Heterotopic corneal grafting in mice: a new approach to the study of corneal alloimmunity

J. Wayne Streilein, James McCulley, and Jerry Y. Niederkorn

Heterotopic grafting of murine corneas to the thoracic cage of recipient mice affords an opportunity to study the alloimmune rejection process in this well-characterized laboratory species. Immune rejection of cornea allografts can be reliably identified by direct visual, slit-lamp, and histologic observations. Virtually intact syngeneic corneal epithelium and stroma survive at the heterotopic site for at least 21 days. Allogeneic corneal epithelium is destroyed by as early as 7 days, and allogeneic corneal stroma is progressively and completely destroyed by an intense fibrovascular infiltrative process. If Descemet's membrane is preserved, integrity of the stroma and epithelium of syngeneic corneal grafts is preserved, whereas when this membrane is broken, progressive stromal deterioration sets in. This property of Descemet's membrane is particularly apparent in allogeneic corneal grafts. (INVEST OPHTHALMOL VIS SCI 23:489-500, 1982.)

Key words: heterotopic corneal grafts, inbred mice, slit-lamp biomicroscopy, allograft rejection, Descemet's membrane

Although allotransplantation of human corneas is more successful than other types of solid-tissue allografts, rejection remains sufficiently common to warrant further investigation into the mechanism of this phenomenon. Experimentation in this field has been hampered by the fact that it is technically very difficult, if not impossible, to accomplish orthotopic corneal allografts in mice; as a consequence, this field of investigation is robbed of the extensive body of immunogenetic and transplantation biologic literature that has developed with the aid of numerous inbred strains of mice. To investigate the role of various histocompatibility antigens and putatively unique properties of corneal tissues, it is important to develop murine models that will allow study of corneal allotransplantation. Recently, we reported that murine corneas transplanted heterotopically to the thoracic wall of allogeneic recipients were rejected if donor and host differed at class I regions of the H-2 complex but not if they differed only at class II loci. In the review of these data and in discussions with experts in corneal biology, questions were raised as to the validity of this approach to the study of corneal transplantation. It seemed appropriate to examine in more detail the possibility that heterotopic grafting of corneas to the thoracic walls of mice can be employed to study the process of immunologic rejection as it applies to this unique ocular tissue. The results of the studies presented in this article confirm that murine corneas can be transplanted successfully to heterotopic sites, representing a model system.
for the study of the alloantigenic basis of corneal graft immunity.

Materials and methods

Mice. Adult BALB/c (H-2b) and C57BL/6 (H-2k) mice, between ages 3 and 6 months, were used for these studies. These animals were either purchased from Jackson Laboratories, Bar Harbor, Me., or obtained from our own breeding facility.

Preparation of cornea grafts. Donor mice were sacrificed, and their globes were enucleated. With a scalpel blade, the cornea was excised at the limbus and placed (endothelial cells down) on sterile filter paper moistened with phosphate-buffered saline (PBS, pH 7.2).

Grafting procedure. Epidermis and dermis were excised with small curved scissors from the skin overlying the thoracic cage of anesthetized mice, exposing the panniculus carnosus muscle layer. These wounds, measuring 2.0 and 1.5 cm, received three corneal grafts, endothelial side down, placed equidistant from each other and away from the margins of the wound. The graft bed was covered with vaseline impregnated gauze, and the thoracic cage was wrapped with plaster of Paris bandages as described elsewhere.4

Graft inspection and scoring. At each observation time, recipient mice were anesthetized with 0.66 mg of ketamine HCl (Vetalar; Parke-Davis, Morris Plains, N.J.); their casts were removed, and PBS was layered over the gauze dressing, which was then carefully removed. After inspection, fresh petroleum jelly-impregnated gauze was applied, and the plaster bandages were replaced. Visual inspections were made with the unaided eye and with a dissecting microscope (8x), noting the number of grafts present, their clarity, their convexity, and whether swelling occurred after exposure to room air. Inspections were also made with the aid of a slit lamp. The following features were scored: clarity, stromal edema, stromal infiltration, stromal neovascularization, retrocorneal debris, and congestion of the graft bed. In addition, the graft beds were examined with cobalt blue-filtered light after fluorescein dye was placed to detect epithelial defects.

For each panel of mice, a rejection index was calculated on the basis of arbitrary scores derived from the gross and slit-lamp observations. Because of the greater reliability of the slit-lamp observations, these scores were preferentially weighted. Each corneal graft was examined and scored (on an arbitrary scale of 0 to 4+) for each of the above features. The mean scores for each group of corneal grafts were calculated. The score, termed the rejection index, provides a numerical rating of the degree of inflammation and/or rejection observed for one group of corneal grafts on each day of observation.

Histologic studies. En bloc excisional biopsies of corneal grafts were performed, and the specimens were fixed in 10% formalin, embedded in paraffin, sectioned, stained with hematoxylin and eosin and with periodic acid-Schiff (PAS) reagent, and examined with a light microscope. At least six sections representing different regions of the excised graft beds were examined for each specimen. At least two graft beds were excised and studied histologically for each observation time.

Experiments and results

Direct visual observations of heterotopic corneal transplants. To establish the validity of this murine model for study of heterotopic corneal transplants, an initial experiment was conducted in which syngeneic, full-thickness corneal grafts were placed on freshly prepared, raw dermal surfaces on the thoracic wall of adult BALB/c mice. Each bed received three corneas, epithelial side up, placed approximately 3 to 4 mm from each other and from the wound edges. The wounds were wrapped in occlusive plaster of Paris bandages that were removed and replaced at each observation time, 4, 7, 10, 12, 14, 18, and 21 days after grafting. Twenty-one corneal grafts on seven recipient mice were observed through the 21 day interval. By direct visualization at 4 days after grafting, the corneal grafts appeared translucent and displayed the convexity characteristics of the stromal contour. Epithelium entirely covered the surface of the bed, including the corneas. Within 10 min of removal of the bandages, the corneal grafts became much more prominent because of a discernible increase in the elevation of the dome. Since the plaster bandages are quasi-pressure dressings, we presume that this exaggerated dome shape represented swelling of the normally hygroscopic stroma. At each subsequent inspection, a similar pattern was observed: the epithelial surface appeared to be intact, the corneas appeared translucent, and their domed contour became exaggerated upon exposure to room air. Little evidence of
inflammation surrounding or beneath the grafts was detected. However, the overall dimensions of the graft beds shrunk gradually until, at the terminal inspection, three distinct, convex corneas were found to be crowded together by a contracted wound margin. One graft on each of three mice could not be identified on days 14, 18, and 21, respectively. Their disappearance was not heralded by clinical signs at antecedent inspections; epithelial defects or eschars were not found in their place. We presume that they were sloughed and destroyed by nonimmunologic processes (vide infra).

Allogeneic corneas were studied next in this heterotopic transplantation model. Three C57BL/6 corneal grafts were placed on freshly prepared dermal beds of four adult BALB/c mice. Bandages were applied and inspections were conducted at subsequent intervals, similar to those described for syngeneic grafts. Twelve allografts on four recipients were studied. At the first observation periods, 4 and 7 days, the appearance of the allografts was essentially indistinguishable from syngeneic grafts; each was convex and swelled on exposure to room air. The entire graft bed was covered with epithelium. However, when inspected at 10 days, the allografts appeared to have lost some of their translucence, and in one graft bed an epithelial defect was observed in the central intercorneal area. Although the grafts displayed a domed contour, there was only a moderate increase in this shape when exposed to room air. On days 12 and 14, a minority of grafts had epithelial defects; the majority of grafts were frankly opaque and swelled when the bandages were removed. On day 18, a dramatic change had taken place. The majority (75%) of grafts were gone, the wounds had contracted around the remaining grafts, and the surfaces were completely epithelialized. In three instances, shadowlike remnants of corneal grafts could be discerned within the contracted scar. The remaining five identifiable corneas were opaque and displayed a domed shape that swelled moderately on exposure to room air. These opaque structures could still be identified on day 21, but by then their tendency to swell was minimal. The cumulative survival of syngeneic and allogeneic corneal grafts placed on heterotopic thoracic wall beds of BALB/c mice. Graft survival was determined by direct visual observation. There were 21 syngeneic corneal grafts on seven mice and 12 allogeneic corneal grafts on four mice.

It would thus appear that it is possible to graft full-thickness corneas heterotopically to sites on the body that lend themselves readily to visual inspection and that allografts of cornea are susceptible to rejection at this site as are other types of solid-tissue grafts.

Slit-lamp biomicroscopic observations of heterotopic corneal transplants. We next conducted a similar set of experiments in which heterotopic corneal transplants were examined with a slit lamp. As expected, this procedure allowed for considerably more detailed observations than did gross direct visualization. With the slit lamp it was possible to observe and quantify corneal clarity (relative ease with which the graft bed could be observed through the overlying cornea); stromal edema, infiltration, and neovascularization; and congestion (engorgement and/or proliferation of vessels in the graft bed). In addition, fluorescein staining of the graft surface followed by illumination with cobalt blue–filtered light was used to describe defects in the epithelial surface.

For syngeneic corneal grafts, the first abnormalities identified through the slit lamp were stromal edema and mild congestion of the graft beds; these changes were noted as
Fig. 2. Composite scores of various pathologic sequelae of allogeneic corneas grafted onto heterotopic thoracic wall beds of BALB/c mice. Scores were based on slit-lamp observations of 21 allogeneic corneal grafts on seven mice (i.e., three corneal grafts per mouse). Each graft was scored on an arbitrary scale from 0 (no pathologic changes compared with syngeneic controls) to +10 (maximum changes). Each point represents the mean score for the surviving corneal grafts. Retrocorneal debris and stromal infiltrate could not be scored on day 21 due to severe corneal opacity.

early as 4 days in a minority of corneas but were present in all syngeneic corneas at 7 days. By day 10 corneal edema was more pronounced, resulting in reduced clarity; however, congestion was less prominent, and infiltration and neovascularization were not detected. Edema and congestion became progressively less prominent throughout the rest of the observation interval. Terminally, minimal evidence of neovascularization was observed in some but not all syngeneic corneal grafts. From the fourth day onward, the wounds were completely epithelialized and epithelial integrity was maintained throughout.

With slit-lamp biomicroscopy, allogeneic corneas appeared vastly different. Although syngeneic and allogeneic corneas were indistinguishable at 4 and 7 days, on day 10 the allografts possessed significant reductions in clarity, due in part to stromal edema, infiltration, and inflammation. Prominent stromal neovascularization was also present by day 10. In some allogeneic corneal grafts, white, flocculent deposits of retrocorneal debris were observed by day 10. Under cobalt blue–filtered light, the fluorescein dye stained the surfaces of approximately 50% of the allogeneic corneas, indicating that epithelial defects were present. At the 12 and 14 day inspec-
Table I. Summary of histologic changes observed in syngeneic and allogeneic corneal grafts

<table>
<thead>
<tr>
<th>Day</th>
<th>Tissue</th>
<th>Syngeneic</th>
<th>Allogeneic</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Epithelium</td>
<td>Thin (2-3 cell layers), nonkeratinized, spreads onto surrounding bed</td>
<td>Thin (2-3 cell layers), nonkeratinized, spreads onto surrounding bed</td>
</tr>
<tr>
<td></td>
<td>Stroma</td>
<td>No inflammation or neovascularization</td>
<td>No inflammation or neovascularization; mild infiltration with PMNs</td>
</tr>
<tr>
<td></td>
<td>Retrocorneal space</td>
<td>Minimal vascularization, PMN infiltrate</td>
<td>Mild PMN infiltration and vascularization</td>
</tr>
<tr>
<td>7</td>
<td>Epithelium</td>
<td>7-8 cell layers, nonkeratinized, except periphery where encroachment with keratinizing trunk epidermis observed</td>
<td>9-12 cell layers; completely keratinized; moderate basilar cell edema</td>
</tr>
<tr>
<td></td>
<td>Stroma</td>
<td>Peripheral 20% moderately infiltrated with PMNs, and neovascularized if Descemet's membrane intact; where membrane broken, new vessels and fibroblasts stream through; central stroma appears normal</td>
<td>Disordered collagen in nonlamellar pattern; gradient of inflammatory infiltrate, most intense at periphery but also present at center; where Descemet's membrane is broken, tremendous ingrowth of vessels and fibroblasts disrupts the stroma; frank hemorrhage beneath Descemet's membrane</td>
</tr>
<tr>
<td></td>
<td>Endothelium</td>
<td>Portions appear intact</td>
<td>None observed</td>
</tr>
<tr>
<td>14</td>
<td>Epithelium</td>
<td>7-8 cell layers, nonkeratinized although more encroachment by keratinizing epidermis; intercorneal surface covered with nonkeratinizing epithelium</td>
<td>Only keratinized epidermis covers the graft bed; occasional clear cells observed in epithelium; basilar edema and infiltration with PMNs and mononuclear cells</td>
</tr>
<tr>
<td></td>
<td>Stroma</td>
<td>Normal except for rare infiltrate and vascularization at periphery</td>
<td>Marked mononuclear cell infiltrate throughout; diffuse neovascularization; enormous fibrovascular scar tissue invades through breaks in Descemet's membrane</td>
</tr>
<tr>
<td></td>
<td>Endothelium</td>
<td>No longer identifiable</td>
<td>No longer identifiable</td>
</tr>
<tr>
<td></td>
<td>Retrocorneal space</td>
<td>Minimal inflammation</td>
<td>Marked mononuclear infiltrate and neovascularization continuous with process invading stroma at periphery.</td>
</tr>
<tr>
<td>21</td>
<td>Epithelium</td>
<td>Nonkeratinizing epithelium remains over areas with intact Descemet's membrane; in other sites, keratinizing epithelium is observed</td>
<td>Epithelial surface is completely keratinized and resembles body-wall epidermis; numerous high level clear cells and basal mitotic figures</td>
</tr>
<tr>
<td></td>
<td>Stroma</td>
<td>Lamellar pattern intact; no infiltrate or neovascularization; where Descemet's membrane is broken, whorls of fibrovascular tissue invade the stroma from beneath</td>
<td>Lamellar pattern is virtually destroyed; moderate mononuclear cell infiltrate, and neovascularization with plump fibroblastic cells; Descemet's membrane fragmented at numerous sites</td>
</tr>
<tr>
<td></td>
<td>Retrocorneal space</td>
<td>Minimal residual fibrovascular reaction</td>
<td>Moderate fibrovascular reaction with mononuclear cell infiltrate</td>
</tr>
</tbody>
</table>

PMN = polymorphonuclear leukocyte.

tions, corneal clarity was reduced further, stromal edema was extreme, neovascularization was prominent, and the graft bed surrounding the corneal grafts was grossly congested. However, at these times no epithelial defects were detected. At 18 and 21 days, neovascularization and congestion were less prominent, but the corneal grafts that remained were opaque, due chiefly to remarkable intrastromal inflammatory infiltration.

To arrive at a semiquantitative assessment of these changes, and in preparation for the next series of experiments, a scoring system was devised, assigning arbitrary numerical scores for quantitative observations. The scoring system is described in detail in Materials and methods. Each individual parameter of observation (clarity, stromal edema, etc.) was evaluated and scored on a 0 to 4+ scale, the latter representing most severe change. These scores were averaged for syngeneic and allogeneic corneal grafts at each observation time. The former were subtracted from the latter and plotted as depicted in Fig. 2. This method of data presentation allows for direct visual comparison of the tempo and intensity of inflammatory reactions observed. Thus the earliest specific change detected in allogeneic corneas was reduction in clarity due to inflammatory infiltration and edema. Later in
Fig. 5. Syngeneic corneal graft, day 7. Note surface covered with nonkeratinizing epithelium that has spread over graft bed (arrows); minimal infiltration of stroma, especially at junction with dermis of graft bed. (PAS; X400.)

the rejection process, stromal edema contributed importantly to the reduction in clarity. It was also possible, by means of a mathematical transformation as described in Materials and methods, to calculate a cumulative score, the rejection index, for each group of corneal grafts at each inspection time. These indices are displayed graphically in Fig. 3 and accurately reflect the general conclusions that syngeneic corneas heal to their graft bed and develop minimal signs of inflammation that evolve slowly over the 21 day observation period. By contrast, heterotopic corneal allografts initiate and sustain intense inflammatory reactions, which reach peak intensity at approximately 12 days after grafting and remain at this level until the corneas are rejected or are rendered into opaque remnants.

Predictive value of clinical observations for heterotopic corneal allograft rejection. In the following experiment, panels of BALB/c mice were grafted heterotopically with corneas from syngeneic (group A) or C57BL/6 (group B) donors. Direct visualization and slit-lamp biomicroscopy were performed without knowledge as to whether the grafts were syngeneic. At the end of an 18-day observation period, rejection indices were calculated for each group, and the code was broken. The purpose of this experiment was to determine whether it was possible by clinical criteria to distinguish allogeneic from syngeneic grafts and to document rejection. The results are presented in Fig. 4. Group A expressed a rejection index pattern typical of syngeneic controls, whereas Group B demonstrated a rejection index similar to that of allogeneic controls. These results clearly indicate that direct visual inspection and slit-lamp biomicroscopy can successfully detect rejection reactions within allogeneic corneas.

Histologic evaluation of heterotopic corneal transplants. Syngeneic and allogeneic (C57BL/6) corneal grafts placed on BALB/c mice were excised at periodic intervals after grafting and subjected to light microscopic analysis. A summary of these observations is presented in Table I. Both syngeneic and allogeneic corneas appeared similar after 4 days.
on thoracic wall beds; nonkeratinized corneal epithelium was intact albeit thin, and there was minimal evidence of stromal inflammation. However, at 7 days striking differences were noted. Syngeneic epithelium had increased in thickness but remained nonkeratinized, although there was some tendency for trunk skin epidermis to overgrow the margins of the cornea. In the intercorneal region of some histologic preparations, nonkeratinizing
Fig. 8. Allogeneic corneal graft, day 7. Arrow marks break in Descemet's membrane through which a massive fibrovascular infiltrate streams into the stroma. Epithelium is completely keratinized. (PAS; ×400.)

Fig. 9. Allogeneic corneal graft, day 14. Note keratinized epithelium, disordered lamellar pattern of stroma, and cellular infiltrate. (PAS; ×500.)

Epithelium had spread over the raw graft bed (Fig. 5). The stroma was intact and relatively acellular except at specific locations where breaks had occurred in Descemet's membrane; at these sites, fibrovascular tissue appeared to stream in from the retrocorneal space, and the infiltrated stroma was locally neovascularized (Fig. 6). The corneal endothelial cells could be identified, although not as a continuous layer. We believe that the breaks in Descemet's membrane were made during preparation of the grafts. At 7 days allogeneic corneas had become markedly infiltrated with inflammatory cells; there ap-
peared to be a gradient of stromal infiltration, most pronounced at the periphery of the graft, and more attenuated as the central stroma was approached (Fig. 7). Where Descemet’s membrane was broken, massive infiltration of the overlying stroma was observed (Fig. 8). At a few places, frank hemorrhage was found between Descemet’s membrane and the stroma. No endothelial cells were observed. The stromal collagen was disordered and nonlamellar, a finding that contrasts sharply with the preservation of ordered collagen in the stroma of syngeneic corneas. Most importantly, the epithelium covering the exterior surface of the majority of grafts was completely keratinized; the most superficial cells contained numerous keratohyalin granules. The epidermis was considerably thickened, compared with syngeneic corneas at this time. In one allografted cornea, nonkeratinized epithelium covered a portion of the graft, that portion that resided above an intact Descemet’s membrane. In the remainder of this graft, keratinizing epithelium prevailed above an extensively infiltrated and vascularized stroma, below which a badly fractured Descemet’s membrane was found. Syngeneic grafts examined histologically at 14 days showed little change from the 7-day findings. The stroma remained intact and was relatively free of infiltrate and neovascularization unless Descemet’s membrane was broken, in which
a modest fibrovascular reaction invaded the deep stroma. The epithelium remained nonkeratinized and no evidence of clear cells in the upper epidermis was found. By contrast, only keratinized epidermis covered the allogeneic corneas at this time, and frequent high-level clear cells were seen (Fig. 9). We presume, but have no direct evidence, that these clear cells are Langerhans cells. The basal layer of epidermal cells showed intercellular edema and was infiltrated with mononuclear cells and neutrophilic leukocytes. The most impressive changes were found in the stroma, which was diffusely neovascularized and in which there was a marked mononuclear cell infiltrate, again most pronounced at the periphery; no normal stromal tissue remained. There were also marked inflammatory cell infiltrates retrocorneally.

Syngeneic corneal grafts examined at 21 days after grafting remained essentially free of acute inflammatory changes. Only at sites where Descemet's membrane was broken was fibrovascular tissue found to invade the deep stroma from the retrocorneal space. In most, but not all, syngeneic grafts, nonkeratinizing epithelium still covered the graft surface. However, in other places, conventional epidermis from body-wall skin had supplanted the corneal epithelium; this phenomenon seemed to be coincident with breaks in Descemet's membrane deeper within the graft. The most striking differences observed with allogeneic corneas that persisted to 21 days were found within the stroma; this tissue was grossly disrupted by heavy neovascularization and mononuclear cell infiltration and by complete distortion of the typical lamellar pattern of collagen. In fact, the appearance of the stroma of late allogeneic grafts resembled that of fibroelastic cartilage rather than corneal stroma. Descemet's membrane was fragmented. The epithelium was completely resurfaced with a thick layer of keratinizing epidermis, frequent mitotic figures were observed in the malpighian layer, and numerous high-level clear cells were observed.

To our surprise, several corneas appeared, upon histologic examination, to have been placed on the graft bed upside down—that is, with the endothelial surface external. The fate of inverted allogeneic cornea grafts was particularly illuminating. If Descemet's membrane was broken, stromal infiltration and neovascularization proceeded as in upright grafts, and the buried epithelium was destroyed. However, in several inverted grafts in which Descemet's membrane was intact, the original corneal epithelium, now lying deep within the graft bed, appeared to form cysts and to survive (Fig. 10). Although inflammatory infiltrates invaded the stroma at its periphery, the region surrounding the encysted epithelium was relatively free of mononuclear cells; moreover, the allogeneic epithelium, which was completely devoid of keratohyalin granules, was well preserved and lacked degenerative changes.

**Discussion**

Syngeneic and allogeneic corneal grafts, when applied to fresh dermal wounds on the thoracic walls of mice, attach successfully and derive nourishment from the host. In fact, syngeneic corneal grafts flourish at this heterotopic site for at least 21 days; examination of these grafts by gross visual inspection, by slit-lamp biomicroscopy, and by light microscopy reveal that: (1) they retain a nonkeratinizing epithelial surface, and (2) the stroma is translucent and retains its domed configuration, and its collagen remains in typical lamellar arrays. Allogeneic corneal grafts do not similarly thrive. They experience a severe destructive inflammatory reaction that leads inexorably to rejection and sloughing in the great majority of instances. Destruction of allogeneic corneal epithelium is complete by as early as 7 days (as determined by light microscopy). The stroma of allogeneic grafts becomes massively infiltrated and intensely neovascularized, as detected by slit-lamp examination. Ultimately, the architectural integrity of the stroma disintegrates and fragments. In the minority of grafts that remain on the bed at 21 days, the tissue consists predominantly of fibrovascular scar. By gross visual inspection, these graft remnants (unlike their syngeneic counterparts) fail to swell when their pressure dressings are removed, an indi-
cation of deterioration of an important physiologic property of corneal stroma.

These dramatic changes are easily detected, giving us confidence that this heterotopic corneal grafting model can be employed to considerable advantage with inbred strains of laboratory mice. It should thus be possible to answer numerous vexing questions concerning corneal allografts: (1) What is the relative immunogenicity of the various corneal cell types in inducing allograft immunity? And what is the relative vulnerability of these same cell types to the rejection reaction? (2) Which H-2 alloantigens are important to induction and expression of corneal allograft immunity? Our laboratory has claimed that class II (Ia) antigens are unimportant, since normal mouse corneas lack Langerhans cells, the only epidermal cells known to express these antigens.4-6 Conflicting results have been reported by Chandler.7 This controversy should be resolvable readily with the method described in this article. (3) Which immune effector mechanisms dominate in corneal graft rejection—T lymphocytes, B lymphocytes, natural killer cells, and/or specific antibody? (4) Which factors promote or initiate neovascularization of corneal stroma—lymphokines, complement, plasminogen, collagenase, and/or other hydrolyases?

Although these studies on heterotopic corneal allografts are newly reported in laboratory mice, corneal transplants, either conducted orthotopically or heterotopically, have long been studied in other species, especially the rabbit.8-11 It has been known that all three layers of the cornea—epithelium, stroma, and endothelium—express transplantation alloantigens and are capable of inducing transplantation immunity, as well as serving as targets of the rejection reaction. Now that a contemporary description of the major histocompatibility complex (MHC) has been achieved in mice and in man, the differential expression of MHC gene products on different types of tissues warrants reinvestigation of the relative immunogenicity of the various components of the cornea.

The most striking new insight gained from the results of this series of experiments is realization of the pivotal role Descemet's membrane plays in immune reactions directed at corneal tissues and in neovascularization of the stroma. Random and capricious breaks in Descemet's membrane, probably resulting from inadvertent injury at the time of corneal grafting, allow fibroblasts and endothelial buds from the graft bed to penetrate into central regions of the corneal stroma. A similar infiltrative process takes place at the periphery of the grafts. In syngeneic grafts, these infiltrations invade the stroma very slowly. But in allogeneic corneas, invasion by fibrovascular tissue proceeds at a rapid pace and is especially prominent as it streams through breaks in Descemet's membrane. As a consequence, the stroma is reduced to a nonfunctional scarred mass. By contrast, in the unusual situations where corneal allografts were placed (incorrectly) upside down on the graft bed and Descemet's membrane was unbroken, fibrovascular replacement of the stroma was remarkably retarded. In two such specimens examined histologically at 21 days, healthy-looking, nonkeratinized allogeneic epithelium characteristic of corneal epithelium was seen to survive as an encysted mass beneath the stroma. These observations suggest that integrity of Descemet's membrane may act to prevent immune effectors from attacking allogeneic tissues behind its barrier. We are particularly interested in pursuing this intriguing conclusion, which addresses the notion that the cornea may be an immunologically privileged tissue.12

We were somewhat disappointed, although not surprised, to be unable to identify corneal endothelial cells beyond 7 days in syngeneic cornea grafts. From one standpoint, the vulnerability of these cells to experimental manipulation conditioned us to expect that they would be unlikely to withstand the trauma of grafting. However, we are not convinced absolutely that all endothelial cells did succumb. A more careful search for these cells with more probing markers than we have used is essential before making this conclusion. In some ways, the attractiveness of this murine model for corneal heterotopic grafting would be enhanced if it were possible to achieve
survival of all three layers of the cornea in the grafted tissue.

We are deeply appreciative of expert technical assistance of Ms. Lanya Lonsberry, Ms. Elizabeth Mayhew, and Ms. Ann Fields, and of Ms. Helen Patterson in preparation of the manuscript.

REFERENCES