The human eye is approximately 1 inch in diameter and is composed of a remarkable array of cells, many of which are found nowhere else in the body. The neurons of the retina process signals through the optic nerve at a rate equivalent to over 1 billion bits of computer data per second. The eye is an extension of the brain, and like the brain, its oxygen and energy requirements are staggering. Since both organs have limited regenerative properties, inflammation of either the brain or eye can have devastating consequences for the survival of the host. Thus, both organs go to extraordinary lengths to reduce immune-mediated inflammation—a phenomenon known as "immune privilege."

One of the first demonstrations of immune privilege was reported over 150 years ago by the Dutch ophthalmologist van Dooremaal. In an attempt to induce experimental cataracts, van Dooremaal placed a variety of foreign objects into the eyes of experimental animals and, among other things, noted that mouse skin transplanted into the anterior chamber of the dog eye enjoyed a prolonged survival. Further evidence that the eye offered a fertile ground for transplants came with the first report of a successful human corneal transplant in 1905. This occurred even before the mammalian immune system had been characterized, and anti-rejection drugs were only a distant reality that did not come into use until the first successful heart and renal transplants in the 1960s. It was not until 1948 that the unique immunologic properties of the eye were fully appreciated by the preeminent immunologist Sir Peter Medawar, who observed that rabbit skin allografts enjoyed significantly extended survival in the anterior chamber (AC) of allogeneic rabbit hosts.

WHAT ARE THE MECHANISMS OF IMMUNE PRIVILEGE?

Immune privilege is the product of multiple anatomical, physiological, and immunoregulatory processes that restrict the induction and expression of immune-mediated inflammation. These include (1) the unique anatomical properties of the eye, (2) immunosuppressive and anti-inflammatory molecules residing in ocular fluids and decorating cells lining the interior of the eye, and (3) regulatory T cells that suppress the induction and expression of immune-mediated inflammation. Many of the blood vessels in the anterior segment of the eye are nonfenestrated and as a result they limit the extravasation of macromolecules and leukocytes from the blood vessels into the AC.

Although major histocompatibility complex (MHC) molecules are displayed on the surface of most nucleated cells in the body, they are either conspicuously absent or weakly expressed on cells in the eye that possess little or no regenerative properties such as the corneal endothelium and the neural retina. MHC class I molecules display viral epitopes and serve as "docking stations" for CD8+ cytotoxic T lymphocytes (CTLs) that kill virus-infected cells. Thus, the absence of MHC class I molecules renders corneal endothelial and retinal cells invisible to the destructive effects of virus-specific CTLs. Although this condition protects corneal endothelial cells and the neural retina from CTL-mediated injury, it creates a potential "immunologic blind spot" for viral infections. The strategy of silencing the expression of MHC class I molecules is also employed by other tissues and organs that cannot tolerate misguided CTLs. For example, the villous trophoblast in humans protects the allogeneic fetus from attack by allospecific CTLs and is crucial for maintaining immune privilege at the maternal-fetal interface.

The aqueous humor that occupies the AC is a cocktail of immunosuppressive and anti-inflammatory molecules that dampen immune-mediated inflammation within the eye and also promote the generation of T regulatory cells (Tregs) that suppress T cell activity in the eye. The cells lining the AC are decorated with cell membrane-bound molecules such as FasL, PD-L1, and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) that either induce apoptosis or suppress the activation of immune cells entering the eye.

Antigens entering the AC, either by direct injection or sloughed from the corneal endothelium during penetrating keratoplasty, induce an alteration of the conventional immune response termed anterior chamber-associated immune deviation (ACAD). ACAD is characterized as a deviation from the prototypic immune response to one in which T cell-mediated
Corneal Nerve Ablation and Ocular Immune Privilege

WHAT IS THE RAISON D’ETRE OF IMMUNE PRIVILEGE?

Why is the eye designed to harbor an "immunologic blind spot"? Three explanations come to mind. The first explanation suggests that by limiting inflammation, immune privilege permits the unfettered transmission of light images from the external environment to the retina and thus preserves vision. A second explanation posits that the corneal endothelium and elements of the neural retina are amitotic and cannot regenerate. Unrestrained inflammation of these tissues would be blinding. When my mentor Wayne Streilein and I first described ACAID 35 years ago, we proposed that the selective downregulation of DTH by ACAID was an adaptation to silence immune-mediated inflammation that was notorious for producing ischemic necrosis and extensive damage to innocent bystander cells. In the eye, such unrestrained inflammation would have blinding consequences. For example, immunogenic mouse tumor cells that fail to induce ACAID elicit robust DTH responses that rid the eye of these tumors but culminate in ischemic necrosis and complete atrophy of the affected eye—a condition termed phthisis bulbi.

Herpes simplex virus (HSV) keratitis provides a compelling example of why in certain circumstances it is beneficial to terminate immune privilege. Studies of HSV keratitis in mice have shown that viral replication is not the direct cause of corneal diseases; instead, corneal tissue injury and blindness are largely due to the immune response to the viral antigens in the cornea. Elegant studies by Metcalf in the mid-1960s showed that HSV corneal infections in athymic nude mice, which cannot develop normal T cell immunity, resolve and leave the cornea clear. Thus, the blinding effects of HSV viral infections of the cornea are T cell-dependent. However, the preservation of vision in T lymphocyte-deficient nude mice comes at a heavy cost, as these mice die from viral encephalitis. Thus, a compromise between the eye and the immune apparatus exists in which microorganisms confronting the eye are perceived by the immune apparatus and a decision is made as to whether they represent a threat to survival or if they are harmless.

One of the possible cues for sounding an alarm to terminate immune privilege and activate a robust immune response is transmitted by pathogen-associated molecular patterns (PAMPs) that are expressed on bacteria and viruses and are recognized by Toll-like receptors (TLRs), which are expressed on cells of the innate immune system such as macrophages and dendritic cells. Engagement of TLRs sets the innate immune apparatus into motion and also activates the adaptive immune system, which ultimately rids the eye of the pathogen. However, robust antimicrobial adaptive immune responses can produce extensive collateral injury to cells in the cornea. In addition to microbial elements, endogenous, host-derived molecules such as the neuropeptide substance P (SP) can terminate immune privilege (discussed later).

EVEN BLIND MICE CAN TELL DAY FROM NIGHT

We have previously proposed that immune privilege was primarily designed to preserve vision by extinguishing inflammation within the eye. However, immune privilege may have an equally important role in preserving circadian rhythm. An ever-growing body of evidence indicates that circadian rhythm affects almost every aspect of human biology and even influences our microbiome. Disruptions of circadian rhythms have been linked to numerous maladies including inflammation, obesity, depression, bipolar disorder, and seasonal affective disorder. It is well recognized that the eye plays a key role in maintaining circadian rhythm, which is coordinated by a master clock located in the suprachiasmatic nuclei (SCN) within the hypothalamus. The eyes are the only known light input pathway to the SCN and for photoentrainment. Although the image-forming rods and cones affect photoentrainment, they are not required for maintaining normal circadian rhythms. That is, mice that are homozygous for the retinal degeneration gene (rd/rd) lack a functional repertoire of rods and cones and are completely blind, yet have normal circadian responses to light. The preservation of photoentrainment in rd/rd mice is due to a subpopulation of retinal ganglion cells (RGCs) that are not affected by the rd/rd mutation and express melanopsin, a non–image-forming photopigment that supports normal circadian rhythm. However, enucleating the eyes of rd/rd mice removes the RGCs and abolishes circadian responses. Thus, preserving the integrity of retinal rods, cones, and RGCs is crucial not only for vision but also for preserving circadian rhythm.

It is noteworthy that ACAID protects the eye from experimental ocular inflammatory diseases. For example, AC injection of retinal S antigen induces ACAID, mitigates inflammation of the retina (i.e., experimental autoimmune uveitis), and preserves the retinas in mice. Investigations by Ferguson and coworkers found that exposure to light was required for the induction of ACAID. Mice maintained in the dark and ostensibly denied normal photoentrainment resisted the induction of ACAID. Could it be that the requirement of light for the induction of ACAID is an adaptation for protecting retinal elements from immune-mediated injury and is an essential element for preserving circadian rhythm (which also requires light exposure)?

CORNEAL ALLOGRAFTS ARE BENEFICIARIES OF IMMUNE PRIVILEGE

Corneal transplantation is the oldest, most common, and arguably the most successful form of solid tissue transplantation. Zirm performed the first successful human corneal transplant over a century ago, at a time when anti-rejection drugs were not even contemplated and almost a half-century before the discovery of transplantation antigens. In the ensuing 100 years, corneal transplants have emerged as the most common and arguably the most successful form of solid tissue transplantation. In uncomplicated first-time settings, over 90% of corneal transplants will succeed even in the absence of human leukocyte antigen (HLA) histocompatibility matching and without the use of systemic anti-rejection drugs.

Many of the factors contributing to the immune privilege in the AC are also responsible for the remarkable success of corneal transplants and include (1) the absence of lymph vessels draining the corneal graft bed, (2) the induction of Treg cells that suppress antigen-specific immune effector responses, and (3) the selective silencing and purging of immune elements at the graft–host interface. All three of these conditions must be present for the long-term success of corneal transplants. Corneal allograft survival is jeopardized in conditions in which peripheral lymph vessels invade the corneal graft bed, which invariably culminates in the immune rejection of corneal allografts. A compelling body of evidence in rodent models of penetrating keratoplasty.
indicates that corneal allograft survival relies heavily on the generation of CD4+CD25+ Tregs that actively suppress immune responses directed at the foreign histocompatibility antigens expressed on corneal transplants. Rodent studies have also revealed the importance of apoptosis-inducing ligands Fasl and PD-L1 that are expressed on the corneal epithelium and endothelium and serve to silence immune lymphocytes at the graft-host interface. Corneal grafts failing to express either functional Fasl or PD-L1 invariably undergo immune rejection. Although immune privilege did not evolve with ophthalmologic surgeons in mind, corneal transplants are nonetheless the beneficiaries of immune privilege.

The absence of lymph and blood vessels in the corneal graft bed has long been recognized as an important factor for the success of corneal transplants in both humans and experimental animals. Although it was long believed that the presence of blood vessels in the graft bed facilitated the egression of histocompatibility antigens expressed on the corneal transplant to the immune apparatus, animal studies have provided compelling evidence that the lymph vessels that accompany blood vessels are the primary conduit for delivering antigens and host antigen-presenting cells to regional lymph nodes. Selectively blocking lymph vessels while preserving blood vessels in the corneal graft bed has a profound effect in preventing immune rejection of corneal allografts and confirms that the blood vessels do not play a significant role in promoting corneal graft rejection.

Preexisting diseases such as HSV keratitis and atopic dermatitis are also important risk factors for the immune rejection of corneal transplants. Mouse models of penetrating keratoplasty have shown that the presence of either allergic asthma or allergic conjunctivitis produces a steep increase in the incidence and tempo of corneal allograft rejection. The Th2 cytokine IL-4 that is elaborated in the course of either allergic conjunctivitis or allergic asthma was found to disable Tregs that are normally induced by orthotopic corneal allografts. Thus, the abrogation of immune privilege of corneal allografts that occurs in allergic diseases is a systemic, rather than a local, effect that uncouples the suppressive function of corneal allograft-induced Tregs.

The highest incidence of rejection occurs in patients who have received two or more corneal transplants. The incidence of rejection soars to 80% in patients receiving a third transplant. On first blush, one might conclude that the skyrocketing incidence of rejection in hosts receiving two or more corneal transplants was the result of immunologic sensitization by the foreign histocompatibility antigens on the previous corneal transplants. However, in the United States, HLA matching is not routinely performed, and corneal donor buttons are selected based on the quality of the graft endothelium with little regard for the histocompatibility genotype of the donor. Thus, the likelihood of encountering the same array of alloantigens on second and third transplants would seem remote. The availability of a mouse model of penetrating keratoplasty paved the way for prospective studies to explore this issue in a prospective setting.

**SYMPATHETIC LOSS OF IMMUNE PRIVILEGE (SLIP)**

We used a well-characterized mouse model of penetrating keratoplasty to test the hypothesis that a first corneal transplant abolishes the immune privilege for subsequent grafts, even those from genetically different donors. The C57BL/6 inbred mouse strain differs from the BALB/c mouse strain at all known histocompatibility gene loci, and thus transplants exchanged between these two mouse strains mimic the condition that typically occurs in human penetrating keratoplasty. In this mouse model, approximately 50% of the C57BL/6 corneal allografts undergo immune rejection in naïve BALB/c hosts. In patients, a 90% acceptance rate is the usual outcome for first-time, uncomplicated corneal transplants, yet the acceptance rate is 50% for mice. It should be noted that immune privilege in transplant patients is typically induced by topical corticosteroids while mouse studies do not employ steroids. However, when topically applied steroids are used in mouse penetrating keratoplasty studies, acceptance is well above 90% and thus recapitulates the human counterpart (Niederkorn et al., unpublished data, 2019).

To test the effect of a first corneal transplant on the fate of subsequent corneal grafts, we transplanted corneas from C3H donors onto the right eyes of BALB/c mice. Sixty days later, C57BL/6 corneal allografts were transplanted to the left eyes of the mice that were previously grafted with C3H corneae on the opposite eyes. C57BL/6 and C3H mice differ at all known histocompatibility gene loci and thus do not share any histocompatibility antigens that could “cross-immunize” the BALB/c mice that had previously received C3H corneal allografts. First-time C57BL/6 corneal allografts consistently underwent rejection in approximately 50% of the naïve BALB/c hosts; however, BALB/c hosts that received C3H corneal allografts in the right eye rejected 100% of the C57BL/6 corneal allografts placed into the left eyes. This is sharply different from the 50% incidence of rejection of the C57BL/6 corneal allografts that is routinely observed in first-time BALB/c recipients. The likelihood that this dramatic increase in the incidence of rejection was the result of immune sensitization and represents a recall response is remote since the BALB/c, C57BL/6, and C3H mouse strains do not share any histocompatibility antigens and thus the possibility of “cross immunization” is obliterated. To confirm this in a more stringent setting, BALB/c corneas were transplanted to the right eyes of syngeneic BALB/c mice. Since the BALB/c mouse strain has been subjected to inbreeding for over a half-century, the histocompatibility genotype is homogeneous and thus these grafts are not recognized by the BALB/c hosts as foreign and are termed “syngeneic.” Sixty days after receiving BALB/c syngeneic corneal grafts in the right eyes, the same mice received C57BL/6 corneal allografts transplanted into the opposite eye. Over 90% of the C57BL/6 corneal allografts underwent rejection in hosts that harbored long-standing clear syngeneic BALB/c corneal grafts in their opposite eyes. These results reveal two important insights. First, the transplantation procedure and not the presence of foreign histocompatibility antigens abolishes immune privilege for a second transplant. Second, the loss of immune privilege extends to the opposite unmanipulated eye. This SLIP is reminiscent of a previously described condition called sympathetic ophthalmia (SO) that sometimes occurs in patients who have experienced penetrating injuries to one eye and subsequently experience inflammation in the opposite “sympathizing” eye. SO was recognized by ancient Greeks and was mentioned by Hippocrates in his writings. SO is still poorly understood, but it is widely believed that trauma to one eye causes the release of retinal antigens that elicit a systemic immune response that affects both eyes, including the opposite eye that was not injured. However, unlike SO, SLIP is not the result of sensitization by antigens expressed on the corneal transplant. We would later learn that SLIP was antigen nonspecific and was the result of a disabling of Tregs that are necessary for corneal allograft survival.

What is it about penetrating keratoplasty that denies immune privilege to subsequent corneal transplants? Two explanations come to mind. The first is the widely recognized observation that suturing the cornea induces an intense ingrowth of lymph vessels and virtually guarantees that corneal...
grafts placed into a vascularized graft bed will undergo immune rejection. Although it seemed unlikely that suturing one eye would affect lymph vessel growth in the opposite eye, we tested this hypothesis nonetheless. As anticipated, suturing the right eyes of BALB/c mice induced luxuriant corneal vascularization in that eye, but had no effect on the fate of C57BL/6 corneal allografts placed into the left eye. This left the surgical incision step of penetrating keratoplasty as the most logical explanation for SLIP. Accordingly, we used a 2.0-mm surgical trephine to make shallow circular incisions in the cornea epithelium of the right eye and placed a C57BL/6 corneal allograft in the left eye. In multiple experiments we observed that 90% to 100% of the corneal allografts transplanted under these conditions underwent immune rejection.

What was it about the shallow corneal incisions that abrogated immune privilege in both eyes? One of the remarkable features of the cornea is its dense innervation. It has been estimated that the density of cornea nerves is 300 times greater than that of the skin. We entertained the hypothesis that it is the severing of corneal nerves that abolishes immune privilege in both eyes. We found that circular incisions produced a rapid dissipation of corneal nerves (Fig. 1) while “X”-shaped incisions had only a minor effect on the corneal nerves. Moreover, “X”-shaped incisions in one eye had no effect on corneal allograft survival in the opposite eye, while circular incisions led to >90% rejection of corneal allografts placed into the opposite eye.

Neuropeptides are known to have a profound effect on immune privilege in the AC. About this time we became aware of the elegant studies from Lucas and coworkers, who found that laser retinal burns to one eye prevented the induction of ACAID in the opposite eye and that the neuropeptide SP was involved in the loss of immune privilege. Accordingly, we interrogated the anterior segments of both eyes following circular corneal incisions and found a steep upregulation of SP in both eyes. Further investigation revealed that blocking the SP receptor (NK1-R) at the time of trephining the corneas prevented SLIP. That is, mice subjected to trephining in one eye and simultaneously treated with Spantide II, an antagonist of NK1-R, displayed the typical 50% incidence of rejection that is known to occur in mice receiving a first corneal transplant. Interestingly, treatment with Spantide II did not enhance immune privilege for first-time corneal allograft recipients not subjected to trephining of the opposite eye. That is, corneal transplants underwent rejection in 50% of the naive mice treated with Spantide II. Thus, SP released following nerve injury affects immune privilege for future corneal allografts but does not jeopardize the fate of a first-time corneal transplant. Just as a train ticket allows one to ride a train the first time, the punched ticket does not permit additional train rides. Tregs are analogous to the train ticket, and the release of SP is analogous to the punch in the ticket that denies repurposing of the Tregs.

**CONTRASUPPRESSOR CELLS MEDIATE SLIP**

SLIP is not restricted to corneal transplantation, but is extended to the AC and specifically affects the induction and expression of ACAID. A series of investigations showed that maneuvers that induced SLIP such as corneal nerve ablation, injection of SP, or keratoplasty prevented the induction of ACAID. Remarkably, a single bolus intravenous injection of as little as 0.1 pg SP prevented the induction of ACAID. SP is clearly a pivotal player in the abolition of immune privilege in at least two other models of immune tolerance. As mentioned earlier, retinal laser burns to one eye prevent the induction of ACAID in the opposite eye by a SP-dependent mechanism. Likewise, 180° circumferential corneal incisions to one eye prevent the induction of mucosal tolerance to OVA antigen applied topically to the opposite eye. This abrogation of mucosal tolerance is dependent and can be blocked by topical application of a SP receptor antagonist.

The SP receptor NK1-R is expressed on a wide variety of cells including antigen-presenting dendritic cells (DCs). In searching for the NK1-R cells that might contribute to SLIP, CD11c+ DCs caught our attention based on their strategic location in the region immediately juxtaposed to where trephine incisions are made prior to orthotopic transplantation. Moreover, previous findings indicated that DCs stimulated via the NK1-R inhibit IL-10 production and promote the generation of Th1 immune responses—two conditions associated with loss of ocular immune privilege.

In vivo experiments revealed that CD11c+ cells isolated from mice subjected to corneal nerve ablation (i.e., “trephining”) and adoptively transferred to naive recipients prevented
the induction of ACAID. Moreover, the CD11c⁺ cells expressed “contrasuppressor cell” activity that blocked the suppressive properties of ACAID Tregs in vivo. Further analysis revealed that severing corneal nerves elicits the release of SP in the immediate location where CD11c⁺ DCs reside and at the site where the corneal allograft is placed. In vitro exposure to SP converts naïve CD11c⁺ cells to antigen nonspecific contrasuppressor (CS) cells that block the induction of ACAID and also disable ACAID Tregs. Additional experiments confirmed that ocular surface CD11c⁺ cells were the precursors for SLIP CS cells. We have previously shown that subconjunctival injection of liposomes loaded with clodronate depletes CD11b⁺ DC, CD11c⁺ DC, and Iba⁺ macrophages at the ocular surface. Using this approach, ocular surface CD11c⁺ cells were depleted prior to corneal nerve ablation. Although corneal nerve ablation normally prevents the induction of ACAID, depletion of ocular surface CD11c⁺ DC prevented the development of SLIP and allowed the development of ACAID and the normal generation of Tregs.

Collectively these results confirmed that CD11c⁺ CS cells are the underlying cell population that mediates SLIP (Fig. 2). That is, CD11c⁺ isolated from mice subjected to trephining were shown to disable Tregs in vivo. Moreover, in vitro conditioning of naïve CD11c⁺ cells with SP converts them to CS cells that block Tregs in third-party hosts. Depletion of ocular surface CD11c⁺ cells prior to corneal nerve ablation prevents the generation of CS cells and allows the full expression of Treg activity and restores immune privilege.

THE EYE SEES EYE TO EYE WITH THE IMMUNE SYSTEM

It might seem counterintuitive that the eye would have such an elaborate system of checks and balances to silence immune-mediated inflammation, yet injury to one eye terminates immune privilege in both eyes. If immune privilege is intended to protect the eye from the ravages of inflammation, what is the benefit of ablating immune privilege in an unperturbed eye (i.e., the opposite eye)? We propose that termination of ocular immune privilege is an adaptation to protect the eye from life-threatening infections. The eye and the immune system establish a compromise in which either noninfectious agents and nominal antigens confronting the eye are ignored by the immune system or they elicit a suppression of the immune response that ensures that inflammation will not be invoked. In making the decision as to whether a foreign entity represents a threat, the immune system perceives “danger signals” that lead to termination of immune privilege. Danger signals occur in different forms. Corneal nerve injury, alkali burns to the ocular surface, or infectious agents stimulate the release of SP that leads to the generation of CS cells and the termination of immune privilege. Alkaline burns to the cornea in one eye prevent the induction of immune tolerance in the opposite eye by a SP-dependent process. Two of the major causes of infectious keratitis and blindness, HSV and Pseudomonas aeruginosa, are associated with the elaboration of SP during corneal infections. SP serves as a “danger signal” in both HSV keratitis and Pseudomonas keratitis and allows the full expression of antimicrobial immunity even if the cost is blindness. In the absence of an immune response these infections can produce a fatal outcome. We propose that the immune system anticipates that an infection in one eye will eventually occur in the opposite eye and thus the full array of immune responses are unleashed to rid the eye of the life-threatening infectious agent.

It is noteworthy that at least one form of immune privilege (i.e., ACAID) is not expressed in mice reared in the absence of light—a condition in which neither vision nor phototainment (i.e., circadian rhythm) is present. Under these conditions immune privilege is unnecessary but protecting the retina from a potentially lethal infection assumes a higher priority. Is it possible that immune privilege is terminated under these conditions as an adaptation for reducing the risk of life-threatening infections?

Acknowledgments

Countless friends and colleagues have profoundly impacted my career and life during the past 40 years. I thank my graduate students who inspired me, my postdoctoral fellows who challenged me, and my technical staff who supported me. I deeply appreciate...
the unwavering support of Research to Prevent Blindness and the stability provided by 35 years of NIH grant support. My postdoctoral mentor Wayne Streilein shaped my development as a scientist and as a person. He taught me that sometimes it is just as important to be kind as it is to be correct. I am a better person and scientist because of his mentoring. My chairman Jim McCulley has been a collaborator, colleague, leader, and most of all a dear friend who has provided unwavering support throughout my entire career. My daughter Jennifer and my son Jason and their families have been a source of love and support through the years. Most of all, my wife Jean has been my most loyal supporter, best friend, and the love of my life.

Supported by National Institutes of Health Grants EY007641, EY030413, and an unrestricted grant from Research to Prevent Blindness.

Disclosure: J.Y. Niederkorn, Immunezey, LLC (S)

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