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Corneal Transplantation and Immune Privilege

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Abstract

Corneal transplants have been successfully performed in human subjects for over 100 years and enjoy an immune privilege that is unrivaled in the field of transplantation. Immune privilege is defined as the reduced incidence and tempo in the immune rejection of corneal allografts compared to other categories of organ allografts performed under the same conditions. Skin allografts transplanted across various MHC or minor histocompatibility barriers undergo rejection in approximately 100% of the hosts. By contrast, orthotopic corneal allografts experience long-term survival in 50% to >90% of the hosts, depending on the histocompatibility barriers that confront the host. The capacity of corneal allografts to evade immune rejection is attributable to multiple anatomical, physiological, and immunoregulatory conditions that conspire to prevent the induction and expression of alloimmunity.

Keywords

Corneal Transplantation; Immune Privilege; Immune Tolerance; Keratoplasty; T regulatory cells

Introduction

The potential for restoring vision with a corneal transplant was proposed almost 250 years ago by Charles Darwin's grandfather, Erasmus Darwin. The first reported attempt at corneal transplantation can be traced to the mid-nineteenth century when an Irish ophthalmic surgeon, Samuel Bigger, transplanted a cornea to a pet gazelle (1). The first documented case of corneal transplantation in a human subject was reported in 1838 by Kissam, who transplanted a pig cornea onto a patient using only two interrupted sutures and without the use of anesthesia (2)! As might be expected, this and other efforts to transplant pig or rabbit corneas to human subjects failed, almost certainly due to acute xenograft rejection. However, in 1905 Edward Zirm successfully transplanted a corneal allograft to a patient who had been blinded by lime burns (3). The full impact of this achievement was not recognized at the time, as histocompatibility antigens had not yet been discovered and their importance as barriers for transplant acceptance would not be appreciated for another 30 years. Since Zirm's initial success in corneal transplantation, hundreds of thousands of transplants have been performed on patients and in the United States alone over 40,000 corneal transplants are performed each year (4). Zirm's landmark success in corneal transplantation was followed by seminal studies by Billingham and Medawar who noted the enhanced survival of corneal allografts placed onto the ocular surface and skin allografts placed into the anterior chamber of the rabbit eye (5, 6). Medawar noted the significance of these observations and coined the term "immune privilege" to describe the unique properties

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of the eye and the corneal allograft (6). These and subsequent observations in animal models led to the inescapable conclusion that corneal allografts were endowed with remarkable properties unlike any other tissue that had been used for transplantation.

What are the ground rules for immune privilege?

Immune privilege is a widely recognized, but often misunderstood phenomenon. One common misconception is that immune privilege is a universal exemption from immune recognition and immunological attack. This has led some clinicians to dismiss immune privilege out of hand based on their personal experiences with patients who have rejected their corneal transplants. Indeed, it is often noted that the survival rate for corneal allografts is 74% at five years and 62% at 10 years, which is comparable to the survival rates for renal, cardiac, and liver transplants (7). How then can one claim that corneal allografts possess immune privilege? The answer lies in the fact that corneal transplants differ from renal, cardiac, and liver transplants in two fundamental ways. First, MHC-matching is not routinely performed, yet 90% of the first-time, uncomplicated corneal transplants are accepted. Second, systemic immunosuppressive drugs are normally not administered and instead, topical corticosteroids are the only anti-rejection medications that are used. In order to legitimately compare the survival rates of corneal allografts with other categories of allografts, one should perform the transplants under the same conditions; that is, without MHC matching and in the absence of systemic immunosuppressive drugs. We and others have performed such studies in the mouse and rat models of penetrating keratoplasty (8, 9). Skin allografts that are mismatched with the host at the entire MHC complex and all known minor histocompatibility (H) loci undergo immune rejection in virtually 100% of the cases, while corneal allografts transplanted across the same barriers experience a 50% long-term acceptance rate (8, 9). The differences in the survival of corneal allografts and skin allografts is even more impressive when the donor and recipient are mismatched only at MHC class I loci. Under these conditions, 65% of the corneal allografts survive indefinitely, while none of the skin grafts escape immune rejection (10). Moreover, corneal allografts that only confront the host with foreign MHC class II antigens undergo rejection in <10% of the hosts, while 100% of the skin grafts are rejected (11).

Studies in both the mouse and rat models of penetrating keratoplasty have allowed investigators to define the ground rules for immune privilege and to test current and past dogmas regarding the immunobiology of corneal transplantation. Immune privilege is terminated in virtually any condition in which inflammation, neovascularization, or trauma is present at the ocular surface (8, 12–14). In order to understand how these conditions lead to corneal allograft rejection we must first characterize the induction and expression of immune privilege itself.

Mechanisms of Immune Privilege for Corneal Allografts

Three fundamental processes are crucial for the establishment and maintenance of immune privilege of corneal allografts: a) blockade of the induction of the immune response (afferent blockade); b) deviation of the immune response to a state of immune tolerance; and c) elimination of immune effector elements at the host-graft interface.

Afferent Blockade of the Immune Response

One of the first explanations to account for the immune privilege of corneal allografts was based on the observation that the graft bed into which corneal allografts are placed is normally devoid of blood vessels and conditions that provoke the in growth of blood vessels invariably lead to corneal graft rejection. It was widely assumed that the presence of blood vessels in the corneal graft bed promoted the induction of alloimmunity and provided a

conduit for immune elements to gain access to the graft. It was noted over 40 years ago that stimuli that induced corneal neovascularization also promoted the invasion of lymph vessels that drained the cornea (15). However, 30 years would pass before the significance of lymph vessels in corneal allograft rejection would be appreciated (16). Yamagami and Dana reported that removal of the ipsilateral draining lymph node prior to corneal transplantation prevented the immune rejection of corneal allografts in mice (16). By contrast, removal of either the contralateral lymph node or the spleen did not prevent corneal allograft rejection. Inducing corneal neovascularization by inserting sutures into the corneas of mice several days prior to orthotopic corneal transplantation creates a “high-risk” condition in which 100% of the corneal allografts undergo immune rejection. However, immune privilege of such graft beds can be restored by selectively blocking the generation of lymph vessels while leaving blood vessels intact. Dietrich and co-workers administered either a small molecule antagonist of α5β1 integrin or anti-VEGFR-3 antibody to block lymphangiogenesis without affecting hemangiogenesis (17). Blockade of lymphangiogenesis dramatically reduced corneal allograft rejection, even in the face of patent blood vessels that penetrated the graft bed. Other studies have shown that administration of soluble VEGFR-3 suppresses both lymphangiogenesis and hemangiogenesis (18). Interestingly, corneal epithelial and stromal cells secrete a soluble form of VEGFR-2, which blocks VEGF-C and inhibits lymphangiogenesis in the cornea, but does not affect hemangiogenesis (19). The significance of VEGFR-2 in maintaining immune privilege of corneal allografts was shown in experiments in which the administration of soluble VEGFR-2 inhibited lymph vessel penetration into “high-risk” graft beds that had been pretreated with sutures to induce hemangiogenesis and lymph angiogenesis. This resulted in a doubling of the survival time of orthotopic corneal allografts placed into the vascularized graft beds (19). The *in situ* production of VEGFR-2 might explain how the normal cornea maintains its alymphatic status and prevents lymph vessel invasion. This in turn, may lead to therapeutic strategies for restoring immune privilege in the “high-risk” host.

The cornea also produces at least one other anti-angiogenic molecule that is important for maintaining immune privilege of corneal allografts. Endostatin is a proteolytic fragment of collagen XVII and inhibits both hemangiogenesis and lymphangiogenesis (20). Corneal allografts and syngrafts produce endostatin, but in the case of corneal allografts, endostatin production dissipates and VEGF synthesis increases with the onset of immune rejection (21). Moreover, subconjunctival injection of exogenous endostatin significantly prolongs corneal allograft survival in mice. Thus, the regulation of lymphangiogenesis in the cornea is a cornerstone for maintaining immune privilege of corneal allografts.

Role of T Regulatory Cells in Maintaining Immune Privilege of Corneal Allografts

Orthotopic corneal allografts are placed over the anterior chamber (AC) and are in direct contact with the various anti-inflammatory and immunosuppressive cytokines found in the aqueous humor. Moreover, it is inevitable that during the process of transplantation, corneal antigens, including alloantigenic cells, are sloughed and enter the AC. These events are significant, as antigens introduced into the AC induce a unique spectrum of systemic immune responses that culminate in the down-regulation of antigen-specific delayed-type hypersensitivity (DTH) and a deviation in isotype switching that favors the production of non-complement-fixing antibodies (22–24). This anterior chamber-associated immune deviation (ACAID) was described over 30 years ago and has been implicated in corneal allograft survival (8, 25–27). Several observations support the notion that ACAID is intimately involved in maintaining the immune privilege of corneal allografts. Mice bearing long-term corneal allografts display an antigen-specific suppression of DTH responses to the corneal donor’s alloantigens that is similar to that found in ACAID (28). Many of the

manipulations that abolish ACAID also provoke corneal allograft rejection (25, 29, 30). Likewise, induction of ACAID by AC injection of alloantigenic cells prior to corneal transplantation produces a significant enhancement of corneal allograft survival in both the mouse and rat models of penetrating keratoplasty (31–33).

A growing body of evidence suggests that orthotopic corneal allografts induce a T regulatory cell (Treg) population that is distinctly different from ACAID (34). Several conditions that abolish immune privilege of corneal allografts do not adversely affect the induction and expression of ACAID (Table 1). Moreover, there is evidence that T regs are generated within the corneal allograft through a glucorticoid-induced tumor necrosis factor receptor family-related protein ligand (GITRL)-dependent process (35). Administration of a blocking antibody to GITRL results in 100% corneal allograft rejection, yet does not prevent the induction of ACAID(35). IL-17 is also known to be required for the long-term survival of corneal allografts and for the generation of Tregs by orthotopic corneal allografts, yet is not needed for the induction and expression of ACAID (34, 36, 37). There is compelling evidence that the Tregs induced by corneal allografts are $CD4^+CD25^+Foxp3^+$ cells that suppress donor-specific DTH and act within the corneal allograft (34–38).

Efferent Blockade of the Immune Response

The cornea is decorated with an array of cell membrane-bound molecules that disarm immune effector elements including T cells and components of the complement cascade. FasL (CD95L) is expressed on multiple ocular cells, including the corneal endothelium (39). Corneal allografts prepared from donors with defective expression of FasL (gld/gld mutant of the C57BL/6 mouse) display a two-fold increase in the incidence of immune rejection compared to their wild-type counterparts (40, 41). A similar condition occurs with programmed death ligand-1 (PD-L1). PD-L1 is expressed on corneal cells and when it engages its receptor, PD-1, on T lymphocytes it inhibits lymphocyte proliferation, induces T lymphocyte apoptosis, and blocks T lymphocyte production of the proinflammatory cytokine, IFN-γ(42, 43). Corneal allografts devoid of PD-L1 experience a sharp increase in the incidence and tempo of immune rejection compared to wild-type corneal allografts (42, 43). Although the cornea also expresses tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) (44, 45), which like FasL and PD-L1, induces apoptosis of T lymphocytes, there are no published reports indicating that TRAIL contributes to the immune privilege of corneal allografts. Efforts to correlate corneal allograft survival with the expression of TRAIL have failed to reveal an association (Niederkorn, unpublished results).

The role of antibody in corneal allograft rejection remains a contentious topic. Corneal allografts can induce the generation of alloantibodies that are capable of mediating complement-dependent cytolysis of corneal cells *in vitro* (46). Two studies have demonstrated that under some conditions passively transferred alloantibody can promote corneal allograft rejection in mice (46, 47). However, induction of corneal allograft rejection by passively transferred antibody is far more inconsistent than rejection mediated by adoptively transferred T lymphocytes (46, 48–50). This may be due in part to the neutralizing effects of complement regulatory proteins (CRPs) that are expressed on the cell membranes of corneal cells and soluble CRPs present in the aqueous humor that bathes the corneal endothelium (51–53). Although murine corneal endothelial cells do not express cell membrane-bound CRPs, they are bathed in aqueous humor that contains multiple CRPs that protect them from complement-mediated cytolysis *in vitro* and presumably, *in vivo* as well (46, 54). In addition to disabling the activation of the complement pathway and complement-mediated cytolysis, CRPs may contribute to the immune privilege of corneal allografts by disturbing antigen presentation to T lymphocytes (55). Decay accelerating factor (DAF) is a CRP that has been shown to modulate interactions between antigen-

presenting cells (APC) and T lymphocytes (56, 57). The absence of DAF on either the donor cornea or in the recipient's graft bed abolishes immune privilege and culminates in the rejection of male corneal grafts placed onto female mice (55). Although DAF is known to disrupt complement activation, neither DAF-deficient corneas nor normal corneal grafts displayed any evidence of complement deposition, suggesting that dysregulation of complement activation did not promote antibody-mediated graft rejection in the DAF-deficient hosts or with DAF-deficient corneal grafts (55). Instead, the absence of DAF led to expansion of donor-reactive IFN-γ-producing $CD4^+$ and $CD8^+$ lymphocytes and a simultaneous down-regulation of immunosuppressive cytokines, IL-10 and TGF-β suggesting that the absence of DAF prevents the generation of Tregs.

The corneal endothelium, like many cells in the central nervous system, expresses little or no MHC class Ia molecules. This is particularly problematic as natural killer (NK) cells are programmed to kill any cell, normal or neoplastic, that fails to express MHC class I molecules (58). This places the corneal endothelium at risk for attack by NK cells, which can be found in the aqueous humor of rats bearing corneal allografts (59). However, the aqueous humor contains at least two molecules that inhibit NK cell-mediated cytolysis: macrophage migration inhibitory factor (MIF) and TGF-β MIF is present in the aqueous humor in concentrations that produce an immediate inhibition of NK cell-mediated cytolysis (60–62). TGF-β is also present in the aqueous humor in biologically relevant concentrations and exerts an inhibitory effect that peaks at 18–20 hr (63). Thus, the aqueous humor that bathes the corneal endothelium contains factors that produce immediate and prolonged inhibition of NK cell activity and presumably shield the corneal allograft from NK cell-mediated rejection.

Conundrums and Misconceptions

Immune privilege is a relative, not an absolute condition

The concept of immune privilege has given birth to several misconceptions and conundrums. Perhaps the most common misconception is that immune privilege represents a universal exemption from immune recognition and immune attack. This has led some clinicians to dismiss the concept of immune privilege of corneal allografts out of hand based on their experience with corneal allograft rejection in their high risk patients. However, when corneal allografts are compared to other forms of organ transplantation on a level playing field - in the absence of MHC matching and without the use of immunosuppressive drugs- there is little doubt about the capacity of corneal allografts to escape immune rejection. However, corneal allografts can undergo immune rejection and it is these exceptions to the rule that provide clues for unraveling the mystery of immune privilege.

The fundamental principle that multiple mechanisms and molecules contribute to the immune privilege of corneal allografts has led some to ask which of these is most important for maintaining immune privilege and why are some many different mechanisms associated with immune privilege? This question is analogous to asking which of the wheels of your car is the most important? The answer is that all of them are important because they function as a unit. An equally compelling question is why has immune privilege of the cornea been preserved throughout evolution and what purpose does it serve? One of the most attractive explanations was offered by the late J. Wayne Streilein, who proposed that immune privilege was "a dangerous compromise between the eye and the immune system" in which the eye is shielded from immune-mediated inflammation, but at the risk of infection (64). Indeed, some of the leading causes of infectious blindness - trachoma, herpes simplex virus keratitis (HSVK) and river blindness – are immune-mediated diseases that occur when immune privilege is terminated (65). Without immune intervention HSV infections of the cornea are fatal. That is, corneal HSV infections in athymic nude mice, which lack a

function T cell repertoire, do not produce blinding keratitis, but prove fatal due to viral encephalitis (66). Thus, immune privilege is terminated in conditions in which the immune system senses a compelling “danger signal” and “decides” that preservation of life trumps preservation of vision.

Role of Th1 and Th2 CD4$^+$ T Cells in Corneal Allograft Rejection

Studies in rodent models of penetrating keratoplasty have demonstrated that CD4$^+$ T cells play a crucial role in the immune rejection of corneal allografts. *In vivo* depletion of CD4$^+$ T cells through the administration of anti-CD4 antibody dramatically reduces the incidence of immune rejection of corneal allografts in both the mouse and the rat (67, 68). Adoptive transfer of CD4$^+$ T cells to nude mice results in the swift rejection of corneal allografts in T cell deficient nude mice (36, 48). Moreover, there is a close correlation between the development of CD4$^+$ T cell-dependent DTH responses and the immune rejection of corneal allografts (9, 31–33, 69). These findings have led many investigators to proclaim that CD4$^+$ T cells are absolutely required for corneal allograft rejection. However, on closer inspection it is apparent that CD4$^+$ T cell-independent mechanisms are also involved in the immune rejection of corneal allografts. While it is true that *in vivo* treatment with anti-CD4 antibody significantly reduces the incidence of rejection, it is noteworthy that corneal allografts undergo rejection in 36% of the rats and 33% of the mice treated with anti-CD4 antibody (67, 68). Moreover, 45% to 50% of corneal allografts undergo immune rejection in CD4$^{-/-}$ mice, which indicates that CD4-independent mechanisms can mediate corneal allograft rejection (49, 69). Further investigations have revealed that either CD4$^-$CD8$^+$ T cells or CD4$^-$CD8$^-$ T cells are capable of mediating corneal allograft rejection when adoptively transferred to T cell-deficient recipients (49). Although CD4$^+$ T cell-mediated immunity is the most likely default pathway for corneal allograft rejection, it is important to note that CD4-independent pathways exist and are invoked when the CD4$^+$ T cell population is disabled.

One of the early dogmas in transplantation immunology posited that CD4$^+$ Th1 cells were the dominant, if not sole, mediators of allograft rejection (70). Th1 cells preferentially produce IFN-γ and mediate DTH, both of which are associated with allograft rejection (70). Since Th2 cells produce a distinct array of signature cytokines, including IL-4, which can cross regulate Th1 cells, it was believed that biasing the alloimmune response toward a Th2 pathway would silence the CD4$^+$ Th1 cell arm of the immune response and promote corneal allograft acceptance (14). Initial studies in which corneal allografts were placed in hosts with a Th2-deviated systemic immune response have produced conflicting results (14, 71). However, subsequent investigations have clearly shown that either wild-type mice treated with anti-IFN-γ antibody or IFN-γ$^{-/-}$ mice, have a strong Th2-biased immune response and display an increased, not decreased, incidence of corneal allograft rejection (36, 72). Moreover, mice with strong Th2-mediated allergic conjunctivitis or allergic airway hyperreactivity experience a sharp increase in the incidence and tempo of corneal allograft rejection (71, 73, 74). Thus, the weight of evidence suggests that if anything, tilting the systemic immune response toward a Th2 pathway exacerbates rather than mitigates corneal allograft rejection.

Are Th17 Cells Universally Inflammatory?

IL-17 and Th17 cells have captured the attention of immunologists in recent years due to their putative involvement in a variety of autoimmune diseases, including ocular diseases that were initially attributed to Th1 cells (75–78). It is often assumed that IL-17 and Th17 cells are uniformly proinflammatory, and this has led some to suggest that IL-17 is involved in corneal allograft rejection. However, three separate laboratories have shown that corneal allografts undergo rejection in 100% of IL-17$^{-/-}$ mice or wild-type mice treated with anti-

IL-17A monoclonal antibody (34, 36, 37, 79, 80). Moreover, there is compelling evidence that in some circumstances, IL-17 may play a role in dampening immune-mediated inflammation in EAU and in promoting the generation of $CD4^+CD25^+Foxp3^+$ Tregs that sustain corneal allograft survival (36, 37, 81).

Conclusions

There is overwhelming evidence that corneal allografts possess immune privilege. However, there is a temptation to overly simplify the underlying mechanisms that sustain immune privilege and attribute corneal allograft survival to a single process. The initial finding that FasL contributed to immune privilege in the eye led some investigators to erroneously assume that they could recapitulate ocular immune privilege by simply engineering allografts to express FasL(82). This of course failed and in fact, exacerbated immune inflammation and rejection of allografts transplanted to non-ocular sites. Immune privilege of corneal allografts is the sum total of multiple physiological, anatomical, and immunoregulatory properties of the corneal allograft and the host graft bed. Immune privilege most likely arose as an adaptation to discourage immune responses to non-infectious environmental antigens while allowing the development of a measured immune response to pathogens at the ocular surface. Although an overly robust immune response will rid the eye of a pathogen, it does so at the risk of inflicting irreparable injury to the cornea and jeopardizing vision. Indeed the three leading causes of infectious blindness – trachoma, river blindness, and HSV keratitis – have underlying immune-mediated pathology. In the case of severe HSV keratitis the “danger signal” is of sufficient strength to terminate immune privilege and restrain viral infection to the anterior segment of the eye. The wisdom of this immunological decision is confirmed by experiments in which HSV infections of the corneas of T cell-deficient mice do not produce blinding keratitis, but ultimately result in fatal viral encephalitis (66). Under non-pathological conditions, such as in keratoconus, the corneal allograft and the graft bed do not transmit “danger signals” and as a result, immune privilege is preserved.

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Table 1

Differences between ACAID Tregulatory cells and Tregulatory cells induced by corneal allografts.

T regulatory cell activity	ACAID	Corneal Allografts	Reference
Abolished by anti-CD25	yes	yes	(34)
Abolished by anti-CD8	yes	no	(34)
Abolished by allergic diseases	no	yes	(34)
Abolished by cyclophosphamide	yes	yes	(34)
Abolished by anti-IL-17	no	yes	(34, 36, 37)
Abolished by anti-GITRL	no	yes	(35)
Abolished by anti-PD-L1	no	yes	(42)
Abolished by anti-IFN-γ	yes	yes	(37, 72, 83–85)