Characterization of Cell-Mediated Immune Responses Elicited by Orthotopic Corneal Allografts in Mice

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Purpose. Corneal allografts placed orthotopically induce a unique and unusual response in recipient mice. More orthotopic corneal allografts are accepted indefinitely than similar skin allografts. Of the rejected corneal grafts, class I major histocompatibility complex (MHC)-incompatible grafts are rejected less frequently than grafts that express only minor histocompatibility complex (minor H) or MHC plus minor H alloantigens. To describe the spectrum of T cells activated (or not) by orthotopic corneal grafts, the authors examined the development of delayed hypersensitivity (DH) to donor-specific alloantigens.

Methods. Recipient BALB/c mice received orthotopic corneal allografts from donor mice that were MHC incompatible at MHC loci only, multiple minor H loci only, or MHC plus multiple minor H loci. These groups of mice were examined to determine when alloantigen-specific DH developed.

Results. The authors report that all mice, whether they accept or reject grafts, acquire donor-specific DH within 4 weeks of engraftment. This reactivity is primarily directed at minor H, rather than MHC-encoded, alloantigens. Through time, spontaneous DH reactivity disappears in all mice, and thereafter, donor-specific DH can be induced by cognate immunization only in mice that have rejected their cornea grafts.

Conclusions. These results can be explained in the context of “direct” and “indirect” pathways of alloreognition. Because normal corneas lack passenger leukocytes, the potential for direct recognition of alloantigens on orthotopic corneal grafts is small. Therefore, T cells activated by orthotopic corneal allografts must recognize donor-derived antigens primarily on recipient antigen presenting cells, that is, through the indirect pathway of alloreognition. Because minor H antigens are the dominant cellular proteins in grafts, it is proposed that minor H determinants are the most immunogenic alloantigens in orthotopic corneal grafts because they are the major source of peptides that will be loaded onto recipient class II molecules for T-cell recognition. We further predict that long-term acceptance of corneal allografts is promoted when recipient mice acquire anterior chamber associated immune deviation (impaired and suppressed DH) directed at minor H alloantigens of the grafts. Invest Ophthalmol Vis Sci. 1995;36:427-434.

Although keratoplasty for the treatment of visual impairment secondary to corneal disease is often a successful clinical procedure, a significant number of operations fail. Frequently, immune rejection of the corneal graft is held to be responsible. For this reason, it is important to increase our knowledge and understanding of the mechanisms by which the immune system recognizes and responds to histoincompatible corneas grafted orthotopically. Until recently, experimental analysis of immune responses to corneal grafts has been conducted in rabbits and other large laboratory animals. Maumane was the first to point out that orthotopic corneal allografts performed in rabbits enjoyed prolonged survival, compared to solid tissue grafts placed at other orthotopic sites in the body. Based on studies of this type, it has been suggested that immune privilege is an important factor in the relative success experienced by orthotopic corneal allografts in man. Approximately 10 years ago, a method was devised for accomplishing orthotopic corneal
other tissue grafts and that minor H antigens from immunogenetic rules that govern the fate of or-
confront the recipient with minor H antigens. Al-
are rejected more acutely than are grafts that only H antigens and that, therefore, MHC-disparate grafts
grafts that displayed class I MHC alloantigens were
orthotopic corneal allografts. For example, corneal
for other solid tissue grafts do not appear to
compatibility complex (MHC, H-2), and combinations
class II antigens encoded by the murine major histo-
the cornea are more immunogenic than antigens en-
nea placed orthotopically in eyes of normal mice.4
neal grafts readily develop DH when cells genetically
in the cornea. We wanted to determine whether and when alloantigen-specific, cell-mediated immunity devel-
oped in mice grafted with histoincompatible corneas and to discern whether delayed hypersensitivity might be implicated in early graft failure and rejection.

MATERIALS AND METHODS

Mice

Six- to 12-week-old mice were obtained from the Uni-
versity of Miami breeding colony. All animals were
treated according to the ARVO Statement for the Use
of Animals in Ophthalmic and Vision Research. The
following inbred strains were used: BALB/c, C57BL/6,
B10.D2, BALB.K.

Method of Orthotopic Corneal Allografts

As described elsewhear, donor corneas were excised
by trephination by using a 2-mm bore and were placed
in chilled phosphate-buffered saline before grafting.
Recipient mice were deeply anesthetized with 0.66 mg keta-
mime hydrochloride (Vetalar; Parke-Davis; Detroit,
MI) administered intramuscularly. The graft bed was
prepared by trephining a 2-mm site in the central
cornea of the right eye and discarding the excised
cornea. The donor cornea was placed in the recipient
bed and secured with 12 interrupted sutures (11-0
nylon, 50-μm diameter needle, Sharpoint; Vanguard,
Houston, TX). Grafted eyes were examined after 72
hours; at that time, grafts with technical difficulties
(hyphema, infection, loss of anterior chamber) were
excluded from further consideration. At 9 days, the
sutures were removed.

Evaluation and Scoring of Orthotopic Cornea
Transplants

Grafts were examined by slit lamp microscopy at
weekly intervals, as described elsewhere.4 A scoring
system was devised to describe semiquantitatively the
extent of opacity (0 to 5+), as follows: 0 = clear graft;
1+ = minimal superficial (nonstromal) opacity; 2+
= minimum deep stromal opacity; 3+ = moderate
deep stromal opacity; 4+ = intense deep 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 scores of 4+ or
greater at 2 weeks never cleared and were also re-
garded as rejected.

Assay for Delayed Hypersensitivity Reaction in
Mice Bearing Corneal Grafts

At the appropriate time after grafting, 1 × 10^6 irrad-
ated (2,000 rads) spleen cells from donors syngeneic
with the corneal graft were injected in 10 μl of Hanks’
balanced salt solution into the right pinnae. The left
grafts in rats, and within the past few years, this
method has been adapted for use in laboratory mice.3
Using this model system, we have recently reported
that immune privilege is extended to allogenic cor-
neas placed orthotopically in eyes of normal mice.4
Irrespective of the degree of immunogenetic disparity
between donors and recipients of histoincompatible
cornea grafts, a significant proportion of grafts was
accepted by recipient mice, often for indefinite time
periods. In these studies, the fate of grafts expressing a
diversity of alloantigens was examined, including
multiple minor transplantation antigens, class I and
class II antigens encoded by the murine major histo-
compatibility complex (MHC, H-2), and combinations
to this rule,6 in general these rules apply to most solid
tissue allografts. Based on our study of orthotopic cor-
neal allografts in mice, we have proposed that the
immunogenetic rules that govern patterns of rejection
for other solid tissue grafts do not appear to apply to
orthotopic corneal allografts. For example, corneal
grafts that displayed class I MHC alloantigens were
rejected infrequently, whereas grafts that displayed mi-
nor transplantation antigens were rejected more of-
ten. In other solid tissue allografts, immunogenetic
rules have been deduced6 that state that MHC-en-
coded antigens are more immunogenic than minor
H antigens and that, therefore, MHC-disparate grafts
are rejected more acutely than are grafts that only
confront the recipient with minor H antigens. Al-
though there are several well-documented exceptions
to this rule,6 in general these rules apply to most solid
tissue allografts. Based on our study of orthotopic cor-
neal allografts in mice, we have proposed that the
immunogenetic rules that govern the fate of or-
thotopic corneal allografts are inverted compared to
other tissue grafts and that minor H antigens from
the cornea are more immunogenic than antigens en-
coded within the MHC.

In a recent study,7 we determined that indefinite
acceptance of orthotopic corneal allografts in mice
correlates positively with the development of anterior
chamber associated immune deviation (ACAID). That
is to say, mice that have accepted histoincompatible
corneal allografts in excess of 2 months are unable
to acquire delayed hypersensitivity (DH) to antigens
expressed on the grafts, even when attempts are made
to immunize the mice to donor antigens by nonocular
routes. By contrast, mice that have rejected their
corneal grafts readily develop DH when cells genetically
identical to the graft are injected subcutaneously.
The conclusion that mice with accepted corneal grafts have
ACAID was bolstered by our demonstration that the
spleens of these mice contain antigen-specific suppres-
or lymphocytes that inhibit the expression of DH in
adoptive transfer recipients.

The studies that form the basis of this report were
initiated to gain insight into the early phases of host
immune responses to alloantigens on orthotopic cor-
ear pinnae was inoculated with an equal volume of Hanks’ balanced salt solution only. 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 (Mitutoyo, MTI, Paramus, NJ). Ear swelling was expressed as follows: specific ear swelling = (24 hours measurement of right ear – 0 hours measurement of right ear) – (24 hours measurement of left ear – 0 hours 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 tested animal, and as group mean $\pm$ SEM. The DH data were obtained from groups of mice ear challenged at the postgrafting time designated in the Results section. Ear-challenged mice in which DH responses were determined were sacrificed; no mice were rechallenged.

Statistical Analyses

Ear swelling measurements were evaluated statistically by using a two-tailed Student’s $t$ test. All $P$ values $< 0.05$ were deemed significant. Corneal graft rejection was evaluated using a two-tailed Fisher’s exact test. All $P$ values of $<0.05$ were deemed significant.

RESULTS

Normal adult BALB/c mice received orthotopic grafts of corneas obtained from donors representing different degrees of immunogenetic disparity: C57BL/6 grafts confront BALB/c recipients with MHC plus multiple minor H incompatible alloantigens; B10.D2 grafts present only minor H alloantigens to BALB/c; BALB.K grafts display only MHC-encoded alloantigens to BALB/c. A large series of orthotopic grafts was performed, and Figure 1 displays a summary of the fate of these grafts 8 weeks after grafting, as judged by clinical examination with a slit lamp. As reported previously, graft failure (defined as corneal opacity $\geq 2+$) can be determined as early as 4 weeks after grafting. Irreversible failure was diagnosed when an orthotopic allograft displayed 2+ or greater opacity for 2 or more weeks and was opaque at 8 weeks after engraftment. BALB/c mice failed to reject syngeneic corneal grafts ($n = 15$, 0% rejected). BALB/c mice that received MHC plus minor H disparate ($n = 18$, 44% rejected) or minor H only disparate grafts ($n = 17$, 41% rejected) rejected these grafts at a frequency significantly greater than syngeneic controls ($P = 0.018$ and 0.031, respectively). By contrast, BALB/c mice that received MHC only disparate corneal grafts rejected these grafts at a reduced frequency ($n = 15$, 20% rejected) that was not significantly different from syngeneic control grafts ($P = 0.233$). The following series of experiments examined panels of orthotopic corneal graft recipients subjected to various experimental protocols to determine whether and when antigen-specific DH developed.

Onset of Delayed Hypersensitivity in Mice Bearing Orthotopic Corneal Allografts

BALB/c mice bearing corneal allografts from C57BL/6 donors were first tested for DH at 2 weeks after grafting. At this time, approximately 30% of grafts displayed opacity of 2+ or greater, whereas the remainder displayed little evidence of inflammation. It was too early to predict at 2 weeks which grafts would eventually succumb to rejection; we previously found that some grafts with 2+ opacity scores at 2 weeks cleared within the next few weeks, whereas grafts clear at 2 weeks became opaque during the next few weeks and remained so indefinitely (considered to be rejected). DH reactions of grafted mice were compared with responses of the positive control group, specifically with sensitized normal BALB/c mice that received subcutaneous injections of C57BL/6 spleen cells ($10 \times 10^6$) and ear challenged with C57BL/6 alloantigens 2 weeks later. As displayed in Figure 2 and Table 1, the positive control group demonstrated significant DH (specific ear swelling of $57 \times 10^{-3}$ mm $\pm 9$). When the ears of cornea-grafted mice were challenged with irradiated C57BL/6 spleen cells at 2 weeks after grafting, none of the ears developed significant swelling ($11 \times 10^{-3}$ mm $\pm 3$) compared with naive mice ($35 \times 10^{-3}$ mm $\pm 6$). Thus, if orthotopic corneal allografts are capable of inducing delayed hypersensitivity, these results indicate that at 2 weeks after grafting, the sensitization process had not yet reached levels that could be detected by this assay.

![Figure 1](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933410/)
Donor-specific delayed hypersensitivity in BALB/c mice bearing orthotopic C57BL/6 corneal allografts for 2 weeks. Ears of grafted and primed, and naive mice received injections of $1 \times 10^6$ irradiated (2000R) C57BL/6 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 response for the group. Delayed hypersensitivity response of grafted mice was not significantly different from naive control mice.

The next time at which DH was assessed in grafted mice was 4 weeks after keratoplasty. Based on our previous experience with murine orthotopic corneal allografts, we anticipated that approximately 25% of C57BL/6 grafts on BALB/c recipients at this time would be opaque (score of 2+). Almost invariably, these grafts can be expected to remain opaque and never resolve, that is, they are irreversibly rejected. The remaining grafts (75%) at 4 weeks after grafting express little or no evidence of opacity or edema, even though a few of these grafts may undergo failure and rejection during the subsequent weeks. Two groups of mice were assayed for DH at 4 weeks after grafting: one panel consisted of mice with clear corneas (termed "acceptors"), and the other panel consisted of mice with opaque corneas (scores of 2+, termed "rejectors"). The ear swelling responses to intrapinnae challenge with irradiated C57BL/6 cells are presented in Figure 3 and are summarized in Table 1. The findings indicate that DH activity was lost in mice that accept their corneal grafts at 8 and 20 weeks after grafting. By contrast, mice with rejected corneal grafts displayed an intermediate DH response at 8 weeks, in which some individual mice still displayed significant DH activity whereas others lost the ability to generate graft-specific DH (Fig. 4). Graft-specific DH reactivity waned over the next 3 months. At 5 months after keratoplasty, all but a single mouse failed to display DH, whether the graft was judged to be healthy or was rejected at the time. Thus, the DH reactivity evoked by orthotopic allogeneic corneal grafts proves to be short-lived and correlates poorly with clinical assessment of graft outcome.

**TABLE 1. Time Course of Donor-Specific Delayed Hypersensitivity After Orthotopic Corneal Transplantation**

<table>
<thead>
<tr>
<th>Time After Grafting</th>
<th>Acceptors</th>
<th>Rejectors</th>
<th>Positive Controls*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 weeks</td>
<td>–</td>
<td>–</td>
<td>++</td>
</tr>
<tr>
<td>4 weeks</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>8 weeks</td>
<td>–/±</td>
<td>–</td>
<td>++</td>
</tr>
<tr>
<td>20 weeks</td>
<td>–</td>
<td>–</td>
<td>++</td>
</tr>
</tbody>
</table>

C57BL/6 corneas were grafted orthotopically to BALB/c mice; recipients were ear challenged with C57BL/6 (10 X 10^6) spleen cells at appropriate times after grafting. Ear swelling responses (DH) are listed as positive or negative, based on data presented in Figures 2, 3, 4, and 5.

* DH response of mice immunized for the corresponding length of time by subcutaneous inoculation of allogeneic spleen cells.
† At 2 weeks after grafting, it was not possible to distinguish "acceptor" from "rejector" mice.
‡ At this time, some mice were positive for DH, whereas others were negative.


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the relative contributions these different types of alloantigens make to the acquisition of DH in grafted mice, the following experiments were performed. First, a panel of BALB/c mice received orthotopic cornea grafts from BALB.K donors; the latter confront the recipients only with MHC alloantigens. The ears of these mice were challenged with BALB.K spleen cells 4 to 10 weeks later, and the DH response was measured (Fig. 6). The positive control group consisted of an equal number of mice primed to the alloantigens for the corresponding length of time (4 to 10 weeks). The results indicate that mice that accept MHC-only disparate corneal allografts fail to generate MHC-specific DH (32 × 10^{-3} mm ± 3) compared with the positive control group (80 × 10^{-3} mm ± 11). However, among mice that rejected corneal grafts, although 2 of 6 displayed MHC-specific DH, the mean response (50 × 10^{-3} mm ± 12) was not significantly different from naive mice (24 × 10^{-3} mm ± 3).

Second, a panel of BALB/c mice received corneal allografts from C57BL/6 donors. Four weeks after grafting, the ears of these mice were challenged with B10.D2 spleen cells, which only share minor H antigens with C57BL/6. The DH responses of these mice are presented in Figure 7. The results indicate that all grafted mice (rejector and acceptor) mount minor H antigen-specific DH responses. The magnitude of the responses by rejector (103 × 10^{-3} mm ± 12) and acceptor (75 × 10^{-3} mm ± 9) mice were equivalent in intensity to the response displayed by positive control mice (67 × 10^{-3} mm ± 8). Third, BALB/c mice received orthotopic grafts from B10.D2 mice, a circumstance in which the graft presents only minor H anti-
FIGURES 7 and 8. Donor-specific delayed hypersensitivity in BALB/c mice bearing orthotopic corneal allografts (minor H only disparate allografts). Recipients of C57BL/6 grafts (Fig. 7, left) or B10.D2 grafts (Fig. 8, right) were ear challenged with irradiated B10.D2 spleen cells (1 × 10⁶), and their ears were measured. Results are presented as described in the legend to Figure 2. *Mean DH is significantly different from naive mice at P < 0.05.

gens to the recipient. When the ears of these mice were challenged with B10.D2 spleen cells 4 weeks later (Fig. 8), positive DH response were detected in mice that had accepted their minor H-disparate grafts (64 × 10⁻³ mm ± 9) and in mice that had rejected their grafts (60 × 10⁻³ mm ± 14). These responses were significantly greater than negative control mice (24 × 10⁻³ mm ± 8) and were equivalent to the positive control group (49 × 10⁻³ mm ± 5). These results are summarized in Table 2. In the aggregate, these findings imply that minor H antigens, rather than MHC-encoded antigens, are more successful inducers of DH in mice bearing orthotopic corneal allografts.

DISCUSSION

Unlike orthotopic, MHC-disparate skin allografts, orthotopic corneal allografts are not uniformly rejected in mice. In the former, substantial experimental evidence indicates that full-thickness skin allografts activate a diverse spectrum of immune T-cell effector modalities. As a consequence, recipients of these grafts acquire donor-specific T cells that mediate delayed hypersensitivity, T cells that are primed to lyse appropriate target cells, and memory T cells. The results presented in this article, along with previously reported data, indicate that a distinct, incomplete, and temporally limited spectrum of T-cell effectors is activated by orthotopic corneal allografts. T cells that mediate delayed hypersensitivity and regulatory T cells that suppress expression of delayed hypersensitivity are detected.

Donor-specific DH was detectable in corneal allograft recipients as early as 4, but not 2, weeks after engraftment. This is of interest because mice that are grafted orthotopically with allogeneic skin display donor-specific DH within 7 days (ref. 7 and unpublished observations, 1993). It is thought that the primary mode of sensitization after skin grafting, and an important reason for the rapidity with which sensitization is evoked, is the release of so-called "passenger leukocytes" from the skin at the time of grafting. The passenger cells of skin include Langerhans cells within the epidermis and dendritic cells and macrophages in the dermis. The superior sensitizing ability of "passenger cells" within skin grafts has been ascribed, on the one hand, to their ability to migrate from the graft and travel to draining regional lymph nodes and, on the other hand, to their ability to migrate from the graft and travel to draining regional lymph nodes and, on

TABLE 2. Specificity of Delayed Hypersensitivity Induced by Orthotopic Corneal Allografts

<table>
<thead>
<tr>
<th>Graft Donor</th>
<th>Recipient</th>
<th>Ear Challenge</th>
<th>Type of Alloantigen</th>
<th>Delayed Hypersensitivity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6</td>
<td>BALB/c</td>
<td>C57BL/6</td>
<td>MHC + Minor H</td>
<td>++</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>BALB/c</td>
<td>B10.D2</td>
<td>Minor H only</td>
<td>++</td>
</tr>
<tr>
<td>B10.D2</td>
<td>BALB/c</td>
<td>B10.D2</td>
<td>Minor H only</td>
<td>++</td>
</tr>
<tr>
<td>BALB.K</td>
<td>BALB/c</td>
<td>BALB.K</td>
<td>MHC only</td>
<td>++</td>
</tr>
</tbody>
</table>

* Ear challenge conducted at 4 weeks after grafting.
† DH response of mice immunized by subcutaneous inoculation of allogenic spleen cells of the appropriate alloantigen.
the other hand, to the powerful array of accessory signals with which dendritic cells activate T cells. 

This pathway of allorecognition has been called "direct" because recipient T cells recognize donor alloantigens directly on donor-derived cells. 

Comparing skin to cornea as a graft, the central region of the normal cornea possesses virtually no passenger cells, neither Langerhans cells within the corneal epithelium nor dendritic cells or macrophages in the stroma. 

We suspect, but have no direct evidence, that the delay in onset of donor-specific DH in cornegafted mice is due to the lack of passenger cells (i.e., Langerhans cells) in the donor cornea. In support of this suspicion, Peeler and Niederkorn have demonstrated that the capacity of heterotopic cornea grafts to induce DH in mice is related to the graft's content of Langerhans cells. 

When normal corneas, which lack Langerhans cells, are grafted to the thoracic wall they fail to elicit graft-specific DH, whereas cornea grafts, forced by experimental artifice to contain Langerhans cells, evoke vigorous DH when grafted to the thoracic wall of allogeneic recipients.

Despite the fact that the normal corneas we used for grafting were deficient in Langerhans cells, recipients of these allografts eventually acquired donor-specific DH. Sensitization in these mice indicates that allorecognition of corneal antigens can occur by the "indirect" pathway, that is, recipient T cells recognize donor alloantigens after they have been processed and presented as peptides on host MHC molecules. 

We have examined orthotopic C57BL/6 corneal grafts 21 to 28 days after engraftment into BALB/c eyes and have found numerous La" mononuclear and dendritic cells within the central epithelium and stroma (unpublished observations, 1994). This suggests that recipient antigen-presenting cells have invaded the graft, which raises the possibility that these cells can provide the cellular basis for indirect pathway recognition of minor H and MHC alloantigens on the graft. 

Based on their considerations, it is tempting to speculate that the immune system can only recognize alloantigens from orthotopic corneal grafts through the indirect allorecognition pathway. If true, it would be worthwhile to devise experimental strategies that can preferentially inhibit this pathway, rather than the direct pathway.

We were surprised to learn that all cornea-grafted mice displayed donor-specific DH at 4 weeks and that this reactivity subsequently disappeared spontaneously whether the cornea grafts were accepted or rejected. Moreover, it was of interest to learn that the evanescent DH reactivity displayed at this time was directed primarily at minor H, rather than MHC-encoded, alloantigens. It is generally thought minor H antigens represent polymorphic cellular proteins that must be processed into peptides that are then loaded onto class II molecules before they can be recognized by T cells. 

Thus, by definition, minor H antigens are recognized through the indirect pathway. Because there may be more minor H-type proteins in cells than there are MHC-encoded antigens, minor H antigens may be the major source of peptides recognized through the indirect pathway. Perhaps this is why the immunogenetic rules of transplantation are inverted for orthotopic corneal allografts. 

According to this construction, T cells have little opportunity to recognize corneal alloantigens by direct pathways because the grafts lack passenger leukocytes. Therefore, we predict that when recipient antigen-presenting cells infiltrate the graft, minor H antigens represent the major source of donor-derived proteins processed and presented through the indirect pathway to T cells. If this prediction is correct, it is possible that minor H antigens may act as immunodominant alloantigens in orthotopic corneal allograft rejection. Experiments will be conducted to test this prediction directly.

There may be a clinical counterpart to the proposal that corneal alloantigens are recognized by T cells through an indirect antigen presentation pathway, and it is related to the controversy in ophthalmology concerning the role of HLA antigens in corneal allograft failure. The evidence that HLA matching improves clinical corneal transplant survival is incomplete and contradictory. 

To resolve this controversy, a multi-center Collaborative Corneal Transplantation Study was recently conducted in the United States. 

One conclusion reached from this study is that HLA matching does not improve the survival rate of keratoplasties, even in so-called high-risk situations. Surprisingly, another conclusion reached by this study is that matching for the human ABO isohemagglutinin locus, I, does improve corneal graft survival. 

Although the ABO antigens are carbohydrates and can be detected by antibodies, the antigens are synthesized intracellularly by glycosyl transferases that are polymorphic. As such, these allotypic proteins can provide peptides that could be loaded onto MHC molecules and recognized by T cells. We speculate that corneal rejection may occur in humans because T cells recognize MHC-associated peptides derived from polymorphic glycosyltransferases encoded at locus I. In other words, an indirect pathway of allorecognition may be the basis of rejection of ABO-incompatible corneal allografts.

We previously reported that mice with longstanding, accepted orthotopic corneal allografts have donor-specific ACAID; they cannot be induced to mount DH reactions when primed against donor alloantigens. 

By contrast, sensitization leading to donor-specific DH can be readily induced in mice bearing irreversibly rejected orthotopic corneal allografts. We report here that all cornea-grafted mice acquire donor-
specific DH and that this response disappears in mice that accept or reject their grafts. ACAID, the stereotypic, deviant immune response acquired by acceptor mice, has been closely linked to the mechanisms responsible for immune privilege in the eye. This suggests that ACAID induction is critical to the success of orthotopic corneal allografts. The observation that ACAID is detected after donor-specific DH has been induced in cornea recipients reveals that ACAID can be imposed on previously sensitized animals. Experiments that confirm this prediction were recently obtained.

Key Words: corneal transplantation, immune privilege, immunoregulation, transplantation, ACAID

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References