Fate of Orthotopic Corneal Allografts in Eyes That Cannot Support Anterior Chamber-Associated Immune Deviation Induction

Yoichiro Sano, Bruce R. Ksander, and J. Wayne Streilein

Purpose. Corneal allografts placed in human eyes at high risk often fail, and immune rejection is thought to be a major pathogenic factor. To understand the immunologic factors responsible for rejection in this instance, the authors have created “high-risk” eyes in mice by inducing corneal neovascularization. The authors then examined the fate of orthotopic corneal grafts placed in these beds and assessed the development of donor-specific delayed hypersensitivity (DH) in recipient mice.

Methods. Three interrupted sutures were placed in the central cornea of recipient BALB/c mice to induce corneal neovascularization. Two weeks later, when corneal vessels occupied more than two quadrants of the cornea, mice received orthotopic corneal grafts from donor mice expressing alloantigens encoded by major and minor histocompatibility loci. Corneal allografts were evaluated by slit-lamp examination after grafting, and recipient mice were examined at the time of the rejection to determine whether they had acquired DH to alloantigens expressed on the corneal grafts.

Results. Compared to grafts placed in normal eyes, a much higher incidence of rejection was observed among corneal allografts placed in neovascularized eyes (96.7% versus 46.7%). Moreover, grafts in neovascularized beds were rejected much more swiftly (2 weeks versus >3 to 4 weeks). Rejection of corneal allografts in high-risk eyes coincided temporally with development of intense donor-specific DH, and the specificity of this immune response was directed solely at minor H antigens (not major histocompatibility complex-encoded antigens) on the graft.

Conclusions. These results indicate that eyes rendered high risk by virtue of corneal neovascularization fail to provide immune privilege for orthotopic corneal allografts. In this circumstance, the grafts rapidly induce intense donor-specific DH that is readily detectable within 2 weeks of engraftment, at which time the grafts are acutely and universally rejected. The recipient DH response is directed exclusively at minor H antigens on the graft, which is consistent with the view that neovascularization creates graft beds in which recipient antigen-presenting cells infiltrate the graft and carry antigenic information by lymphatics to draining lymph nodes. In this manner, anterior chamber-associated immune deviation is avoided, and potentially allodestructive DH is promoted. Invest Ophthalmol Vis Sci. 1995;36:2176-2185.

Among solid tissue transplants in humans, orthotopic corneal allografts show the greatest success. In uncomplicated cases, the 2-year survival rate for initial corneal allografts placed in normal avascular corneal beds is estimated at more than 90%.1-3 However, the success rate of corneal allografts placed in so-called high-risk eyes is much reduced (only 35% to 65% of grafts produce acceptable outcomes).2,4 Recipient eyes can be regarded as high risk for many reasons: stromal vascularization, uncontrolled glaucoma, decreased corneal sensation, presence of persistent active intraocular inflammation, and associated ocular abnormalities, such as eyelid disease, abnormal conjunctiva, or dry eye syndromes.5 Corneal allografts in these eyes fail repeatedly. A recent multicenter collaborative study reported that the rate of graft failure increased from 17% in patients with no previous grafts to 53%
in patients with two or more previous grafts. Although the reasons for frequent corneal allograft failure in high-risk situations have not been completely elucidated, it is suspected that immunity directed at alloantigens on the transplant is a major cause of graft failure in high-risk eyes. Therefore, it is important to understand the immunopathogenesis of corneal allograft rejection, especially in eyes at high risk.

Immune privilege of the normal eye is thought to be one of the primary reasons for the high rate of success when corneal allografts are placed in avascular, normal graft beds. The early experimental work of Maumanee demonstrated that orthotopic corneal allografts performed in normal eyes of rabbits had prolonged survival, compared to solid tissue grafts placed at other orthotopic sites in the body. Our laboratory and others have found also that a significant proportion of corneal allografts placed orthotopically in normal eyes of rats and mice succeed, even when the grafts confront recipients with the greatest extent of histoincompatibility. These results are thought to reflect the immune privileged status of the corneal surface of the normal eye.

Several microanatomic features of the cornea have been considered to be important for establishing and maintaining immune privilege: The central cornea is virtually devoid of antigen-presenting cells (APCs); the epithelium lacks Langerhans cells, and the stroma displays no bone marrow-derived cells that are candidates for antigen presentation; the cornea lacks vascular connections to the blood and lymph systems and, therefore, can neither send nor receive immunologically important information; and corneal cells secrete factors that suppress certain immune and inflammatory responses within the anterior segment of the eye. These features of the cornea are important in ocular immune privilege and contribute to the capacity of the normal anterior chamber to support the induction of anterior chamber-associated immune deviation (ACAID). Experimentally manipulated eyes in which the cornea contains Langerhans cells in the epithelium cannot support ACAID induction. Moreover, if corneal neovascularization is induced in murine eyes by placing a suture through the central cornea, the tissue loses its capacity to secrete immunosuppressive factors. Consequently, protein antigens injected into the anterior chamber of sutured eyes fail to induce ACAID.

Because ACAID is important in ocular immune privilege and because ACAID does not occur in neovascularized eyes, we wanted to determine the fate of corneal allografts placed in neovascularized eyes and to determine the nature of the recipient immune response to alloantigens expressed on these grafts. In this article, we report that the vast majority of allografts placed in high-risk neovascularized mouse eyes suffer acute rejection and that rejection correlates with the development of donor-specific delayed hypersensitivity (DH). These findings strongly support the view that ACAID and immune privilege are powerful forces in promoting the success of corneal allografts and that, in their absence, such grafts are highly susceptible to irreversible immune rejection.

**MATERIALS AND METHODS**

**Mice**

Six- to 12-week-old mice were purchased from Taconic Farms (Germantown, NY). All animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The following inbred strains were used: BALB/c (H-2b), C57BL/6 (H-2b), C57BL/10 (H-2b), BALB.B (C.B10-H2b) (H-2b), BALB.K (C.C3-H2k) (H-2b), A/J (H-2b), and B10.D2 (H-2b).

**Induction of Corneal Neovascularization**

Three interrupted sutures (11-0 nylon, 50-μm-diameter needle, Sharpoint; Vanguard, Houston, TX) were placed in the central cornea of one eye of normal BALB/c mice. As described previously, these sutures induce corneal neovessels from the limbus that can be detected as early as 5 days; after 2 weeks, neovessels occupy more than 2 quadrants of the cornea, including the central area. In these experiments, corneal neovascularization was induced by sutures that were in place for 2 weeks. These mice with neovascularized graft beds then served as recipients of orthotopic corneal transplants.

**Corneal Transplantation and Grafting**

All donor corneas were excised by vannas scissors after marking the central cornea with a 2-mm diameter microcurette; corneal grafts were placed in chilled phosphate-buffered saline. Recipients were anesthetized with intramuscular injections of ketamine (3 to 4 mg/reipient) and xylazine (0.1 mg/reipient). The graft bed was prepared by excising with vannas scissors a 2-mm site in the central cornea of the right eye and discarding the excised cornea. The donor cornea was then placed in the recipient bed and secured with eight interrupted sutures (11-0 nylon, 50-μm-diameter needle, Sharpoint; Vanguard). In the neovascularized graft beds, it was more difficult to appose the edges of the graft to the graft beds because of corneal edema of host tissue. Antibiotic ointment was placed on the corneal surface for 2 weeks after surgery. All grafted eyes were examined after 72 hours; at that time, grafts with technical difficulties (hyphema, infection, or loss of anterior chamber) were excluded from further consideration. When the grafts were next examined, at 9 days after transplantation, all sutures were removed.
BALB/c mice were used as recipients in all grafting experiments.

**Evaluating and Scoring of Orthotopic Cornea Transplants**

After corneal transplantation, grafts were examined by slit lamp microscopy at weekly intervals. At each time point, the grafts were scored for opacity and neovascularization. A scoring system was devised to describe in semiquantitative terms the extent of opacity (0 to 5+), as follows: 0 = clear graft; 1+ = minimal superficial (nonstromal) opacity; 2+ = minimal deep (stromal) opacity; 3+ = moderate stromal opacity; 4+ = intense stromal opacity; 5+ = maximum stromal opacity. Grafts with opacity scores of 2+ or greater at 8 weeks were considered to have been rejected; grafts with score of 3+ or greater at 2 weeks never cleared and also were regarded as rejected.

**Assay for Delayed Hypersensitivity Reaction in Mice Bearing Corneal Allografts**

At 2 weeks after grafting, $1 \times 10^6$ irradiated (2000 rad) spleen cells from the appropriate allogeneic strain were injected in 10 µl of Hank's balanced salt solution into the right pinnae. As a positive control, a similar number of spleen cells was injected into the ear pinnae of mice immunized by subcutaneous injection of $10 \times 10^6$ spleen cells of the appropriate allogeneic strain. After 24 hours, ear thickness was measured with a low-pressure engineer micrometer (Mitsutoyo; MTI Corporation, Paramus, NJ). Ear swelling was expressed as follows: specific ear swelling = (24-hour measurement of right ear – 0-hour measurement of right ear) – (24-hour measurement of left ear – 0-hour measurement of left ear) $\times 10^{-3}$ mm. Ear swelling responses at 24 hours after injection are presented as individual values ($10^{-3}$ mm) for each animal tested and as group mean ± SEM. Delayed hypersensitivity data were obtained from groups of mice that were ear challenged with spleen cells from the allogeneic strains designated in the results section. After mice were ear challenged and the DH response was measured, the mice were killed; no mice were challenged a second time.

Ear swelling measurements were evaluated statistically by using a two-tailed Student’s $t$ test. $P < 0.05$ was considered significant.

**RESULTS**

**Fate of Syngeneic Orthotopic Corneal Grafts in Neovascularized Graft Beds**

To establish the clinical criteria for corneal allograft rejection in neovascularized graft beds, we first examined the fate of syngeneic corneal transplants placed in graft beds that displayed suture-induced neovascularization. Figure 1 displays photographs of a BALB/c cornea containing neovessels induced by sutures placed 2 weeks earlier. Corneal buttons harvested from normal eyes of BALB/c donors were grafted into eyes such as this, and the grafts were examined by slit lamp microscopy and evaluated clinically using a scoring system described previously. As presented in Figure 2, all syngeneic grafts displayed moderate to intense stromal edema and opacity during the first week after surgery. Thereafter, this response began to recede, and at 3 weeks after surgery, only 4 of 12 grafts showed minimal superficial (but not stromal) opacity; the remaining 8 grafts showed no evidence of edema or opacity. It is worth pointing out that we have reported that syngeneic grafts placed in normal graft beds experience a similar postoperative course. Thus, syngeneic cornea grafts, which can elicit no alloimm-
Corneal Allografts in 'High-Risk' Eyes

Weeks after grafting, with 33.3% (10 of 30) of these grafts having been rejected at 2 weeks (Fig. 3). By contrast, all but one corneal allograft placed in high-risk graft beds developed corneal stromal opacity, with scores of 3+ or greater at 2 weeks; these grafts never cleared throughout the remainder of the observation period (Fig. 4). Thus, the rejection rate of corneal allografts in high-risk neovascularized graft beds was 96.7%. Compared to grafts rejected in normal graft beds, the tempo of rejection in grafts placed in neovascularized beds was considerably faster (Table 1). The photograph in Figure 5 shows a rejected corneal allograft in a neovascularized graft bed, demonstrating intense opacity and vascular invasion at 2 weeks after grafting. These results indicate that corneal allografts placed in high-risk neovascularized graft beds are much more susceptible to immune rejection than grafts placed in normal beds and that the intensity and tempo of rejection is decidedly more acute.

TABLE 1. Fate of Orthotopic Corneal Allografts in Neovascularized Graft Beds

<table>
<thead>
<tr>
<th>Graft Beds</th>
<th>Graft Failure at 2 Weeks (%)</th>
<th>Graft Failure at 8 Weeks (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal-vascular</td>
<td>10 (33.3)</td>
<td>14 (46.7)</td>
</tr>
<tr>
<td>Neovascularized</td>
<td>29 (96.7)</td>
<td>29 (96.7)</td>
</tr>
</tbody>
</table>

C57BL/6 corneas were grafted orthotopically to BALB/c with either normal-vascular graft beds or neovascularized graft beds. The pattern of survival of corneal allograft in each type of graft bed is presented in Figures 3 and 4.
Delayed Hypersensitivity Response to Donor Antigens in Recipients of Allografts in Neovascularized Graft Beds

We have reported\(^2^0\) that systemic immunity (delayed hypersensitivity) directed at donor alloantigens developed between 2 and 4 weeks after grafting when MHC plus minor H disparate corneal grafts were placed orthotopically in normal eyes. Moreover, in this circumstance, all mice acquired donor-specific DH, irrespective of whether the graft eventually was rejected, and irreversible graft rejection typically occurred between 3 and 8 weeks after engraftment. Because the incidence of rejection of corneal allografts in high-risk graft beds was much higher and because rejection occurred within 2 to 3 weeks, we designed the following experiments to determine whether graft rejection in neovascularized beds was directly and temporally correlated with the acquisition of DH to donor alloantigens. In these experiments, BALB/c mice received orthotopic corneal allografts from MHC plus multiple minor H disparate C57BL/10 mice. The majority of these grafts was judged to have undergone rejection by 2 weeks, and, at that time, the DH response of the recipients to donor alloantigens was measured by challenging their ear pinnae with irradiated C57BL/10 spleen cells. Delayed hypersensitivity reactions of these grafted mice were compared, on the one hand, with the responses of positive control mice—that is, specifically sensitized BALB/c mice that received subcutaneous injections of C57BL/10 spleen cells (10 × 10^6) 2 weeks earlier—and, on the other hand, with responses of BALB/c recipients of C57BL/10 corneal allografts placed in normal ocular graft beds. The results are presented in Figure 6 and indicate that mice in the positive control group displayed significant DH (specific ear swelling of 66.8 × 10^-3 mm ± 7.4). In confirmation of previously reported results,\(^2^0\) challenged ears of mice bearing corneal allografts in normal graft beds displayed little or no significant swelling (12.0 × 10^-3 mm ± 1.8) compared with naive mice (7.6 × 10^-3 mm ± 1.9). However, mice that received corneal allografts in high-risk graft beds displayed intense DH (78.8 × 10^-3 mm ± 7.6) comparable in intensity to that of the positive controls. These results indicate that DH to donor alloantigens emerges 2 weeks after corneal allografts are placed in high-risk graft beds. Thus, emergence of donor-specific DH coincides with rejection of corneal allografts in high-risk graft beds, raising the possibility that the two events are causally related.

Specificity of Donor-Directed Delayed Hypersensitivity Displayed by Mice Bearing Orthotopic Corneal Allografts in Neovascularized Graft Beds

We have previously reported that donor-specific DH developed between 2 and 4 weeks after corneal allo-

grants were placed in normal eyes.\(^2^0\) There was no correlation between DH reactivity and graft rejection because donor-specific DH was detected in all grafted mice, irrespective of whether the graft was rejected or accepted indefinitely. Surprisingly, in those experiments, the specificity of the DH detected at 4 weeks after grafting was directed against donor minor H alloantigens rather than MHC alloantigens. Because only 50% of grafts placed in normal graft beds were rejected, whereas virtually all grafts placed in neovascularized beds were destroyed immunologically, we wondered whether the higher incidence of rejection in the latter instance might be related to the type of donor antigens to which DH reactivity was directed. The following experiments were designed to determine whether donor-specific DH detected in recipients of corneal allografts placed in high-risk graft beds was directed at minor H alloantigens, MHC alloantigens, or both. First, to detect DH against donor-derived MHC alloantigens only, a panel of BALB/c mice with neovascularized ocular graft beds received orthotopic corneal allografts from C57BL/10 donors. Two weeks later, the ears of these mice were challenged with BALB.B spleen cells (this strain possesses the H-2 haplotype of C57BL/10 but shares no minor H antigens with this donor). Instead, the minor H antigens of BALB/c and BALB.B are identical. The results are presented in Figure 7 and summarized in

![Figure 6. Donor-specific delayed hypersensitivity in BALB/c mice bearing orthotopic C57BL/10 corneal allografts for 2 weeks. Ears of recipients bearing grafts in normal avascular (a) and neovascularized graft beds (b). Primed (c) and naive (d) mice received intrapinna injections of 1 × 10^6 irradiated (2000 rads) C57BL/10 spleen cells. Ear swelling responses were measured by micrometer after 24 hours. Each data point represents the response of a single animal. The bar indicates mean ear swelling responses for the group. Only delayed hypersensitivity responses of primed mice and animals bearing grafts in neovascularized graft beds were significantly greater than naive control. *Mean delayed hypersensitivity is significantly different from that of naive mice at P < 0.0005.](image-url)
Corneal Allografts in 'High-Risk' Eyes

Table 2. As positive control, DH responses were induced in normal BALB/c mice by immunizing them with C57BL/10 spleen cells; 2 weeks later, the ears of these mice were challenged with BALB.B spleen cells. Despite the intensity and rapidity of corneal allograft rejection in neovascularized beds, recipients mice failed to display MHC alloantigen-specific DH (13.3 x 10^{-3} mm ± 1.7) compared with the positive control group (60.7 x 10^{-3} mm ± 8.4).

Second, to detect DH directed at minor H alloantigens only, BALB/c mice with high-risk graft beds received corneal allografts from C57BL/10 donors. Two weeks later, their ears were challenged with irradiated B10.D2 spleen cells. This strain expresses minor H alloantigens identical to C57BL/10 but does not express the H-2^k haplotype of C57BL/10. In this manner, B10.D2 cells can only elicit DH directed at minor H antigens. The DH responses of these mice, which are presented in Figure 8 and summarized in Table 2, indicate that all recipients of C57BL/10 corneal grafts in neovascularized beds acquired minor H antigen-specific DH responses (81.1 x 10^{-3} mm ± 10.7) that were comparable in intensity to positive controls (85.2 x 10^{-3} mm ± 7.8). Taken together, these two sets of experiments reveal that when allogeneic corneal grafts that express both MHC and minor H antigens are placed in neovascularized eyes, systemic DH is evoked, and the specificity of the response is directed exclusively at minor H antigens, not at antigens encoded within the MHC.

Because mice bearing corneal allografts in high-risk eyes showed vigorous DH responses when tested with cells bearing minor H alloantigens similar to those expressed on the grafts, it was important to determine whether the DH evoked by such grafts was donor specific. To examine this point, ears of BALB/c mice with corneal allografts from C57BL/10 in neovascularized graft beds were ear challenged with two types of third-party cells at 2 weeks after grafting. In one experiment, the ears of recipient mice were challenged with BALB.K spleen cells. This strain expresses antigens encoded by an H-2 haplotype (H-2k) unrelated to graft donors or recipients. In another experiment, the ears of BALB/c mice recipients of C57BL/10 corneal allografts in neovascularized beds were challenged with A/J spleen cells. This strain expresses

Table 2. Specificity of DH Response at 2 Weeks in Animals With Corneal Allografts in Neovascularized Graft Beds

<table>
<thead>
<tr>
<th>Graft Donor</th>
<th>Recipient</th>
<th>Ear Challenge</th>
<th>Type of Alloantigen</th>
<th>DH Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/10</td>
<td>BALB/c</td>
<td>C57BL/10</td>
<td>MHC + minor H</td>
<td>++</td>
</tr>
<tr>
<td>C57BL/10</td>
<td>BALB/c</td>
<td>BALB.B</td>
<td>MHC only</td>
<td>--</td>
</tr>
<tr>
<td>C57BL/10</td>
<td>BALB/c</td>
<td>B10.D2</td>
<td>Minor H only</td>
<td>++</td>
</tr>
<tr>
<td>C57BL/10</td>
<td>BALB/c</td>
<td>BALB.K</td>
<td>MHC third party</td>
<td>--</td>
</tr>
<tr>
<td>C57BL/10</td>
<td>BALB/c</td>
<td>A/J</td>
<td>Minor H third party</td>
<td>--</td>
</tr>
</tbody>
</table>
third-party minor H alloantigens. The results are presented in Figures 9 and 10 and are summarized in Table 2. No animals with grafts in neovascularized graft beds displayed DH responses that could be elicited by alloantigens (MHC or minor H) not expressed by tissues of C57BL/10 mice. Thus, DH responses to minor histocompatibility antigens detected at 2 weeks after grafts are placed in neovascularized eyes are donor specific.

DISCUSSION

In this article, we report that corneal allografts placed in high-risk neovascularized graft beds were rejected more frequently and more swiftly than grafts in normal avascular graft beds, that the appearance of allo-specific DH coincided with the rejection of allografts in high-risk eyes, and that even though donor grafts were disparate from recipients both at MHC and minor H antigens, the DH response in recipients was directed solely at minor H antigens. These results provide important information on the mechanisms responsible for the success of allografts placed in normal graft beds and the failure of allografts placed in high-risk or neovascularized graft beds.

Originally, it was thought that the success of orthotopic corneal transplantation in animals and in humans was caused by a deficiency in the expression of transplantation antigens on the cornea. The normal cornea is devoid of MHC class II bearing cells, and corneal cells express reduced amounts of class I molecules. Nonetheless, it has been shown that corneal allografts transplanted heterotopically into the thoracic wall or subcutaneously in mice and rats induce systemic sensitization to transplantation antigens. Moreover, our results demonstrated that whereas significant numbers of corneal allografts survive in normal graft beds, corneal allografts are rejected rapidly from high-risk graft beds. Because rejection coincided with the appearance of DH directed at donor alloantigens, we conclude that corneal grafts express sufficient transplantation antigens to sensitize recipients. Therefore, the success or failure of corneal allografts must be dictated by local or eye-dependent mechanisms that influence the induction and expression of immunity to these transplantation antigens. Perhaps local factors dictate the failure of DH to cause graft rejection when the graft is placed in normal graft beds.

It has been shown that antigens placed in the anterior chamber of the normal eye elicit a deviant systemic immunity that is selectively deficient in antigen-specific DH (ACAID). In orthotopic corneal transplantation, the corneal allograft forms the anterior surface of the anterior chamber and, therefore, displays transplantation antigens in the anterior chamber. It has been proposed that ACAID might play a role in the high level of acceptance of orthotopic corneal allografts. To that end, we have reported that mice with longstanding, accepted orthotopic corneal allografts placed in normal eyes have donor-specific ACAID; they cannot be induced to mount DH reactions when immunized with donor alloantigens. This finding suggests that ACAID induction is critical to the success of orthotopic corneal allografts placed in normal eyes. However, we have found that if corneal neovascularization is induced in murine eyes by placing sutures in the central cornea, soluble antigens (bo-
Corneal Allografts in ‘High-Risk’ Eyes

vine serum albumin) inoculated into the anterior chamber of sutured eyes fail to induce ACAID. In a similar manner, we describe here that donor-specific DH was detectable in corneal allograft recipients with neovascularized graft beds as early as 2 weeks after engraftment. At that time, all grafts displayed intense and irreversible opacity as a sign of rejection. Therefore, the failure to acquire donor-specific ACAID in neovascularized eyes may be responsible for the high rate of corneal allograft rejection by eliciting destructive DH response to donor antigens.

Experimental evidence indicates that ACAID plays a prominent role in establishing and maintaining immune privilege within the eye. The failure to induce ACAID and the failure of corneal allografts to survive in high-risk neovascularized eyes suggests that immune privilege is disrupted in these eyes. Several features observed in eyes with suture-induced corneal neovascularization can be considered as important factors for the destruction of immune privilege. First, the sutured cornea contains blood vessels, which, as an effenter limb of the immune response, can allow immune effector cells and molecules to gain access into the graft and to destroy the graft tissue. Second, suture-induced corneal neovascularization in mice is accompanied by Langerhans cell migration into the central corneal epithelium. Thus, these recipients' corneal beds already contain significant numbers of APCs when corneal allografts are transplanted. These APCs are thought to play an important role in sensitization. In this circumstance, recognition of alloantigens occurs through “indirect pathway” (see below), that is, recipient APCs infiltrate the graft and pick up and carry donor antigenic information to draining lymph nodes. We have examined the frequency of Iaα+ mononuclear and dendritic cells within the central epithelium of C57BL/6 grafts placed in either normal or neovascularized graft beds of BALB/c mice. We observed significantly greater numbers of Iaα+ dendritic cells in neovascularized graft beds compared with normal beds 7 to 28 days after grafting (unpublished observation, 1995).

Because normal corneas are devoid of lymphatic vessels and lymphatic drainage pathways, it is unclear how APCs that migrate to corneal allografts might escape from the cornea and present reprocessed alloantigen to recipient T cells. However, Collin et al have reported the existence of lymphatic vessels in neovascularized rabbit cornea. These investigators clearly showed lymphatic vessels in corneas with neovascularization, induced by injection of alloxan monohydrate into the corneal stroma. Therefore, we suspect, but have no direct evidence, that murine corneas with suture-induced neovascularization possess lymphatic vessels and that, through this novel pathway, recipient APCs can carry antigenic information from the graft to draining lymph nodes.

In other types of solid organ transplantation, such as skin, heart, kidney, and liver, highly disparate allografts are rejected within 2 weeks. In these cases, MHC-encoded antigens are considered to be the most immunogenic antigens as well as the primary targets of the rejection reaction. We were surprised to learn that when corneal allografts placed in neovascularized graft beds were uniformly and intensively rejected within 2 weeks, DH responses were directed only at minor H antigens, not at MHC alloantigens. In most other types of solid organ transplants, the grafts contain so-called passenger leukocytes (Langerhans cells in the skin, Kupffer cells in the liver, and so on), which express high levels of MHC class II molecules, are highly mobile, and sensitize recipient T cells by migrating from the graft to the draining lymph nodes. This pathway of allorecognition has been called direct because recipient T cells recognize donor alloantigens directly on donor-derived cells. Because normal corneas contain virtually no passenger leukocytes—neither Langerhans cells in the epithelium nor dendritic cells or macrophages in the stroma—sensitization of recipients must occur via indirect pathways in which recipient APCs infiltrate the graft, acquire donor antigens, and present them to T cells. It has been shown that MHC-encoded alloantigens and minor H alloantigens can be recognized through the indirect pathway by which they are loaded onto class II molecules of recipient APC and presented to T cells. In fact, we previously reported that mice that reject grafts placed in normal eyes acquire MHC-specific DH. Because corneal tissue has been reported to have reduced expression of class I and class II MHC antigens, there may be more minor H type proteins in corneal cells than MHC-encoded antigens, and, therefore, minor H antigens may be the major source of cornea peptides that are recognized by the indirect pathway.

Our findings agree with the recent report from the Collaborative Corneal Transplantation Study, in which it was reported that matching for HLA-A, HLA-B, and HLA-DR antigens had no effect on overall graft survival even in high-risk eyes. Instead, this study has reached the conclusion that ABO blood group matching improved corneal graft survival. Because ABO antigens are synthesized intracellularly by glycosyl transferases that are polymorphic, these allotypic proteins can act as minor H antigens, providing peptides that can be loaded onto MHC molecules and recognized by T cells through indirect pathway.

In these experiments, we obtained evidence suggesting that corneal allografts placed in high-risk graft beds are rejected by a donor-specific DH response. Our results imply that a failure of neovascularized corneas to maintain immune privilege contributes to the formation of high risk and that graft rejection in eyes at high risk may be prevented by the induction of
ACAID and the restoration of immune privilege. Recently, our laboratory has discovered that alloantigen-specific ACAID can be induced when allogeneic peritoneal exudate cells are cultured with transforming growth factor-beta in vitro and injected intravenously. This approach may be useful in creating donor-specific ACAID in mice with neovascularized corneas, and in this manner ACAID may prevent corneal allograft rejection in high-risk eyes. Experiments to examine this possibility are under way.

**Key Words**

anterior chamber-associated immune deviation (ACAID), corneal neovascularization, corneal transplantation, delayed hypersensitivity, immune privilege

**References**

31. Austyn JM, Larsen CP. Migration patterns of dendritic