Ocular Resurfacing and Alloepithelial Rejection in a Murine Keratoepithelioplasty Model

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Purpose. To determine definitively the epithelial origin of corneal resurfacing and to elucidate the immunologic mechanisms of epithelial rejection in a murine keratoepithelioplasty (KEP) model.

Methods. After corneal epithelial removal and peritomy, donor corneal lenticules were grafted around the limbus (KEP procedure). The process of corneal reepithelialization was observed with 0.25% methylene blue staining. The origin of the renewed epithelium was determined by immunofluorescence. Syngeneic corneal lenticules were grafted to BALB/c mice. C3H/He, C57BL/6, BALB.K, DBA/2, and B10.D2 allogeneic corneal lenticules were grafted to BALB/c mice, and A.SW and A.TL allogeneic corneal lenticules were grafted to A.TH mice. Alloepithelial rejection was evaluated on the basis of clinical findings and histologic changes in grafted corneas.

Results. All KEP grafts were reepithelialized entirely at 3 days after surgery. The renewed epithelium proved to be derived from the lenticules in BALB/c eyes receiving C3H/He lenticules. In syngeneic grafts 5 days after KEP, the cornea recovered clarity and smoothness, which persisted to the end of the study. After complete reepithelialization, all allogeneic grafts also experienced a short duration of clear cornea. Then followed four characteristic phases of inflammatory epithelial response: initial phase, acute phase, chronic phase, and rejected phase. Histologic examination confirmed the progress and severity of inflammatory response. The mean onset times of initial phase in assorted grafts with mismatched histocompatibility antigens were: 7.9 ± 1.8 days for both major and minor disparity grafts, 9.5 ± 3.8 days for major disparity grafts, 18.2 ± 5.5 days for major histocompatibility class I disparity grafts, 25.6 ± 7.2 days for major histocompatibility class II disparity grafts, and 9.2 ± 2.2 days for multiple minor disparity grafts.

Conclusions. In donor corneal lenticule grafting to host eyes with corneal epithelium removed and conjunctival peritomy, the ocular surface was reepithelialized by lenticule-derived epithelium. Alloepithelial rejection in this model displayed characteristic manifestations and well-defined processes, enabling easy and precise evaluation of onset and intensity of graft rejection. Both major and minor histocompatibility antigens are related to corneal epithelial rejection. Invest Ophthalmol Vis Sci. 1995;36:2623–2633.
success of such transplants depends on the availability of healthy autologous conjunctival or limbal tissues, these techniques cannot be used in patients with bilaterally damaged ocular surfaces. Additionally, in patients with chronic ocular surface disorder, the use of conventional penetrating keratoplasty or lamellar keratoplasty usually offers an extremely poor prognosis. 8-10 For these reasons, Thoft 7 in 1984 first developed a new corneal transplantation procedure, known as keratoepithelioplasty (KEP), in which corneal lenticules from cadaver donor eyes are grafted to the recipient limbus of the superficially keratectomized cornea to reconstruct a new ocular surface. 7,11 Since then, several clinical studies have reported the application of KEP for treating ocular surface disorders, including chemical or thermal burns, 7,11-13 Stevens–Johnson syndrome, 11 band keratopathy, 11 aniridia, 11 atopic disease, 11 benign hereditary intraepithelial dyskeratosis, 11 and Mooren’s ulcer. 14 Despite reports of its notable efficacy, the KEP procedure has not been used extensively. This is because, as Kaufman 15 suggested in 1984, it is not clear whether the grafted corneal lenticules actually are able to reepithelialize the denuded corneal surface and whether the alloepithelial graft increases antigenicity and results in immunologic rejection. Still, few basic studies to date have been performed to clarify the mechanism of KEP, and no firm evidence has been obtained to prove that corneal surface is reepithelialized by the grafted corneal lenticules.

Clinically, the renewed epithelium in most KEP grafts has been demonstrated to be steady in maintaining the reconstructed ocular surface under attentive postoperative care, combined with comparative long-term use of corticosteroid. 11,14-16 However, certain patients exhibited intense postoperative epithelial responses that could not be controlled by immunosuppressive agents. 16 Although those responses were considered to be immunologic allograft rejection, this judgment was generally based on the clinical observation of human eyes. The mechanisms of this type of “epithelial rejection” and whether these responses are actually immunologic reactions remain unclear. We have used the KEP procedure to establish a rat KEP model and found a similarity between intensive epithelial responses in rats and humans. 17 To determine definitively the origin of renewed corneal epithelium by KEP graft and to elucidate the immunologic mechanism of epithelial rejection, we report here the establishment of a murine KEP model. Varieties of histocompatibility antigens influencing alloepithelial rejection were investigated through tissue antigen mismatching by different murine strain combinations.

**MATERIALS AND METHODS**

**Animals**

The following strains of 8- to 10-week-old C3H/He, C57BL/6, DBA/2, B10.D2, and BALB/c mice were purchased from Japanese SLC Inc. (Osaka, Japan). BALB.K mice were kindly provided by Dr. Hiroshi Yamamoto of the National Institute of Neuroscience (Tokyo, Japan), and A.TH, A.TL and A.SW mice were kindly provided by Dr. Kazuo Moriwaki of the National Institute of Genetics (Mishima, Japan). The strain combinations with mismatched histocompatibility antigens are presented in Table 1. All animals in our studies were female. The animals were handled in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**Mice Keratoepithelioplasty Grafting**

The methods of KEP grafting in mice were modified from the procedures previously described in rats. 17 In detail, the donor animal was anesthetized with a mixture of ketamine 0.2 mg and xylazine 0.2 mg in a 0.4 ml phosphate-buffered saline solution (PBS). The full-thickness cornea was excised along the limbus and cut into four 1.0 mm X 2.5 mm lenticules with Vanas long-neck scissors (Inami, Tokyo, Japan). The lenticules were kept in RPMI 1640 medium (Nikken Biological Medical Laboratory, Kyoto, Japan) until use.

The cornea of the anesthetized recipient was then prepared. First, the corneal epithelium extending from limbus to central cornea was scraped off carefully with a multisided sclerotome (Becton Dickinson Acute Care, Franklin Lakes, NJ); 560° peritomy was then performed to remove 1.0 to 1.5 mm of adjacent limbal conjunctiva. Complete debridement of the corneal epithelium, including the limbus, was confirmed with 0.25% methylene blue staining in the KEP recipients (Fig. 1A), and further confirmed histologically in three euthanized animals, with the same epithelial removal technique as in the KEP recipients (data not shown). The donor lenticule was grasped at the edge with a Born iris forceps (Inami, Tokyo, Japan); three lenticules were secured to the limbus of the recipient eye, each with two interrupted 11-0 nylon sutures (Alcon Laboratories, Ft. Worth, TX) (Fig. 1A). At the end of surgery, ofloxacin antibiotic ointment was placed in the conjunctival sac of the operated eye, and the eyelids were closed with one 11-0 nylon suture. Only the right eye of each recipient received grafts.

**Postoperative Care and Observation**

The eyelid-closing suture was removed 3 days after grafting; no further antibiotic ointment was applied. Lenticule-securing sutures were not removed after surgery. The eyes were inspected every 2 days for 30 days and once a week afterward for a total of 120 days. If not specified, no immunosuppressive agents were used.

Reepithelialization of the KEP-grafted cornea was confirmed by staining with 0.25% methylene blue. After complete reepithelialization, subsequent epithelial edema, corneal opacity, or both were graded 0 to 4.
Antibodies and Immunofluorescent Staining

After being rewashed and mounted, the sections were observed under Olympus (Tokyo, Japan) fluorescent microscope. Cryostat sections of naive C3H/He mouse keratoepithelioplasty model served as a positive control for anti-H-2Kk staining, and sections of naive BALB/c cornea as a positive control for anti-H-2Dd staining. Mouse IgG constituted negative control for antibodies of anti-H-2Kk and anti-H-2Dd.

Histologic Evaluation

Eyeballs with KEP grafts were enucleated at different time intervals and fixed in 10% formalin. Paraffin-embedded tissues were sectioned to 6-μm thickness and stained with hematoxylin and eosin for histologic evaluation under conventional light microscopy.

RESULTS

Of 150 recipients of KEP grafts, 10 were excluded from the study because of limbal corneal perforation and anterior chamber hemorrhage during surgery, and nine were excluded because of suture loosening and lenticule sloughing during the observation period.

Reepithelialization of Corneal Surface by Keratoepithelioplasty Grafts

To observe corneal reepithelialization by KEP grafts, the eyelid-closing suture was removed 24 hours after surgery in six animals, two with syngeneic grafts of female BALB/c to female BALB/c, the other four with allogeneic grafts (two C3H/He to BALB/c and two B10.D2 to BALB/c). Figure 1 presents the exemplary processes of corneal reepithelialization. One day after surgery, one-third of the corneal surface was reepithelialized (Fig. 1B). Reepithelialization was observed to proceed from the grafted lenticules on the limbus, toward the central cornea. More than two-thirds of the corneal surface was reepithelialized 2 days after KEP (Fig. 1C), and the entire corneal surface was reepithelialized 3 days after KEP (Fig. 1D).

To determine whether the renewed epithelium originated from the grafted lenticules, we used strain

### TABLE 1. Summary of Strain Combinations and Epithelial Rejection States in Mouse Keratoepithelioplasty Model

<table>
<thead>
<tr>
<th>Donor</th>
<th>Recipient</th>
<th>Disparity</th>
<th>N</th>
<th>Time of Rejection Onset (Initial Phase)</th>
<th>Final Clear Cornea* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB/c</td>
<td>BALB/c</td>
<td>None</td>
<td>12</td>
<td>No rejection</td>
<td>12 (100)</td>
</tr>
<tr>
<td>C3H/He</td>
<td>BALB/c</td>
<td>H-2d, multiple minor H</td>
<td>16</td>
<td>7.7 ± 2.0 days</td>
<td>0 (0)</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>BALB/c</td>
<td>H-2d, multiple minor H</td>
<td>14</td>
<td>8.3 ± 1.5 days</td>
<td>0 (0)</td>
</tr>
<tr>
<td>BALB/K</td>
<td>BALB/c</td>
<td>H-2a haplotype</td>
<td>11</td>
<td>9.5 ± 3.8 days</td>
<td>2 (18)</td>
</tr>
<tr>
<td>A.SW</td>
<td>A.TH</td>
<td>H-2 Dd</td>
<td>8</td>
<td>18.2 ± 5.5 days</td>
<td>1 (12)</td>
</tr>
<tr>
<td>A.TL</td>
<td>A.TH</td>
<td>H-2 1a</td>
<td>9</td>
<td>25.6 ± 7.2 days</td>
<td>1 (11)</td>
</tr>
<tr>
<td>DBA/2</td>
<td>BALB/c</td>
<td>Multiple minor H</td>
<td>12</td>
<td>9.0 ± 2.3 days</td>
<td>0 (0)</td>
</tr>
<tr>
<td>B10.D2</td>
<td>BALB/c</td>
<td>Multiple minor H</td>
<td>13</td>
<td>9.5 ± 2.2 days</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

* Final corneal opacity score <2.

(0 = clear cornea; 1 = lenticular and regional corneal epithelial edema, opacity, or both; 2 = diffuse epithelial edema, corneal opacity, or both, obscuring iris vessels; 3 = diffuse epithelial edema, corneal opacity, or both, obscuring pupil margin; 4 = anterior chamber invisible caused by epithelial edema, corneal opacity, or both). Neovascularization also was graded 0 to 4 (0 = no vessels in lenticule and the cornea; 1 = vessels limited to around sutures or in lenticules; 2 = one-quarter of cornea vascularized; 3 = half of cornea vascularized; and 4 = entire cornea vascularized). After complete corneal reepithelialization, when the sum of the scores for each eye was >2, graft rejection onset was recorded.

Antibodies and Immunofluorescent Staining

Mouse monoclonal antibodies of anti-mouse H-2Kk (clone no. AF3-12.1) and anti-mouse H-2Dd (clone no. 34-2-12) were purchased from Pharmingen (San Diego, CA). Antibody of fluorescein isothiocyanate-conjugated goat anti-mouse IgG (γ + L)-F(ab')2 fraction was from Tago, Inc. (Burlingame, CA); mouse IgG was from Zymed Laboratories (San Francisco, CA).

Eyeballs of BALB/c mice grafted with C3H/He or BALB/c corneal lenticules were enucleated at 5 days after KEP, frozen in Tissue Tek II OCT compound on dry ice, and stored at −70°C. Cryostat sections 6-μm thick on gelatin-coated slides were then made. The sections, air-dried for 30 minutes and fixed in acetone for 5 minutes, were washed in cold 0.1 M PBS three times for 30 minutes and then primarily overlaid by 10-fold-diluted mouse monoclonal antibodies of anti-H-2Kk or anti-H-2Dd (antibodies were diluted by 0.1% bovine serum albumin in 0.1 M PBS) at 4°C overnight. The sections were rewarshed in PBS three times for 30 minutes and reoverlaid by 100-fold-diluted secondary antibody of fluorescein isothiocyanate-conjugated goat anti-mouse IgG at 4°C overnight. After being rewarshed and mounted, the sections were observed under Olympus (Tokyo, Japan) fluorescent microscope. Cryostat sections of naive C3H/He cornea served as a positive control for anti-H-2Kk staining, and sections of naive BALB/c cornea as a positive control for anti-H-2Dd staining. Mouse IgG constituted negative control for antibodies of anti-H-2Kk and anti-H-2Dd.

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To determine whether the renewed epithelium originated from the grafted lenticules, we used strain
combinations of C3H/He (H-2k) to BALB/c (H-2d) and BALB/c to BALB/c for KEP grafting. After the entire corneal surface was reepithelialized, the eyes were enucleated 5 days after KEP, and cryostat sections were made. The immunofluorescence method is described in Materials and Methods. Staining results are presented in Figure 2. When anti-H-2Kk was used as the primary antibody, the regained epithelial cells of BALB/c corneas grafted with C3H/He lenticules were stained positively (Fig. 2A), whereas the epithelial cells of BALB/c corneas grafted with BALB/c lenticules were stained negatively (Fig. 2B). By contrast, when anti-H-2Dd was used as the primary antibody, negative staining of epithelium, but positive staining of stroma, were observed in BALB/c corneas grafted with C3H/He lenticules (Fig. 2C), whereas positive staining of epithelial cells and positive staining of stroma were observed in BALB/c corneas grafted with BALB/c lenticules (Fig. 2D). No positive staining was observed in sections using mouse IgG as the primary antibody for replacement of mouse anti-H-2 Kk and anti-H-2Dd monoclonal antibodies (data not shown). These results demonstrate that regained epithelium in the eyes with KEP grafts derived from the grafted lenticules and had the same antigenicity as that of lenticule donors.

Epithelial Responses After Keratoepithelioplasty

Epithelial Responses of Syngeneic Keratoepithelioplasty Grafts. First, we observed the postoperative epithelial responses in 12 eyes with syngeneic KEP grafts in the strain combination of female BALB/c to female BALB/c. The process of corneal reepithelialization after KEP has been described in Reepithelialization of Corneal Surface by Keratoepithelioplasty Grafts. After complete reepithelialization at 3 days, all 12 eyes had slight epithelial edema and lenticular edema, which resolved completely at 5 days after KEP. The clarity and smoothness of the grafted corneas recovered and persisted to the end of the study (Fig. 3). All 12 grafted eyes had slight neovessels around the sutures from day 5. These neovessels did not disappear thereafter, but they never invaded the host cornea. The mice were killed after 4 months of observation, and the enucleated eyes were examined histologically. Results demonstrated only a few infiltrated cells in the lenticular surroundings. In the host side cornea, the renewed epithelium was arranged regularly and the cell layers were almost normal. No inflammatory cells were observed in the epithelial layers—neither in the subepithelial area nor in the stroma (Fig. 4).

Epithelial Responses of Allogeneic Keratoepithelioplasty Grafts. In most allogeneic KEP grafts, we found that postoperative inflammatory response of the renewed epithelium was common. For easier understanding of the characteristics of alloepithelial responses in the KEP grafts, we first delineated these common processes and subsequently the influence of histocompatibility antigens on alloepithelial rejection of different onset times and intensities. After complete reepithelialization and a short duration of clear and smooth cornea, the allografts underwent the following four phases of inflammatory response: initial phase, acute phase, chronic phase, and rejected phase. The representative processes of these responses are shown in Figure 5. The initial phase displayed lenticular edema and regional corneal edema. The margin of regional corneal edema was associated with an obviously elevated line that could be stained by 0.25% methylene blue (Fig. 5A). The elevated edematous line in this model was remarkably similar to the previously defined epithelial rejection line in human eyes with KEP or penetrating keratoplasty grafts. The regional corneal edema and elevated line commenced either from one peripheral side of the cornea to the other (Fig. 5A) or from the entire periphery to the central cornea (data not shown).
FIGURE 3. (top) After reepithelialization of 12 syngeneic graft eyes of female BALB/c to female BALB/c, the corneal clarity and smoothness recovered and were maintained to the end of the 120-day study. Few neovessels were observed around the sutures.

FIGURE 4. (bottom) In the syngeneic grafts to female BALB/c corneas, the 12 grafted eyes were enucleated after 4 months of observation. Histologic examination demonstrated that the renewed epithelium was arranged regularly and cell layers were normal. No inflammatory cells appeared in the corneal epithelial layers or in the anterior stroma. Bar = 50 μm.

FIGURE 5. (A) Regional corneal edema and neovessel invasion were characteristics of alloepithelial rejection at the initial phase. The margin of regional corneal edema was associated with markedly elevated line, stained by methylene blue. (B) Subsequent to the initial phase, diffuse edema of the entire cornea, accompanied by severe invasion and injection of neovessels, indicated initiation of the acute rejection phase. (C) The cornea finally was covered by scarring and neovascularization with irregular ocular surface (the rejected phase). In these eyes, the clarity and smoothness of the grafted cornea finally recovered, and there were only a few neovessels.

For histologic evaluation of allogeneic KEP grafts in different phases, we used seven animals in strain combinations of C3H/He to BALB/c (mismatched in
major histocompatibility complex [MHC] majors and minors) and seven animals in strain combinations of B10.D2 to BALB/c (mismatched in multiple minors only). At different phases of epithelial response, as described above, the eyes were enucleated and examined histologically. At the initial phase, inflammatory cell infiltration occupied the surrounding area of grafted lenticules; however, the lenticular stroma had comparatively fewer inflammatory cells, and the Descemet's membrane of the lenticules was still intact at this time (Fig. 6A). Besides the lenticules, vigorous inflammatory cells appeared in the subepithelial area and anterior stroma of the host cornea. The intensity of cell infiltration was comparatively more severe in the peripheral cornea near the lenticules (Fig. 6A) than in the central cornea (Fig. 6B). Epithelial layers also were partially infiltrated by lymphocyte-like cells, and epithelial cell junctions were destroyed (Figs. 6A, 6C). The clinically observed rejection line at the initial phase presumably resulted from variations in the subepithelial and anterior stromal edema caused by the infiltrated inflammatory cells and by the infiltration of lymphocyte-like cells in the epithelial layers (Figs. 6B, 6C). Progressively in the acute phase, inflammatory cells further infiltrated the lenticular stroma (Fig. 7A). The epithelial layers of the host cornea were devastated, and further infiltration of lymphocyte-like cells was observed in the epithelial layers (Fig. 7B). After the acute phase of rejection, histologic changes in the graft sections showed some recession of inflammatory cells and lessened stromal edema of the lenticules and host cornea, which was consistent with the clinical findings of relieved epithelial rejection. Moreover, after the acute phase, partially retained corneal epithelium was noted in the grafted eyes (data not shown). The donor-derived epithelium retention on the cornea was presumably related to the later recommencement of chronic rejection. After repeated chronic rejection episodes, histologic examination demonstrated corneal surface was replaced by irregular monolayer epithelium, combined with multiple subepithelial neovessels and inflammatory cells (the rejected phase) (Figs. 8A, 8B). Throughout all phases, the inflammatory cells infiltrating the lenticule and host cornea predominantly comprised mononuclear cells and occasionally neutrophils.

**Histocompatibility Antigens on Epithelial Rejection**

All allografts, including the mismatches of entire majors and minors, entire majors only, MHC class I only, MHC class II only, and multiple minors, experienced at least one phase of epithelial response during the 4-month observation period. The results of allografts with different mismatches of histocompatibility antigens are summarized in Table 1. In the groups of C3H/He to BALB/c and C57BL/6 to BALB/c—the
only mismatched group recovered corneal clarity, with extensive recession of invading neovessels.

In the B10.D2 to BALB/c and DBA/2 to BALB/c groups, the allografts with disparity minor antigens only, the initial phase of rejection was 2 to 4 days later than in those with entire MHC major as well as minor disparate grafts; however, more than 90% of these eyes (20/22) experienced the acute rejection phase within 19 days. The chronic phase of rejection also was observed in these allografts, with no eyes recovering corneal clarity.

In the A.SW to A.TH or A.TL to A.TH groups, the allografts with only MHC class I or class II disparity, the initiating rejection phase was significantly later than it was in the grafts with entire MHC major and minor disparity or with minor only disparity. The progress of epithelial rejection in these eyes was slow, but 100% experienced allograft rejection. The onset time of the acute rejection phase in these eyes occurred sporadically over a wide range of the observation pe-

allografts with MHC major as well as minor disparity—100% of the grafted eyes experienced the initial rejection phase within 9 days, and more than 95% (29/30) experienced the acute rejection phase with higher than grade 3 of edema and/or opacity and with severe neovessel invasion within 15 days. After the acute phase of rejection, especially 6 and 9 weeks after KEP, all the eyes experienced the chronic phase of rejection, displaying mild lenticular and regional corneal edema and injection of invading neovessels. Finally, at the end of the study, no grafted eyes recovered corneal clarity (grade of opacity <2). In the allografts with only major disparity, i.e., the BALB.K lenticles grafted to BALB/c eyes, more than 90% (10/11) of the grafted eyes demonstrated the initial phase of rejection within 11 days of KEP, and more than 80% experienced the acute rejection phase within 17 days of KEP. In contrast to the grafts with MHC major as well as minor disparity, 2 of 11 eyes in the majors

FIGURE 7. (A) During the acute phase, two eyes from the group of B10.D2 to BALB/c mice (at 15 and 17 days, respectively, after grafting) and two eyes from the group of C3H/He to BALB/c mice (at 11 and 13 days, respectively, after grafting) were enucleated. Histologically, progressive infiltration of inflammatory cells was observed in the lenticular stroma. Bar = 60 μm. (B) The epithelial layers of the cornea were devastated, and lymphocyte-like cells were observed in the epithelial layers. Bar = 30 μm.

FIGURE 8. (A) At the rejected phase, three eyes from each allogeneic group (total, 15) were enucleated after 4 months of observation. Histologic examination made in the eyes with scarring and neovascularization showed that infiltrated cells persisted in the lenticule and that the lenticular stroma was loose. Bar = 60 μm. (B) The corneal surface was replaced by irregular monolayer epithelium, with neovessels and inflammatory cells in the subepithelial area and anterior corneal stroma. Bar = 50 μm.
DISCUSSION

Our established murine KEP model confirms a previous hypothesis that KEP, a surgical procedure in which donor corneal lenticules are grafted to the recipient limbus with superficial keratectomy and peritomy, can reconstruct the ocular surface through lenticule-originated epithelium.1 Our conclusion is derived from the following evidence: First, the process of corneal reepithelialization of the KEP-grafted eye was initiated from the grafted lenticules to the central cornea. Second, the renewed epithelium by KEP graft had antigenicity identical to that of the donor. Third, when eyes underwent limbal conjunctival and corneal epithelial removal by the same technique used in KEP recipients, but were not grafted with donor corneal lenticules around the limbus, the process of reepithelialization was significantly slower than in the KEP-grafted eyes. More important, these epithelium-removed corneas were not resurfaced by smooth and clear epithelium but by scarring and neovascularization (data not shown). These results demonstrate that donor lenticule grafting to the limbus is exceptionally important for normal reepithelialization of the denuded cornea.

Fourth, in KEP-grafted corneas that did not undergo allograft rejection, such as in syngeneic grafts, the renewed epithelium was steady and was almost as histologically normal as the naive cornea.

Alloepithelium grafted by KEP is sufficiently immunogenic to induce allograft rejection. We consider the alloepithelial responses in our model to be immunological graft rejection, based on the following evidence: First, the antigenicity of renewed epithelium in KEP grafts was identical to that of the donor. Second, after complete reepithelialization, all eyes with KEP grafts experienced a certain duration of clear and smooth corneas. Subsequently, inflammatory epithelial responses ensued in the grafts with mismatched histocompatibility antigens, but not in the syngeneic grafts. Third, the inflammatory epithelial response process was characterized by the progress of lenticular edema—edema of renewed epithelium on the host cornea—succeeded by neovascularization and scarring. These inflammatory responses are the same as the allograft rejection observed in penetrating or lamellar keratoplasty.16–21 Notably, the epithelial response process, accompanied by a typical rejection line in this murine KEP model, was closely similar to that observed in human eyes with KEP grafts16 or penetrating keratoplasty.18–21 Fourth, the inflammatory epithelial responses of edema and neovascularization were suppressed by the immunosuppressant agent FK506, but they did not respond to antibiotics (data not shown). FK506 has been demonstrated recently as efficient in suppressing corneal graft rejection in animal models.22–24 Fifth, the clinical findings of epithelial edema and neovessel invasion were accompanied histologically by inflammatory cell infiltration. The cells infiltrating the lenticules, the epithelial layers, and the anterior corneal stroma of KEP-grafted eyes mainly comprised mononuclear cells.

Alloepithelial rejection unquestionably devastated the refurbished ocular surface with scarring and neovascularization. Histologically, the rejected eye had abnormal monolayer epithelial cells and exhibited partial epithelial defects. The monolayer epithelium was arranged irregularly, with subepithelial neovessels and inflammatory cells, in the rejected eyes. We have not yet investigated whether the rejected ocular surface eventually was recovered by recipient conjunctival epithelial cells. However, histologic evidence suggests that the possibility of conjunctival epithelium finally recovering the ocular surface cannot be excluded.

In our KEP model, we found that the incidence and intensity of alloepithelial rejection were extraordinarily high. All allografts experienced graft rejection. This high incidence might result from: (1) The entire donor cornea, including the limbus, was used for lenticules, thus providing a large amount of antigens. (2) The donor lenticules were sewn along the limbus of the recipient cornea, in closest proximity to each recipient’s antigen-presenting cells (possibly Langerhans cells), which recognized the foreign antigens and triggered immunologic sensitization25,26; the reactive T cells also had easy access to the target cells. This is consistent with the fact that, in human penetrating keratoplasty, if the corneal graft is placed eccentrically or on a neovascularized bed, the incidence of graft rejection is increased dramatically. (3) The corneal lenticules did not contact the aqueous humor of the recipient anterior chamber in the KEP procedure. A recent report by Sonoda and Streilein27 on a murine penetrating keratoplasty model demonstrated that allogeneic corneal grafts bathed in aqueous humor induced anterior chamber associated-immune deviation, which caused suppression of delayed hypersensitivity specific to the corneal graft. The delayed hypersensitivity response suppression thus induced was presumed to have caused the recipient acceptance of allogeneic corneal grafts without rejection. Using anterior chamber inoculation of donor-identical lymphocytes before KEP in the rat, we also demonstrated the suppression of epithelial rejection, in company with suppression of delayed hypersensitivity response.28

The histocompatibility antigens, including major and minor antigens, are related to corneal epithelial rejection. Grafts with complete disparity in major as
well as minor histocompatibility antigens, such as in the strain combinations C3H/He or C57BL/6 to BALB/c, experienced the earliest and most vigorous graft rejection. However, the disparity in minor antigens only also induced intensive graft rejection. These results are in partial agreement with a recently published study by Sonoda and Streilein, who used the penetrating keratoplasty model in mice and indicated that the minor histocompatibility antigens were the major cause of graft rejection. We used the same strain combinations as described by them in our KEP model and found that allografts with MHC class I only disparity (A.SW to A.TH) or class II only disparity (A.TL to A.TH) also experienced a 100% incidence of rejection, even though the onset time was much later than in those with disparity in major as well as minor histocompatibility antigens, or in minor antigens only. However, in the strain combination BALB.K to BALB/c, in which the tissue antigens were mismatched in MHC major antigens only, the onset time and intensity of epithelial rejection were not significantly different from those seen with disparity in major as well as minor antigens or minor antigens only. The delay of graft rejection onset of MHC class I or class II disparate grafts may be explained as follows: Either MHC class I or class II antigen alone has lower response capacity to corneal allograft rejection, or A.TH recipients have lower response capacity to corneal allograft than BALB/c recipients. Our results on the investigation of histocompatibility antigens in the KEP model support the presumption made by Nicholls and coworkers that disparity at a single locus is significantly immunogenic to cause corneal allograft rejection.

The alloepithelial rejection in this model is obviously characterized by distinctive processes. The typical demonstrations of epithelial rejection in this model will enable the easy and precise clinical evaluation of onset and intensity of allograft rejection. The elucidated immunologic mechanisms of alloepithelial rejection in the murine KEP model will help in the understanding of the epithelial response in the human eye with KEP grafts. We think this model will be a valuable tool, either for investigating the immunologic mechanisms of corneal graft rejection or for studying epithelial characteristics after KEP grafting.

Key Words
allograft rejection, animal model, corneal epithelialization, keratoepithelioplasty, mice

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