

Interleukin 1 Receptor Antagonist Suppresses Allosensitization in Corneal Transplantation

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Objective: To delineate the mechanisms by which topical interleukin 1 receptor antagonist (IL-1RA) treatment promotes orthotopic corneal allograft survival.

Methods: Corneal buttons were prepared from eyes of C57BL/6 mice and placed orthotopically in normal or neovascularized (high-risk) eyes of BALB/c mouse recipients. Topical IL-1RA (or vehicle alone) was applied to grafts 3 times daily until the grafted eyes were enucleated. Corneal specimens were evaluated for content of Langerhans cells. A week after enucleation, 1 group of recipients was tested for allospecific delayed-type hypersensitivity elicited by intrapinnae injections of donor splenocytes. In companion experiments, a second group of mice that underwent transplantation, IL-1RA treatment, and enucleation was challenged with orthotopic skin grafts from B10.D2 donor mice (sharing minor H antigens with C57BL/6 mice) to determine whether the second group of mice could reject grafts bearing corneal donor minor H alloantigens in an accelerated fashion.

Results: Mice whose orthotopic corneal allografts were treated topically with IL-1RA acquired neither donor-

specific delayed-type hypersensitivity ($P < .001$) nor the capacity to reject orthotopic donor-type skin allografts in an accelerated manner ($P < .05$), whereas controls treated with vehicle alone developed delayed-type hypersensitivity and rejected B10.D2 grafts in an accelerated manner. Moreover, IL-1RA-treated grafts placed in both high-risk ($P = .01$) and normal-risk ($P = .004$) eyes displayed significantly reduced levels of infiltrating Langerhans cells compared with vehicle-treated controls.

Conclusions: Topical IL-1RA promotes corneal allograft survival in large part by preventing activity of recipient Langerhans cells, and thereby preventing these cells from inducing systemic allosensitization. These data suggest that IL-1 plays a key role in promoting allosensitization when corneal allografts are placed orthotopically.

Clinical Relevance: Suppression of allosensitization by topical IL-1RA may prove a clinically useful method for enhancing corneal transplant survival.

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CORNEAL transplantation is the most successful type of solid tissue transplantation in humans.¹ However, immune rejection remains a significant clinical problem, and many patients lose sight from corneal graft failure.¹ The extraordinary ability of allografts of cornea to survive when placed orthotopically has been ascribed to "immune privilege."^{2,3} Many features of the cornea and the ocular graft bed have been identified as pertinent to the existence of immune privilege.^{4,5} In general, major histocompatibility complex (MHC)-encoded class I and class II molecules expressed on cells within solid tissue grafts play a central role as targets of recipient T-cell-mediated alloimmunity,⁶ and bone

marrow-derived antigen-presenting cells (APCs) located within grafts play a central role in the induction of alloimmunity.^{7,8} Both of these features are unusual in the normal cornea. The MHC class I and class II molecules are poorly expressed on cells of the normal cornea, especially the endothelial cells.^{4,9,10} Moreover, APCs, such as Langerhans cells (LCs), are essentially absent from the cornea,^{4,8} except at the limbus¹¹; hence, donor buttons are devoid of these cells.

Recently, we have shown that topical treatment of murine corneal allografts with interleukin 1 receptor antagonist (IL-1RA) suppresses transplant rejection, whether the recipient corneal beds were normal (ie, avascular) or high risk (ie, neovascularized). Long-standing corneal

MATERIALS AND METHODS

MICE

Male mice aged 8 to 10 weeks were purchased from Taconic Farm (Taconic, NY). Animals with dystrophic or degenerative corneal calcific deposits were excluded from study. All animals were treated according to the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. In corneal transplant experiments, BALB/c (H-2^d) strain mice were used as recipients and C57BL/6 (major and minor allodisparate, H-2^b) or BALB/c (syngeneic) strain mice were used as donors. In skin graft experiments, B10.D2 (minor disparate with BALB/c, H-2^d) tail skin grafts were used.

INDUCTION AND GRADING OF CORNEAL NEOVASCULARIZATION

Corneal neovascularization (high-risk graft beds) was induced by intrastromal sutures as described previously.¹³ Briefly, 3 interrupted 11-0 nylon sutures were placed in the central cornea of 1 eye of a normal BALB/c mouse 2 weeks before transplantation on day -14 using aseptic microsurgical technique performed with an operating microscope. The neovascularized beds then received orthotopic corneal transplants on day 0 as described above (neovascularization-inducing sutures were removed at the time of transplantation).

ORTHOTOPIC CORNEAL TRANSPLANTATION

As described previously,¹² each recipient was deeply anesthetized with an intraperitoneal injection of 3 mg of ketamine hydrochloride and 0.0075 mg of xylazine hydrochloride before all surgical procedures. The central 2 mm of the donor cornea was excised and secured in recipient graft beds with 8 interrupted 11-0 nylon sutures (Sharp-point; Vanguard, Houston, Tex). Antibiotic ointment was applied to the corneal surface, and the eyelids were closed for 24 hours with a tarsorrhaphy using 8-0 nylon sutures. All grafted eyes were examined after 72 hours; no grafts were excluded from analysis because of technical difficulties. Transplant sutures were removed in all mice on day 7.

PHARMACOLOGICAL STRATEGY

In most experiments (unless noted in the "Results" section), topical preparations were applied in a masked fashion to the surface of eyes of BALB/c recipient mice on the day of grafting and 3 times daily thereafter until enucleation. The study medication was composed of human recombinant IL-1RA, 20 mg/mL, in 0.2% sodium hyaluronate in phosphate-buffered saline (supplied by Amgen, Boulder, Colo). Vehicle-treated animals received 0.2% sodium hyaluronate only.¹²

ASSESSMENT OF DONOR-SPECIFIC DELAYED-TYPE HYPERSENSITIVITY

At appropriate times after grafting, 1×10^6 irradiated (2000 rad) spleen cells (in 10 μ L of Hanks balanced salt solution) from donors syngeneic with the corneal graft were

allografts treated locally with IL-1RA displayed decreased inflammation and fewer LCs than did grafts treated with vehicle alone.¹² We hypothesized that the observed suppression of LCs was retarding allosensitization in IL-1RA-treated recipients and that IL-1RA could promote corneal allograft survival, possibly by inhibiting induction of immunity to donor alloantigens by preventing recipient APC from migrating into the graft. We tested this possibility by examining induction of donor-specific immunity in corneal allograft recipients treated topically with IL-1RA. The results reported here indicate that topical IL-1RA inhibits onset of donor-specific allosensitization by a strictly local effect.

RESULTS

EFFECT OF TOPICAL IL-1RA ON ACQUISITION OF DONOR-SPECIFIC DTH

To determine whether topical IL-1RA interfered with the induction of donor-specific DTH by orthotopic allogeneic cornea grafts, we needed first to describe the time at which systemic donor-specific DTH arises after corneal allografts are placed in high-risk and normal graft beds. Donor-specific DTH was assessed in transplant recipients 1 week after enucleation (weeks 1 and 2 after grafting and weeks 2 and 3 after grafting in high-risk and

normal-risk recipients, respectively) and in positive control mice 1 week after subcutaneous immunization. As the results presented in **Figure 1** (top) reveal, donor-specific DTH was detectable in mice exposed to donor corneas grafted to normal avascular eye beds at 3, but not at 2, weeks. Recipients of donor corneas in high-risk eyes (Figure 1, bottom) displayed donor-specific DTH when tested at both 1 and 2 weeks after grafting. Thus, corneal allografts in high-risk eyes induce systemic sensitization 1 to 2 weeks earlier than they do when placed in normal-risk avascular beds.

We next examined the effect of topical IL-1RA on the rapidity of acquisition of donor-specific DTH following orthotopic corneal allografts. Donor C57BL/6 corneas remained in place in normal-risk BALB/c recipients for 3 weeks, and in high-risk eye recipients for 2 weeks, before enucleation of the grafted eyes. Enucleation was necessary to prevent continuing exposure of the recipient immune system to donor antigens. While the grafts were in place, eyes received treatment with topical IL-1RA or vehicle only as described previously. The results are presented in **Figure 2** (top, normal-risk eyes; bottom, high-risk eyes). Unlike positive controls, mice treated topically with IL-1RA displayed weak DTH responses that were indistinguishable from negative controls. We conclude that topical treatment with IL-1RA inhibits both normal- and high-risk orthotopic corneal allografts from inducing donor-specific DTH.

injected into the right pinnae, as described previously.¹⁴ Positive controls were immunized by subcutaneous injection of 10×10^6 spleen cells of the appropriate allogeneic strain 1 week before ear challenge. At 24 and 48 hours after ear challenge, ear thickness was measured in a masked fashion with a low-pressure micrometer (Mitutoyo, MTI Corp, Paramus, NJ). Ear swelling was expressed as follows: specific ear swelling = (measurement of right ear at 24 hours – measurement of right ear at baseline) – (measurement of left ear at 24 hours – measurement of left ear at baseline) in microns. Ear swelling responses at 24 hours after injection are presented as a group mean \pm SE measurement. Since results at 24 and 48 hours were similar in all experiments, only 24-hour data are presented. All panels contained a minimum of 5 animals.

SKIN GRAFTS AND ASSESSMENT OF SKIN GRAFT SURVIVAL

BALB/c mice received an orthotopic skin graft from B10.D2 mice as described previously.¹⁵ Briefly, tail skin grafts (2×4 mm) were placed on graft beds prepared on the thoracic wall of anesthetized mice (halothane and atropine, Vedco, Arcadia, Calif). Petrolatum gauze was placed over the graft site. Antibacterial powder (nitrofurazone) was then applied, and the wound was covered with dry gauze followed by a plaster cast wrapped around the thorax. One week later, the cast was removed, and the grafts were examined every day. Graft rejection was determined when the surface was judged by clinical inspection to be completely denuded of epidermis. In general, normal mice rejected their test skin allografts within 15 to 18 days.

EFFECT OF TOPICAL IL-1RA ON MIGRATION OF RECIPIENT LCs INTO ORTHOTOPIC CORNEAL ALLOGRAFTS

C57BL/6 corneas were grafted into normal- or high-risk eyes of BALB/c mice that were subsequently treated with topical IL-1RA or vehicle. The results are presented in **Figure 3**. In normal graft beds treated with IL-1RA, the epithelium contained significantly decreased levels of LCs, compared with vehicle-treated controls at 2 and 3 weeks after grafting. Similarly, in high-risk graft beds, treatment with IL-1RA reduced the density of LCs within graft epithelium at 1 week ($P = .07$, borderline significance) and at 2 weeks ($P < .01$). Thus, topical IL-1RA suppresses migration of recipient LCs into corneal allografts.

EFFECT OF TOPICAL IL-1RA ON ACQUISITION OF ABILITY TO REJECT ORTHOTOPIC SKIN GRAFTS

Mice with alloimmunity reject orthotopic skin allografts in an accelerated fashion compared with nonimmune controls. Our objective in this series of experiments was to test whether mice treated topically with IL-1RA could reject orthotopic skin grafts, syngeneic at minor alloantigen epitopes with the corneal grafts, in an accelerated fashion. High-risk corneal graft beds of BALB/c mice received C57BL/6 corneal grafts. The eyes were

LC ENUMERATION

Langerhans cell densities within corneal epithelium were assessed by an immunofluorescence assay as previously described.¹⁶ Briefly, each eye was enucleated and the anterior segment dissected under the operating microscope. Topically treated eyes bearing corneal transplants were enucleated at either 2 to 3 weeks postoperatively (normal graft beds) or 1 to 2 weeks postoperatively (high-risk graft beds). The cornea was placed in 20 mmol/L EDTA buffer and incubated for 30 minutes at 37°C after which the epithelium was removed in toto and washed in phosphate-buffered saline at room temperature. The cornea was then fixed with 95% ethanol prior to washing and incubation with 1:20 diluted primary anti-murine Ia^d antibody for 45 minutes at 37°C. The tissue was then washed in phosphate-buffered saline and incubated with a fluorescein isothiocyanate-labeled goat anti-mouse secondary antibody for 30 minutes at 37°C. Negative controls either bypassed this step or were incubated with antibody specific for an unrelated MHC epitope. Sections were then mounted on slides and examined under the fluorescent microscope with a square ocular grid where LCs were enumerated. All panels contained data from at least 5 animals.

STATISTICAL METHODS

Statistical analyses were performed using the Student *t* test for comparison of delayed-type hypersensitivity (DTH) responses and LC population. In addition, we constructed Kaplan-Meier survival curves and used the Breslow-Gehan-Wilcoxon test to compare the rates of skin graft survival. All *P* values less than .05 were deemed significant.

treated topically with IL-1RA or vehicle only as described above. High-risk eyes of positive control mice were treated with vehicle alone. After 1 week, treatment was stopped and the graft-containing eyes were enucleated to prevent ongoing exposure of the recipient immune system to donor-derived antigens. One week later, each recipient received an orthotopic B10.D2 skin graft, and the survival of these grafts was assessed by visual inspection of the epidermis. The results are summarized in **Figure 4**. The median survival time of B10.D2 skin on BALB/c mice whose grafted eyes were treated with topical IL-1RA was 17 days, whereas the median survival time of skin grafts on BALB/c mice whose grafted eyes were treated topically with vehicle was 14 days. The rate of rejection of orthotopic B10.D2 skin grafts on mice that had received a C57BL/6 cornea treated only with vehicle (**Figure 5**, left) was significantly shorter than that of similar grafts placed on mice whose cornea-grafted eyes were treated with IL-1RA (**Figure 5**, right). These results indicate that orthotopic C57BL/6 corneal allografts equip BALB/c recipients with the capacity to reject B10.D2 skin grafts in an accelerated fashion. By contrast, BALB/c mice that encounter B10.D2 alloantigens on orthotopic C57BL/6 corneal grafts under cover of topical IL-1RA reject subsequent B10.D2 skin grafts less aggressively. These findings confirm that topical IL-1RA suppresses corneal allograft rejection by interfering with the induction of donor-specific alloimmunity.

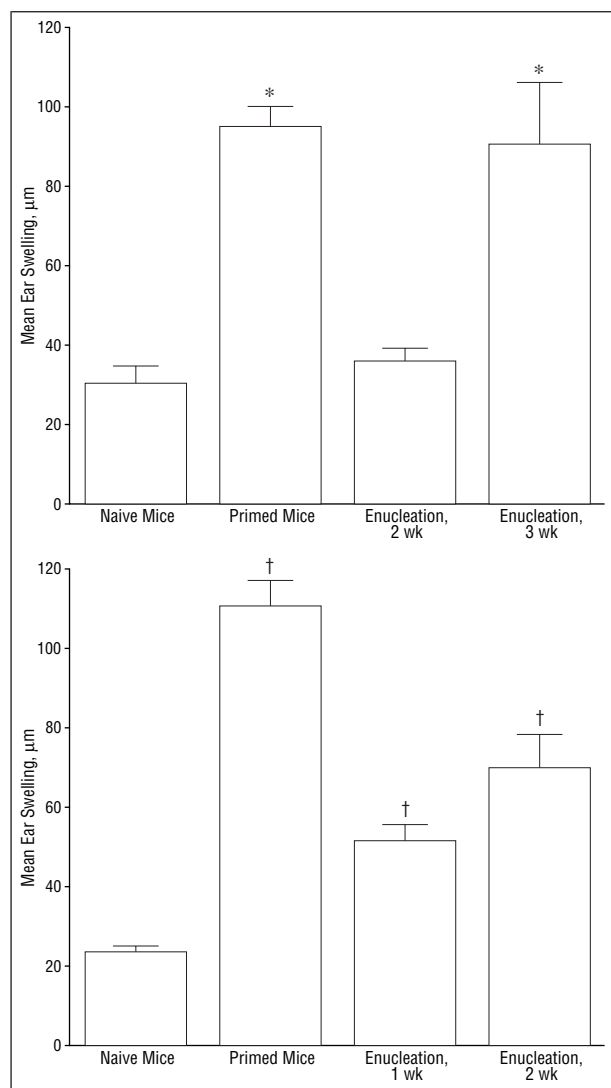


Figure 1. Donor-specific delayed-type hypersensitivity in BALB/c recipients of C57BL/6 corneal transplants placed in normal-risk (top) or high-risk (bottom) eyes. Grafted eyes were retained for 1 to 3 weeks, then enucleated as indicated in the "Materials and Methods" section. Primed mice were immunized subcutaneously with C57BL/6 splenocytes. One week later, recipient ear pinnae were challenged with donor splenocytes. Mean (\pm SEM) ear swelling responses at 24 hours are presented. Symbols indicate responses significantly greater than those in naive controls: asterisk, $P < .02$; dagger, $P < .003$.

POTENTIAL SYSTEMIC EFFECTS OF IL-1RA APPLIED TO THE OCULAR SURFACE

It is possible that topically applied IL-1RA could interfere with immune induction systemically, as well as locally. To evaluate this possibility, syngeneic corneas were grafted onto high-risk beds of BALB/c mice, and the ocular surface was treated with topical IL-1RA or vehicle. Grafted eyes were enucleated 1 week later, and, after an additional week, B10.D2 skin was grafted to the thoracic wall of the BALB/c mice. To assess the impact on acquisition of systemic donor-specific DTH, a subgroup of the BALB/c animals received 10×10^6 spleen cells (from C57BL/6 donors) 7 days after receiving syngeneic corneal grafts that were treated with either IL-1RA or vehicle for 10 days. Seven days thereafter, donor-specific

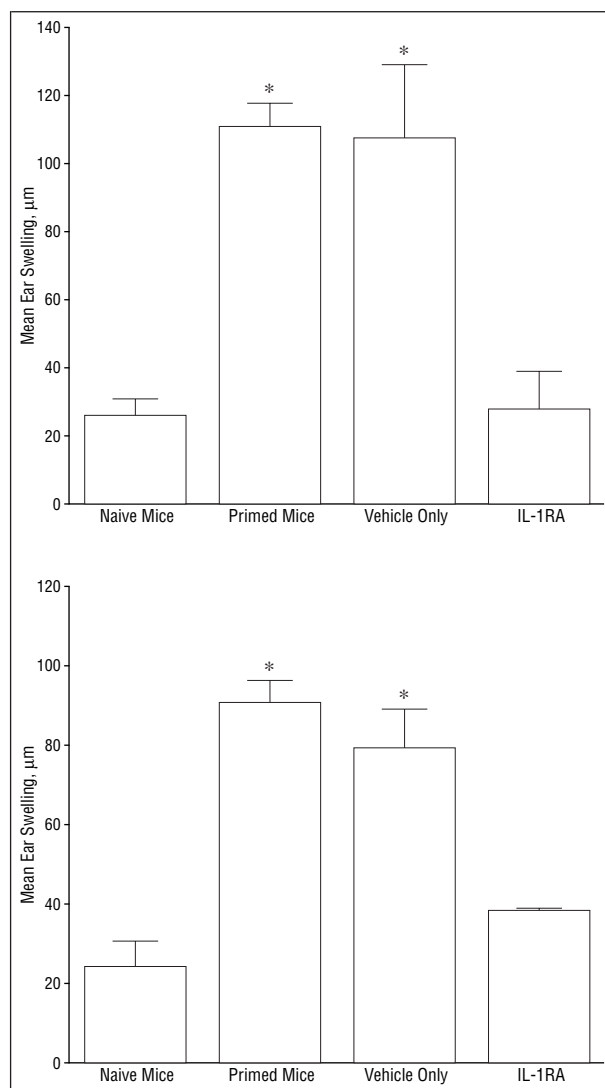


Figure 2. Donor-specific delayed-type hypersensitivity in BALB/c mouse recipients of C57BL/6 corneal transplants placed in normal-risk (top) or high-risk (bottom) eyes that were treated topically with interleukin 1 receptor antagonist (IL-1RA) or vehicle alone. Grafted eyes were enucleated at 3 weeks (normal-risk eyes) or 2 weeks (high-risk eyes). Primed mice were immunized subcutaneously with C57BL/6 splenocytes. One week later, the recipients were ear challenged and swelling responses assessed. Bar indicates SEM. Asterisks indicate responses significantly greater than those in naive controls ($P < .005$).

DTH was assessed as described above. As the results displayed in **Figure 6** indicate, mice treated with topical IL-1RA rejected B10.D2 grafts at the same tempo as vehicle-treated controls, and both groups rejected the grafts more slowly than did mice sensitized subcutaneously with C57BL/6 spleen cells. Moreover, mice treated topically with IL-1RA and then immunized subcutaneously with C57BL/6 spleen cells mounted donor-specific DTH responses comparable in intensity to those of mice treated with vehicle alone before subcutaneous immunization (**Figure 7**). Together, these results demonstrate that topical IL-1RA fails to alter allo-sensitization initiated via nonocular routes. Hence, topical IL-1RA interferes with alloimmunization in the context of corneal transplantation via a local, rather than a systemic, mechanism.

Along with IL-6 and tumor necrosis factor α , IL-1 is regarded as a prototypical inflammatory cytokine,¹⁷⁻¹⁹ and as such plays an important role in a wide array of immunoinflammatory states, ranging from endotoxin-induced septic shock^{18,20} to immune-mediated graft rejection. In the context of ocular immunity, IL-1 appears to play a critical role in initiating immune responses by virtue of its action on APC function. It has been shown²¹ in mice that suppression of IL-1 receptor function with IL-1RA can suppress corneal LC activity and restore immune privilege in eyes subsequent to an inflammatory insult. Moreover, topical IL-1RA can significantly reduce the rate of corneal allograft rejection in normal- and high-risk eyes.¹² Our present results indicate that IL-1 plays a critical role in the induction of alloimmunity by orthotopic corneal transplants. To summarize that evidence, corneal allografts induced vigorous donor-

specific alloimmunity, expressed, on the one hand, as accelerated rejection of orthotopic skin grafts syngeneic with the corneal graft donor and, on the other hand, as donor-specific DTH. Topical application of IL-1RA to eyes bearing a corneal allograft prevented recipient mice from acquiring donor-specific DTH and from rejecting donor-type skin allografts in an accelerated fashion. Together, these findings implicate IL-1 in the process by which an orthotopic corneal allograft alerts the recipient immune system to the graft.

Normally, the central cornea displays little evidence of IL-1-dependent activity.²² While IL-1 levels are very low in normal corneal tissue, its levels can be significantly up-regulated in inflammation.²³ This is in contrast to the significant expression of IL-1RA messenger RNA in normal corneal tissue.²⁴ Along with the report of Niederkorn et al¹⁹ that LCs can be induced to migrate from the limbus into the central cornea by injection of IL-1 into the corneal stroma, our results suggest that IL-1 is an important potential chemoattractant for LCs within the cornea and that endogenous IL-1RA is the natural inhibitor that keeps LCs at the limbus. In the present experiments, topical IL-1RA prevented LCs from migrating into corneal allografts and simulta-

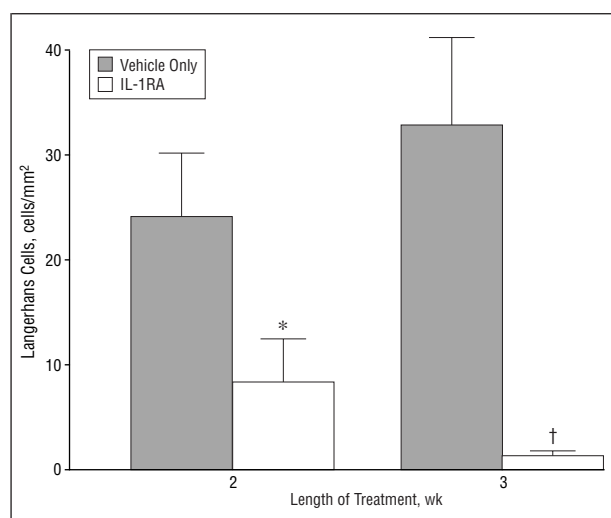


Figure 3. Density of Langerhans cells (LCs) in epithelium of corneal transplants placed in normal-risk beds and treated topically with interleukin 1 receptor antagonist (IL-1RA) or vehicle for 2 or 3 weeks. Bar indicates SEM LC per square millimeter. Solid bars represent corneas treated with vehicle alone; open bars represent corneas treated with IL-1RA. Symbols indicate significant difference between IL-1RA and vehicle-only treatment: asterisk, $P = .01$; dagger, $P = .003$.

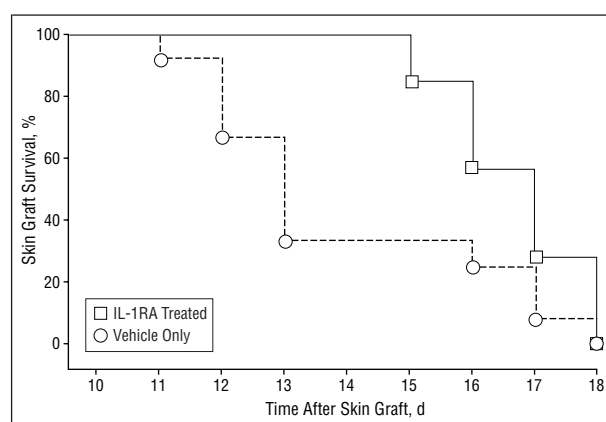


Figure 4. Kaplan-Meier survival curve of orthotopic B10.D2 skin grafts on BALB/c mice that had previously received C57BL/6 corneal allografts and were treated topically with interleukin 1 receptor antagonist (IL-1RA) ($n = 7$) or vehicle only ($n = 12$). Difference between IL-1RA treatment effect and vehicle alone is significant ($P < .03$).

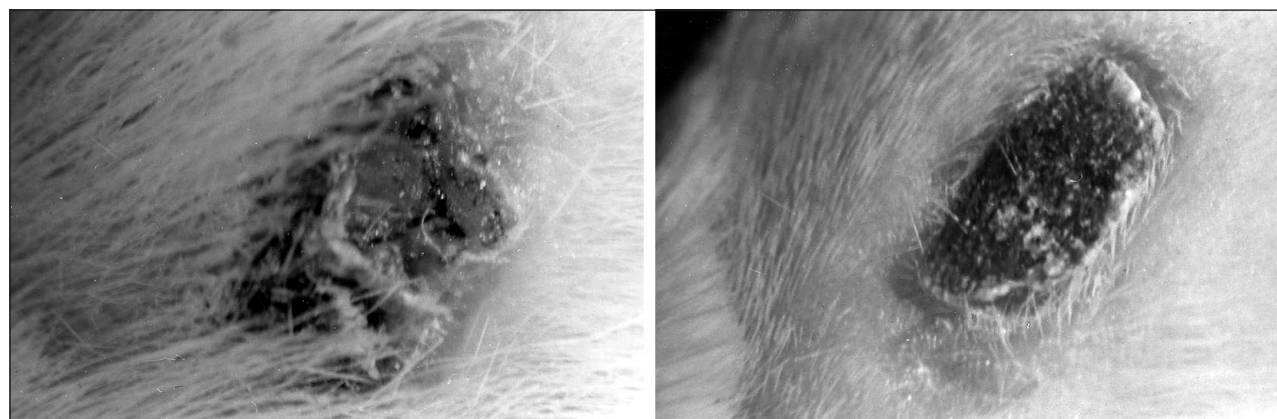


Figure 5. Appearance of B10.D2 tail skin grafts that had been in residence for 14 days on the thoracic wall of BALB/c mice that received orthotopic C57BL/6 corneal allografts 3 weeks' previously. Graft without surviving epidermis (left); corneal grafts of these recipients had been treated topically with vehicle alone. Graft with healthy epidermis (right); corneal grafts of these mice had been treated topically with interleukin 1 receptor antagonist.

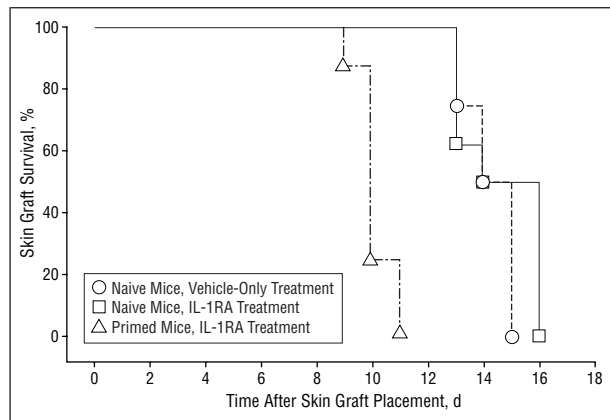


Figure 6. Survival of orthotopic B10.D2 skin grafts on BALB/c mice that had previously received BALB/c corneal grafts and were treated topically with interleukin 1 receptor antagonist (IL-1RA) ($n = 8$) or vehicle only ($n = 8$). Primed mice received orthotopic syngeneic corneal grafts and topical IL-1RA, but were immunized subcutaneously with C57BL/6 splenocytes ($n = 8$). Kaplan-Meier survival curves were constructed based on length of survival (in days) of B10.D2 skin grafts. No significant difference was observed between IL-1RA treatment effect and vehicle alone.

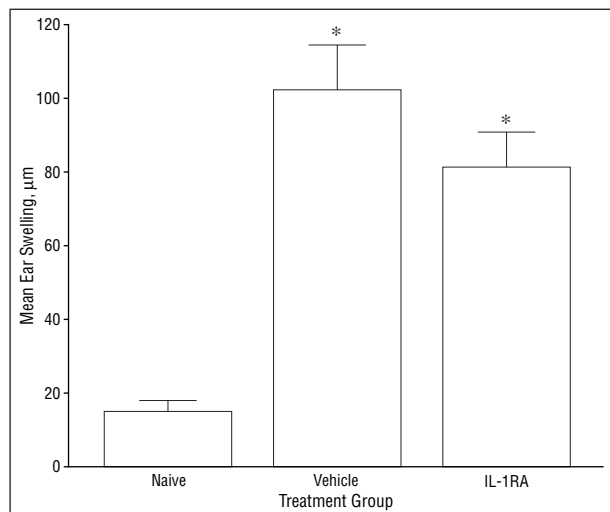


Figure 7. C57BL/6-specific delayed-type hypersensitivity in BALB/c mice that received orthotopic BALB/c corneal grafts treated topically with interleukin 1 receptor antagonist (IL-1RA) or vehicle only. One week later, the mice were immunized subcutaneously with C57BL/6 splenocytes. Topical therapy was stopped 3 days later. Mice were ear challenged with irradiated C57BL/6 splenocytes 7 days after subcutaneous immunization. Mean ear swelling responses at 24 hours are presented as described in the legend to Figure 2. Asterisks indicate responses significantly greater than those in naive controls ($P < .001$).

neously inhibited the acquisition of donor-specific allosensitization.

The importance of LCs to the immunogenicity of corneal tissues cannot be overstated. Heterotopically grafted class II MHC-disparate corneas without LCs are not rejected and are incapable of immunizing recipients to MHC class II alloantigens. By contrast, class II MHC-disparate corneas containing LCs are promptly rejected at heterotopic sites.²⁵ Similarly, LC-containing corneas grafted orthotopically into normal eyes are rejected with a speed²⁶ and intensity comparable with that of LC-deficient corneas grafted into high-risk eyes.¹³ The rapidity with which LCs migrate into orthotopic corneal grafts is enhanced when the graft bed is a high-risk eye,²⁷ and there is a positive cor-

relation between the time when LCs enter the donor graft and when the graft experiences a rejection reaction. For these reasons, we have proposed that IL-1 promotes corneal allograft rejection by attracting LCs into the graft from the limbus, thereby providing the graft with a vehicle for capturing and presenting graft-derived alloantigens to the recipient immune system. Topical IL-1RA promotes graft survival, at least in part, by re-creating the blockade that normally keeps LCs within the limbus and away from the corneal graft. We cannot, however, rule out that IL-1RA's effective suppression of allosensitization may involve, in part, non-LC populations, such as macrophages.

Unlike the cornea, most other solid tissues contain hefty endowments of APCs in the form of dendritic cells and macrophages. When these tissues are grafted, their complement of "passenger leukocytes" is a major source of immunogenicity.²⁸ This population of constitutively expressing MHC class II APCs enables recipient T cells to recognize graft alloantigens via the "direct" pathway of allorecognition, and these are the T cells that mediate acute graft rejection. Graft alloantigens can also be detected by the "indirect" pathway of allorecognition, ie, presentation of graft-derived antigens by infiltrating recipient APCs.²⁹⁻³¹ The normal cornea possesses few bone marrow-derived cells that could function as APCs,^{4,32} and, therefore, lacks passenger cells. This is relevant to recently published evidence^{33,34} that alloantigens expressed on corneal grafts are detected by recipient T cells almost exclusively by the indirect pathway of allorecognition. This pathway requires that recipient APCs infiltrate the graft and acquire, process, and present graft-derived alloantigens to recipient T cells.^{35,36} In this context, we propose that, in orthotopic corneal allografts, endogenous IL-1 entices APCs to migrate into the graft and thereby activate the "indirect" pathway of allorecognition. The value of IL-1RA as an immunosuppressant in this context rests on its ability to thwart the indirect pathway of allorecognition for a tissue that constitutively lacks the ability to activate T cells via the direct pathway.

Other reported approaches^{37,38} to suppression of alloimmune responses have relied on systemic administration of monoclonal antibodies, such as anti-leukocyte function-associated antigen 1 and anti-CD4. To our knowledge, our experiments with IL-1RA are the first example of a topical treatment modality that can promote corneal transplant survival by interfering with the induction of allosensitization. Moreover, this molecular strategy relies on a naturally produced cytokine that largely circumvents the concern of inducing immunogenic responses when foreign (nonself) proteins are used in immune modulation. It is hoped that the development of topical molecular therapies will make it possible in ophthalmology to suppress ocular inflammatory disorders and corneal graft rejection without incurring the undue risks that accompany systemic immunosuppressive therapies.

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100 Years Ago in the ARCHIVES

A look at the past . . .

False glaucoma, concludes DE WEAVER. . . , is often confounded with true glaucoma. When the ophthalmoscope alone is depended upon to make the diagnosis, one-half the cases of simple glaucoma are false glaucoma. This false glaucoma is a primary disease of the nerve-head, requiring medicamentous treatment, while true glaucoma, even when of the chronic simple variety, demands sclerotomy.

Reference: *Arch Ophthalmol*. 1897;26:469.