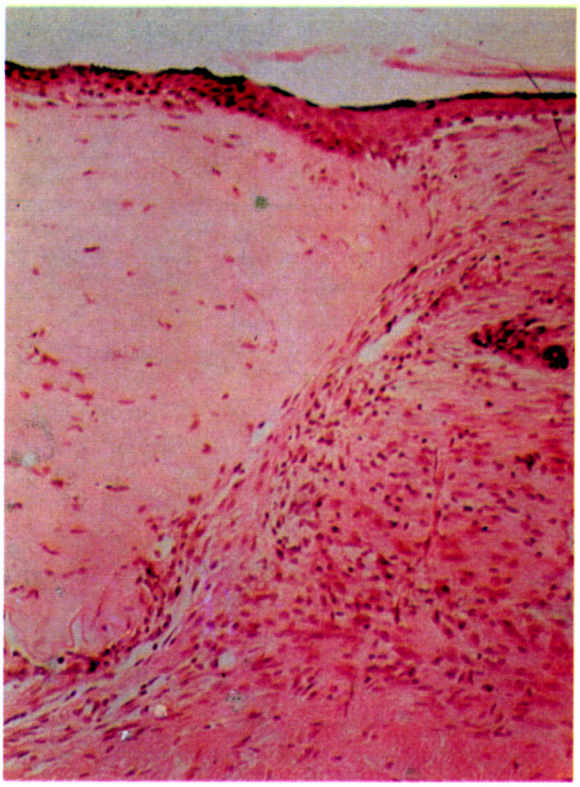

Fig. 6. Hematoxylin-eosin staining of the corneal wound area in the disparate group, 3 weeks postoperatively. There was heavy infiltration of inflammatory cells in the grafted cornea (right field in the picture).

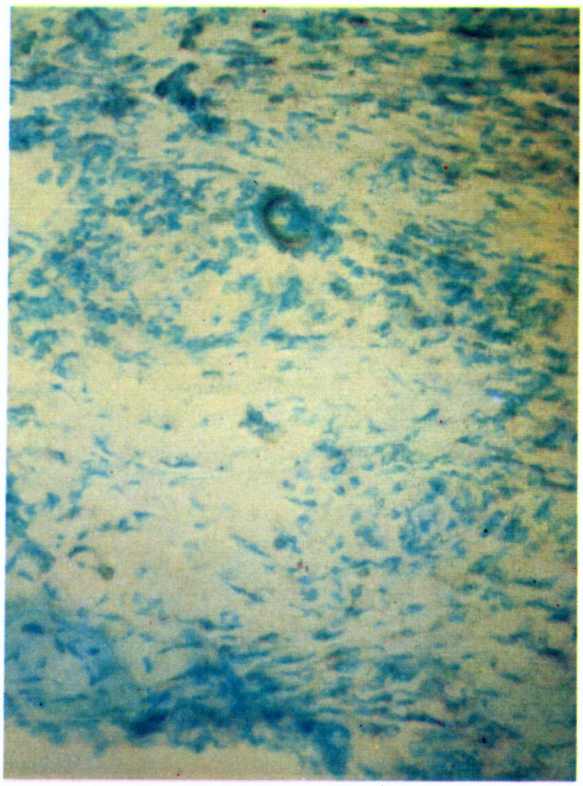

Fig. 7. Immunohistochemical staining of grafted cornea in the disparate group, 3 weeks postoperatively. There was an infiltration of T suppressor/cytotoxic cells.

ntly for the study of corneal graft rejection reaction. This experiment demonstrates that the rat penetrating keratoplasty model is a useful model to evaluate the corneal allograft rejection reaction.

The rabbit is the most commonly used animal in Ophthalmology. There is, however, no pure inbred rabbit line, so that the degree of histocompatability between the donor and recipient animals cannot be controlled. Furthermore, the major histocompatability (MHC) antigen system in rabbit is not fully understood. A model which controls the input of the MHC antigens is heterotopic corneal transplantation in the mouse (Chandler et al., 1982/83). The thoracic wall is commonly used in this model because mouse eye is too small to allow for successful orthotopic grafts. Not being able to assess the endothelial function is a big disadvantage of this model.

Recent advances in understanding rat MHC enabled corneal transplantation studies in the rats. The rat eye is large enough so that orthotopic grafts are possible. Therefore, the rat penetrating keratoplasty model is desirable because of the controlled MHC input, ease of handling the animals, and their relative inexpensiveness. One disadvantage of the rat model is that rat endothelium has regenerative power unlike human endothelium (Tuft et al., 1986). This means that rejected endothelium can be replaced by regenerated recipient endothelium. However, endothelial regeneration did not play a role in our model because endothelium was not seen in the rejected cornea.

In our rat model, we made some modifications. A slight larger (0.5 mm) trephine was used to cut the donor button (The same sized corneal graft was performed in the previously described rat model [Moran et al., 1988 ; Williams and Coster, 1985]). This enabled us to deepen the anterior chamber more easily and produce a more secure wound apposition. Nylon sutures were left during the whole examination period. Sutures usually attract vessels and, in the disparate group, new ves-

sels on the cornea might be one of the factors which increase the rejection rate which is desirable in our studies. The rejection rate of the disparate group was 84.6% compared to 57.0% in previous study which recommended suture removal (Moran et al., 1988). (The rejection rate in our earlier study was 92% [Holland et al., 1987]).

Immunohistochemical evaluation revealed that T-suppressor/cytotoxic cell, T-helper/inducer cell, and macrophage were involved in rejection reaction. T-suppressor/cytotoxic cells seem to play more of a role in rejection reaction considering their dorminant presence in rejected cornea. The cells surrounding the sutures were mainly macrophages. They were more prominent in the syngeneic grafts. It appears that the inflammatory response to the sutures may have palyed a major role in initiating the graft rejection reaction. These findings, however, was those of 3 weeks postoperatively. The study of kinetics of inflammatory cells is needed to evaluate their exact roles in rejection reaction.

Wound disruption occurred during observation. We did not anesthetize the animals at follow up observation because a possible overdose of anesthetics could kill the animals and cause epithelial damage. Sometimes the eyes were squeezed while holding the animals, and then hyphema and wound disruption occurred. Skillful holding is required to decrease wound disruption.

In summary, this orthotopic model for penetrating keratoplasty is a reliable and reproduclble model to evaluate the corneal allograft rejection reaction.

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