Langerhans Cells, Orthotopic Corneal Allografts, and Direct and Indirect Pathways of T-Cell Allorecognition

Yoichiro Sano,1,2 Bruce R. Ksander,1 and J. Wayne Streilein1

PURPOSE. To determine after orthotopic corneal allografting the role of Langerhans cells in activation of T cells via the direct and indirect pathways of allorecognition and the relationship between these pathways and the rapidity of graft rejection.

METHODS. Corneas from eyes of normal mice and from eyes after superficial cauterization were grafted to eyes of major histocompatibility complex (MHC) and/or minor histocompatibility (H)–disparate recipient mice. The grafts were analyzed through time for content of class II MHC–bearing Langerhans cells and for rejection or acceptance. Graft recipients were evaluated for acquisition of delayed hypersensitivity (DH) and cytotoxic T cells (Tc) directed at donor MHC and minor H alloantigens.

RESULTS. Langerhans cells migrated more rapidly into epithelium of cauterized grafts than normal grafts. Unlike normal grafts, the vast majority of cauterized allografts were rejected within 2 weeks. Normal grafts induced neither DH nor Tc directed at donor MHC antigens, whereas cauterized grafts induced both DH and Tc specific for donor MHC. All grafts induced DH directed at donor minor H antigens, but only rejected grafts correlated with acquisition of Tc directed at donor minor H antigens.

CONCLUSIONS. The rapidity of orthotopic corneal allograft rejection correlated with density of Langerhans cells within epithelium and with acquisition of donor-specific DH and Tc. Although recipient-derived Langerhans cells promoted minor H–specific, self-MHC-restricted T cells (indirect pathway) and subacute graft rejection, donor-derived Langerhans cells promoted early, acute rejection in conjunction with allogeneic MHC-specific Tc (direct pathway). (Invest Ophthalmol Vis Sci. 2000;41:1422–1431)

The immune privilege enjoyed by orthotopic corneal allografts is multifactorial.1,2 On the one hand, the site in which the graft is placed (forming the anterior surface of the anterior chamber) is a well-studied immune-privileged site.3–5 On the other hand, cornea possesses inherently the properties of an immune-privileged tissue.6,7 Many features of the cornea contribute to its privileged status: reduced expression of class I MHC molecules, especially on keratocytes and endothelial cells;8,9 constitutive expression of CD95L on corneal parenchymal cells10–12; constitutive secretion of immunosuppressive factors;13–15 the absence of blood vessels and lymph channels; and the absence of class II MHC–bearing, bone marrow–derived cells, such as Langerhans cells and macrophages.16,17 This last property appears to be particularly important in the unique manner by which orthotopic corneal allografts induce donor-specific sensitization. Heterogeneous grafts placed on the thoracic wall of mice readily induce sensitization and are rejected, unless the only histocompatibility antigen difference is at the class II MHC locus.18 If grafts are prepared from corneas into which class II–bearing Langerhans cells have been recruited and are then grafted heterotopically, both class II–specific sensitization and rejection occurs.19 Peeler et al.20 studied the cellular immune responses of recipients of heterotopic cornea grafts with or without Langerhans cells in the epithelium. Allogeneic corneas induced delayed hypersensitivity (DH) whether Langerhans cells were present or not, but cytotoxic T cells directed at donor MHC alloantigens were induced only if Langerhans cells were present in the immunizing cornea graft.

Over the past several years our laboratory as well as others has examined in detail the alloantigens most responsible for corneal allograft rejection.21–25 In both normal (low risk) and neovascularized (high risk) mouse eyes, minor histocompatibility (H) antigens, rather than MHC-encoded antigens, proved to be the more significant barriers to successful cornea engraftment. Moreover, recipients of orthotopic corneal allografts acquired DH and cytotoxic T cells that were directed at self-restricted minor H, rather than MHC, alloantigens.26 Presentation of alloantigens to T cells in the context of self–class I or II MHC molecules is called the “indirect” pathway of allorecognition.27–29 This is the only pathway available for presentation of minor transplantation antigens when donor and recipient share no MHC-encoded class I or II molecules. This pathway also accounts for the presentation of peptides derived from MHC alloantigens. By contrast, intact MHC alloantigens can be

From the 1Schepens Eye Research Institute, Department of Ophthalmology, Harvard Medical School, Boston, Massachusetts; and 2Department of Ophthalmology, Kyoto Prefecture School of Medicine, Kyoto, Japan.

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Corresponding author: J. Wayne Streilein, Schepens Eye Research Institute, 20 Staniford Street, Boston, MA 02115-2500.

waynes@vision.eri.harvard.edu

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recognized directly by T cells with a different set of receptors for antigen, and this is called the “direct” pathway of allore cognition. In solid tissue allografts, numerous class II-expressing, bone marrow-derived cells of the dendritic and macrophage type are present, and these cells, termed passenger leukocytes,30–32 are primarily responsible for the activation of allo reactive T cells through the direct pathway. The absence of passenger cells within the normal cornea correlates very well with the relative inability of orthotopic corneal allografts to activate direct alloreactive T cells. Because corneal allografts activate and may eventually succumb to the effector function of indirect alloreactive T cells, antigen-presenting cells other than passenger leukocytes, that is, of recipient origin, must be responsible for initial T-cell activation.

Various forms of trauma to the cornea, including sutures through the central epithelium,33 mild cauterization of the central corneal surface,34 and injection of polystyrene beads into the corneal stroma,35 result in the centripetal migration of Langerhans cells from the limbus into the central corneal epithelium. This migration may be accompanied by macrophage and monocyte invasion into the stroma. The proinflammatory cytokine, interleukin 1 (IL-1), plays a central role in this migration. Direct stromal injection of IL-1 induces Langerhans cell immigration into the corneal epithelium,35 and topical treatment with IL-1 receptor antagonist suppresses Langerhans cell migration from the limbus after cautery.36 The surgical procedure of orthotopic corneal transplantation is itself a form of trauma and may induce recipient Langerhans cells to migrate from the limbus into the graft after transplantation.

We have conducted a series of experiments in mice designed to determine the extent to which Langerhans cells of donor origin contribute to the capacity of orthotopic corneal allografts to trigger the graft’s rejection and to activate T cells via the direct and/or indirect pathways of allore cognition. The results reveal that recipient Langerhans cells migrate rapidly into corneal grafts, especially if the graft bed is neovascularized, implying that these cells are responsible for activating minor H-2-specific T cells of the indirect alloreactive type. If Langerhans cells of donor origin are present in the donor cornea at the time of grafting, rejection is more frequent and more rapid, and both CD4+ and CD8+ MHC-specific T cells of the direct alloreactive type are activated.

**Materials and Methods**

**Mice**

Seven- to 9-week-old mice were purchased from Taconic Farm (Germantown, NY) or Jackson Laboratories (Bar Harbor, ME). The following inbred strains with the following H-2 alleles were used: BALB/c (H-2b), A BY (H-2d), BALB.B (H-2d), and A/J (H-2k/d). All animals were treated in accordance with the ARVO Statement for Use of Animals in Ophthalmic and Vision Research.

**Corneal Cauterization and Suturing**

Cautery of the corneal surface was accomplished with a handheld Accu-Temp thermal cautery (Concept Inc., Los Angeles, CA). Five light burns were applied to the central third of the cornea.33 To induce corneal neovascularization, three 10-0 nylon sutures were placed superficially through the central cornea as described previously.33

**Langerhans Cells in Corneal Allograft Immunity**

**Langerhans Cell Assay**

To determine the density of Langerhans cells in the cornea, epithelial sheets were prepared by soaking the eyes in EDTA-phosphate-buffered saline and stripping the epithelium off gently with a fine forceps. The epithelial sheets were stained with an immunofluorescence assay using a monoclonal antibody against I-Ak and I-Ak. The epithelial sheets were mounted on glass slides, and the number of Langerhans cells was counted in the central half of the cornea using an ocular grid and observing the sheet through the light microscope.34

**Corneal Transplantation and Grafting**

Orthotopic corneal transplantation was performed as described previously.34 Briefly, donor central corneas (2 mm diameter) were excised by vannas scissors and placed in chilled phosphate-buffered saline. Recipients were anesthetized with intraperitoneal (i.p.) injections of ketamine (3–4 mg/recipient) and xylazine (0.1 mg/recipient). The graft bed was prepared by excising with vannas scissors a 2-mm site in the central cornea of the right eye. The donor cornea was then placed in the recipient bed and secured with eight interrupted sutures (110 nylon). 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. At 9 days after grafting, all sutures were removed.

**Evaluation and Scoring of Orthotopic Cornea Transplants**

After corneal transplantation, grafts were examined by slit lamp microscopy at weekly intervals. At each time point, grafts were scored for opacity and neovascularization by a method previously described.21 Briefly, the 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 sustained opacity scores of 2+ or greater for 5 weeks or at the end of the 8-week observation interval were considered to have been rejected.

**Assay for Cytotoxic T cells**

Recipient mice were killed, and their cervical lymph nodes and spleen were removed. Effector cells from BALB/c recipients (5 × 106) and irradiated stimulator cells from the appropriate mouse strain (5 × 106) were plated together into 24-well plates in 2 ml of culture media containing RPMI 1640 (GIBCO, Grand Island, NY), nonessential amino acids, 0.1 mM sodium pyruvate, 1 mM l-glutamine, 2 mM penicillin, 100 units; streptomycin, 100 mg/ml (GIBCO); HEPES, 5 mM (GIBCO); and 2-mercaptoethanol, 2 × 10-5 M. Cells were cultured at 37°C in a humidified 5% CO2 atmosphere for 3 days. Target cells (spleen cells) from appropriate strains of mice were placed in 10 ml of culture media 3 days before the actual assay, and 5 µg/ml of Concanavalin A (ConA) was added. On the day of assay, these ConA target cells were used in a standard 4-hour 51Cr release assay. Effector cells were plated with 1 × 104 target cells in triplicate at ratios of 6:1, 12:1, 25:1, 50:1, and 75:1. Six wells containing only media and target cells were used to measure spontaneous release, and 6 wells containing 5 N HCl and target cells were used to measure maximal release. The percent
specific cytotoxicity at each dilution was calculated using the following formula: (Experimental mean – Spontaneous release mean)/(Maximal release mean – Spontaneous release mean) × 100.

**Assay for Delayed Hypersensitivity Reactions**

At 2 weeks after grafting, 1 × 10⁶ X-irradiated (2000 rad) spleen cells from the appropriate allogeneic strain were injected in 10 μl Hanks’ 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 (s.c.) injection of 10 × 10⁶ 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) ÷ 10⁻³ mm. Ear swelling response 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.

**Statistical Analyses**

Corneal graft rejection was evaluated using a two-tailed Fisher’s exact test. The percent specific cytotoxicity in cytotoxic T-lymphocyte (CTL) assays, and ear swelling measurements in DH assays were evaluated statistically by using a two-tailed Student’s t-test. P < 0.05 was considered significant.

**RESULTS**

**Migration of Recipient Langerhans Cells into Orthotopic Corneal Allografts**

Cornea grafts were prepared from eyes of normal A.BY mice. These grafts were placed orthotopically in normal BALB/c eyes and in eyes rendered “high-risk” by three sutures that were placed in the central cornea 2 weeks previously. High-risk eyes were intensely neovascularized at the time of cornea grafting. Fifty percent of the grafts placed into normal eyes were rejected, whereas all the grafts placed in high-risk eyes were rejected. Graft rejection occurred between 4 and 8 weeks (normal eyes) and at 2 weeks after grafting (high-risk eyes). Grafts were excised at 7, 14, 21, and 28 days thereafter, and the epithelium was removed and examined for class II Langerhans cells by immunofluorescence microscopy. The density of Langerhans cells/mm² was determined with the aid of an ocular grid. The number of cells/mm² was determined for five mice per group per day. All experiments were performed at least twice. The results of this experiment are summarized in Figure 1. In the normal cornea, the baseline density of Langerhans cells is approximately 5/mm². Virtually all these cells are near the limbus. In cornea grafts placed in normal beds, rejected grafts; (○), normal beds, accepted grafts; (■), neovascularized beds, rejected grafts. *Significantly higher than day 0 controls.

**FIGURE 1.** Migration of recipient Langerhans cells into orthotopic corneal allografts. A.BY corneas for normal eyes were grafted into normal or neovascularized eyes of BALB/c mice. The grafts were excised periodically thereafter, and the density of Langerhans cells (I-A<sup>d</sup>) was determined within epithelial sheets by fluorescence microscopy. Mean density ± SEM of I-A<sup>d</sup> cells are presented. (●), normal beds, rejected grafts; (○), normal beds, accepted grafts; (■), neovascularized beds, rejected grafts. *Significantly higher than day 0 controls.
although the levels remained significantly higher than the levels detected in cornea grafts placed in normal eyes. These findings indicate that recipient Langerhans cells migrated into cornea allografts and that the rate of migration was greater when the graft-bed was neovascularized. We have previously reported that BALB/c mice first acquired and displayed donor-specific DH at 4 weeks after C57BL/6 corneas were grafted into normal eyes, whereas donor-specific DH was detected within 2 weeks when similar allografts were placed in high-risk BALB/c eyes. When viewed in the context of our present findings, we deduce that the tempo with which a cornea graft recipient acquires donor-specific DH is dictated by the rate at which Langerhans cells migrate into the graft.

Fate of Orthotopic Cornea Allografts Containing Donor-Derived Langerhans Cells

The evidence described above makes a strong circumstantial case for a key role of Langerhans cells in the induction of donor-specific immunity in recipients of orthotopic corneal allografts. A similar argument has been advanced by Callanan et al., who studied corneal graft rejection in rats. In an attempt to prove this hypothesis, cornea grafts were prepared from eyes of A.BY mice 2 weeks after the central corneal surface was exposed to mild cautery. We have previously demonstrated that catherization of this type causes limbal Langerhans cells to migrate into the central corneal epithelium, but no neovascularization develops in these corneas. With respect to the densities of I-A<sup>b</sup>-bearing dendritic cells in catherized A.BY corneas of the type used in these experiments, the central epithelium of the normal A.BY cornea contains fewer than 10 class II–bearing Langerhans cells/mm<sup>2</sup>. By contrast, 2 weeks after cautery, the density of Langerhans cells was found to be approximately 58/mm<sup>2</sup> (data not shown).

Grafts from normal and catherized A.BY eyes were prepared and placed orthotopically in normal eyes of BALB/c mice. These mouse strains differ across the MHC and at multiple minor H loci. The opacity scores of the grafts were assessed at weekly intervals thereafter, as described in Materials and Methods, and the results are presented in Figure 2. Similar to results previously reported, grafts prepared from normal A.BY eyes experienced within the first week transient opacity (nonspecific) secondary to the trauma of the grafting procedure (Fig. 2A). Starting at 14 days, a small but increasing number of grafts displayed an opacity score of 2<sup>+</sup> or greater, indicative of irreversible rejection. In this panel, 50% of the grafts were eventually destroyed, and the remainder were accepted and remained clear at 8 weeks. By contrast, a high proportion of grafts prepared from catherized eyes never recovered from the nonspecific opacity that developed within the first week (see Fig. 2B). By 3 weeks, only one such graft was still clear; thereafter, all grafts displayed impenetrable opacity that remained in this state for the remainder of the observation interval. A summary comparison of these two sets of results is presented in Figure 3.

In control experiments, catherized syngeneic BALB/c corneas were grafted into normal BALB/c eyes and observed clinically. The initial opacity that typically followed surgical trauma cleared from these catherized grafts promptly (within 1 week), and the grafts remained clear for the next 8 weeks (data not shown). This result indicates that prior catherization of the corneal surface has no significant deleterious effect on the tissue as an orthotopic graft, unless the graft is placed in an allogeneic recipient. Together, these findings indicate that cornea grafts that already contained donor class II–positive Langerhans cells at the time of grafting experienced acute rejection reactions that led within 2 weeks to irreversible destruction.

Acquisition of Donor-Specific Delayed Hypersensitivity by Recipients of Langerhans Cell–Containing Cornea Allografts

Our next goal was to determine whether recipients of Langerhans cell–containing cornea grafts acquired donor-specific DH and if so, whether the immunity was directed at MHC and/or minor H alloantigens. BALB/c mice received A.BY corneas from eyes treated with cautery 2 weeks previously or from normal A/BY eyes. Two weeks after grafting the ear pinnae of panels of grafted mice were ear challenged with X-irradiated lymphoid cells (1 × 10<sup>6</sup>) from one of three different strains of mice: A.BY; BALB.B, which express the MHC alloantigens of A.BY, but are otherwise syngeneic with BALB/c; and A/J, which express minor H antigens of A.BY, but MHC antigens unrelated to A.BY. Panels of BALB/c mice, serving as positive controls, were immunized s.c. with A.BY spleen cells (10 × 10<sup>6</sup>) and ear-challenged with the three different genetic types of lymphoid cells. Ear swelling responses, indicative of DH were assessed and are presented in Figure 4. Recipients of catherized corneal allografts displayed DH when ear-challenged with cells expressing H–2<sup>b</sup> alloantigens: A.BY and BALB.B (Figs. 4A, 4B, respectively). The level of ear swelling was not significantly different from that of positive controls. No such reactivity was detected in recipients of normal A.BY corneas. We have previously reported that recipients of normal minor H–disparate grafts display reduced DH at 2 weeks. We infer from these findings that the direct allorecognition pathway was operative in the induction of DH by Langerhans cell–containing corneal allografts. This inference is based on the previous finding that corneas that lack passenger leukocytes (normal corneas) only sensitize their recipients to minor H antigens. If reprocessing of MHC alloantigens into peptides that are loaded onto self-MHC molecules were to make a significant contribution to the immunity evoked by corneal allografts, this would have been detected in the DH assays described in Ref. 26. No such MHC-specific DH activity was found. Moreover, recipients of catherized corneal grafts also displayed DH when challenged with A/J spleen cells (see Fig. 4C). These results confirm that corneal allografts activate the indirect pathway of T-cell allorecognition of minor H antigens. Thus, Langerhans cell–containing corneal allografts induce systemic immunity to minor H alloantigens via the indirect pathway, and MHC alloantigens by both the direct and indirect pathways. Moreover, grafts with Langerhans cells induced donor-specific DH within 2 weeks. We reported previously that donor-specific DH first was detectable at 4 weeks when allogeneic corneas normally deficient in Langerhans cells were grafted orthotopically. This indicates that early appearance of donor-specific DH after orthotopic corneal allografts correlates positively with early presence of Langerhans cells within the graft epithelium.

Cytotoxic T-Cell Activity of Lymphoid Cells Obtained from Recipients of Orthotopic Langerhans Cell–Containing Cornea Allografts

We have previously reported that primed donor minor H–specific cytotoxic T cells were detected regularly in recipients
with rejected corneal allografts, but primed donor MHC-specific cytotoxic T cells were never detected in these mice. Because cauterized corneal allografts had rejection much more frequently and swiftly than normal corneal allografts, we hypothesized that the high rate of graft rejection of cauterized corneal allografts might result from priming of donor MHC-specific cytotoxic T cells. The following experiments were designed to test this hypothesis. Five BALB/c mice that received orthotopic corneal allografts from cauterized A.BY eyes were killed at 2 weeks after grafting, and their cervical lymph nodes (ipsilateral to the grafted site) and spleen were removed. Lymph node cells were cultured with irradiated A.BY spleen cells. Three days later, effector cells were harvested and examined for cytotoxic activity on BALB.B target cells. Lymph node and spleen cells from BALB/c mice that received s.c. injections of A.BY spleen cells 2 weeks previously served as positive controls. The results of cytotoxic activity of ipsilateral cervical lymph node cells are shown in Figure 5A. As previously reported, effector cells from s.c.-primed animals developed donor-specific cytolytic capacity within 3 days of culture, lys-
dramatically enhanced if the graft was manipulated to contain Langerhans cells. Prior to grafting, these cells migrated into the corneal epithelium at a rate of approximately 20% per day. They continued to migrate for several days after grafting, with the peak rate of migration occurring at 1 week post-grafting. In the absence of Langerhans cells, the rate of graft rejection was significantly reduced, indicating the role of Langerhans cells in the rejection process.

**DISCUSSION**

The first evidence that Langerhans cells might be important in the immune physiology of the cornea was reported almost 20 years ago. Streilein et al. demonstrated that (1) corneal epithelium, except at the limbus, was devoid of Langerhans cells, and (2) cornea allografts disparate from their recipients only at class II MHC loci were not rejected when grafted heterotopically to the thoracic wall. This information gradually merged with the concept that Langerhans cells are included among the bone marrow–derived cells called "passenger leukocytes." In fact, Callanan et al. reported in 1988 that the tempo of rejection of orthotopic corneal allografts in rats was dramatically enhanced if the graft was manipulated to contain donor-derived Langerhans cells at the time of grafting. This and other evidence indicates that cells of this type confer a high degree of immunogenicity on solid tissue allografts. Within the past decade one important dimension to the role of passenger leukocytes in alloimmunity relates to their capacity to activate direct alloreactive T cells. T cells of this type are present in the periphery at a high clonal frequency, account for the T-cell proliferation observed in vitro mixed lymphocyte reactions, and are the primary mediators of acute rejection of solid organ transplants of skin, kidney, and heart.

The deficit of Langerhans cells in the normal cornea has been correlated with the inability of orthotopic MHC-disparate corneal grafts to suffer acute immune rejection. In fact, the absence of corneal Langerhans cells has been invoked to explain why minor H, rather than MHC, alloantigens are the more significant barriers to acceptance of corneal allografts. By definition, minor H antigens are recognized by a distinct category of T cells, termed indirect, because they detect peptides derived from minor H antigens that are loaded on self-MHC molecules. Indirect alloreactive T cells differ from direct T cells by being present at much lower clonal frequency among peripheral lymphocytes and by failing to proliferate in response to antigen stimulation in vitro. When multiple minor H antigens are expressed by cells of an allograft, indirect T cells may incite acute rejection, but more commonly, T cells of this type reject grafts more slowly, that is, subacute rejection. The slow rate at which orthotopic corneal grafts are destroyed in normal eyes is believed to reflect the activities of minor H–specific, indirect effector T cells. The veracity of this hypothesis is supported by the evidence presented in this article.

Recipient Langerhans cells were observed to migrate into orthotopic corneal transplants before the acquisition of donor-specific DH. In the case of grafts placed in low-risk eyes, Langerhans cells first appeared in graft epithelium between 1 and 2 weeks, and recipient sensitization was not detected until 4 weeks. In the case of grafts placed in high-risk eyes, Langerhans cells migrated into graft epithelium within the first week, and recipient sensitization was perceived within 2 weeks. Moreover, donor grafts that already contained Langerhans cells at the time of grafting also induced donor-specific DH within 2 weeks. Callanan et al. reported a similar observation in the rat corneal allograft model. We interpret these results to mean that the capacity of an orthotopic corneal allograft to sensitize its recipient depends almost exclusively on the presence of Langerhans cells, whether of recipient or donor origin.

Our study has focused on correlating sensitization to donor antigens and graft rejection with the density of class II MHC–bearing dendritic cells in the corneal epithelium. By tradition, these cells have been referred to as Langerhans cells, but, at least in mice and rats, there is no singular marker that enables these cells to be unequivocally identified as Langerhans cells. Thus, we have used the term Langerhans cells in this traditional sense. More important, we have not studied class II MHC–bearing cells of bone marrow origin that might be present in the corneal stroma. Particularly, after cauteryization of the corneal surface (which induces Langerhans cell migration into the central epithelium), it is likely that bone marrow–derived, class II–bearing cells capable of functioning as antigen-presenting cells also migrate into the corneal stroma. However, technical barriers have prevented us from studying these changes quantitatively, and therefore we do not know whether stroma class II–bearing cells might also be relevant to...
our analysis. In any event, class II–bearing, bone marrow-derived cells that infiltrate the epithelium (Langerhans cells) and/or stroma (dendritic cells, macrophages) have similar capacities to function as “passenger leukocytes.” Therefore, the correlation we observed between Langerhans cell density in the corneal epithelium and recipient sensitization and graft rejection may equally apply to similar cells that migrate into the stroma.

Emergence of donor-specific DH in recipients of orthotopic cornea graft–bearing mice correlated strongly with the rapidity and incidence of graft rejection. Grafts that either contained Langerhans cells at the time of surgery or that acquired Langerhans cells promptly thereafter were rejected acutely and uniformly. By contrast, grafts that accumulated Langerhans cells more slowly experienced a lower incidence of irreversible rejection, and when rejection occurred, it was delayed and slowly progressive.

Langerhans cell–containing cornea grafts placed in low-risk eyes and normal corneas placed in high-risk eyes were rejected with comparable intensity and incidence, but the T cells responsible for rejection were not identical. Normal corneal allografts placed in high-risk eyes activated predominately DH T cells of the indirect type. The explanation for this finding that we favor is that recipient Langerhans cells migrated into the graft and presented graft-derived peptides in the context of recipient class II MHC molecules. By contrast, Langerhans cell–containing grafts induced donor MHC-specific DH, indicating that direct alloreactive CD4+ T cells had been activated.
Thus, there appears to be a strict correlation between the type of alloreactive DH T-cell that was activated and whether the sensitizing Langerhans cells are of donor or host origin. Our findings indicate that donor Langerhans cells are required to activate class II–specific, direct alloreactive T cells that mediate DH. Because the bulk of experimental evidence implicates DH rather than cytotoxic T cells as the mediators of acute and subacute rejection of orthotopic corneal allografts,40–42 grafts

![Diagram](image)

**Figure 4.** (Continued)

**Figure 5.** Cytotoxic T-cell responses of lymphoid cells from orthotopic cornea graft–bearing mice. Ipsilateral cervical lymph nodes (A) and spleens (B) were harvested from BALB/c recipients of normal (LC⁺) or cauterized (LC⁻) A.BY cornea grafts at 2 weeks after grafting. Positive control BALB/c mice were immunized by an s.c. injection of 10 × 10⁶ A.BY spleen cells. Single-cell suspensions were obtained and cultured for 72 hours with A.BY stimulators. Effector cells were then harvested and assayed at different effector:target ratios on ⁵¹Cr-labeled, ConA-stimulated A.BY spleen cells. Percent specific lysis is plotted.
that contain donor Langerhans cells must necessarily be destined to succumb to acute rejection. This conclusion may have relevance to the clinic. The catartered mouse corneas we used for grafts were perfectly clear at the time of surgery, and they bore no evidence to suggest that Langerhans cells were present in the epithelium. A similar situation may prevail in humans. Human donor corneas from cadaver sources are not routinely screened for the presence of Langerhans cells before being used for transplantation. Therefore, the possibility exists that the inadvertent presence of Langerhans cells in donor corneas may be an unsuspected cause of acute graft rejection in low-risk human eyes. This possibility has already been addressed by Williams and colleagues,5,44 who assayed Langerhans cells in the unused rim of donor corneas. They claimed that donor rims with elevated densities of Langerhans cells correlated positively with a high rate of rejection reactions in the corresponding corneal allografts.

With respect to cytotoxic T cells, Langerhans cell–containing corneas generated cells of the direct allorecognition type. By contrast, normal corneas placed in low-risk eyes of BALB/c mice never generate such cells. Instead, only indirect alloreactive CTLs emerge from sensitization with these grafts.37 This difference is not trivial when one considers the vulnerability of the graft to CTL-mediated destruction. When donor and recipient share no class I alloantigens, indirect minor H–specific alloreactive CTLs are unable to find suitable target cells in corneal allografts. Because these effector cells are restricted by self–class I molecules and because the parenchymal cells of the graft express different class I alloantigens, no recognition leading to target cell killing can take place. By contrast, direct alloreactive CTL, once activated, can recognize and lyse target cells of the cornea, because corneal cells do express class I alloantigens. We suspect that this is the reason that catartered corneal allografts placed in low-risk eyes were rejected so quickly and so completely. If this suspicion is true, then the inclusion of donor Langerhans cells in cornea grafts carries two serious risks to graft rejection: activation of direct alloreactive T cells that mediate DH and activation of direct alloreactive T cells that are cytotoxic. When this situation applies, immune privilege has little opportunity to protect the graft, and rejection is inevitable. Our analysis would lead to the implication that class I matching of donor corneas that contain passenger leukocytes would lead to a worse outcome. This may explain the lack of benefit from HLA matching in some patients.

References


