Immunologic Research 2002;25/1:1–26

Experimental Corneal Allograft Rejection

Bryan M. Gebhardt
Weiyun Shi*

From the Lions Eye Research Laboratories, LSU Eye Center, LSU Health Sciences Center, New Orleans, LA

Abstract

The major findings regarding corneal allograft rejection in experimental animals are reviewed. The principal anatomic and biological feature of the cornea that determines the immunologic privilege of this tissue is its avascularity. The surgical trauma of transplantation compromises the immunologic privilege, putting corneal allografts at risk for immune rejection. During the past 50 yr, rabbits, rats, and mice have been used extensively in the study of the process of immunologically mediated corneal allograft rejection. It is clear that the inflammation and neovascularization of the graft that occurs following transplantation predisposes a corneal allograft to the classic cell-mediated immune rejection response. The antigenicity of cornea cells has been studied and has been found to be significantly lower compared to other cells and tissues.

Rejection of a corneal allograft is a cell-mediated process directed against major histocompatibility complex antigens involving both CD4+ T helper cells and CD8+ cytotoxic cells. The prevention of corneal allograft rejection depends on the development of topically applied compounds that can prevent inflammation and vascularization and inhibit the activation of T lymphocytes. Considerable progress has been made using immunomodulators, including blocking antibodies and soluble coreceptor blocking agents such as CTLA4-Ig. Combinations of antiangiogenic agents and immunomodulators hold great promise for preventing corneal allograft rejection in patients.

Key Words

Cornea
Allograft
Rejection
Vascularization
Cell-mediated immunity
Immunologic privilege
Rabbit
Mouse
Rat
Immunosuppression

*Present address: Weiyun Shi, MD, Shandong Eye Institute and Hospital, 5 Yanerdao Road, Quingdao, 266071 P. R. China. E-mail: weiyunshi@yahoo.com

Bryan M. Gebhardt, Ph.D.,
LSU Eye Center,
2020 Gravier Street, Suite B,
New Orleans, LA 70112-2234;
E-mail: BGEBHA@LSUHSC.EDU

0257–277X/02/
25/1:1–26/$16.50

Introduction

The cornea is the only part of the eye that is technically feasible to transplant clinically and experimentally. Many thousands of patients have vision restored to blind eyes by having a diseased cornea replaced with a corneal allograft. Protected against immunologic attack by immunosuppressive drugs, 90% of corneal allografts survive for extended periods of time. Given this high rate of success, why do we continue to study corneal allograft immune reactions in experimental animals? The impetus for pursuing the study of corneal allograft immune reactions is that, although the cellular and molecular components of rejection have been characterized, the immune mechanisms whereby corneal allograft cells are destroyed are not understood. Furthermore, in patients who are considered to be at high risk for rejecting a corneal allograft because of a vascularized graft bed or previous graft rejection, it is often impossible to prevent rejection with immunosuppressive drugs.

The immunologic privilege of the cornea is universally appreciated. This privilege derives from the anatomy and cell biology of this tissue: The cornea is avascular and does not contain lymphatic channels, and corneal cells express low levels of histocompatibility antigens. A variety of pathological circumstances result in inflammation and neovascularization of the cornea, eliminating immunologic privilege. When a cornea becomes vascularized it is subject to the same rules of transplantation immunology as other tissues and organ grafts. Corneal allograft rejection has all the hallmarks of a cell-mediated immune response to alloantigens; the death of irreplaceable graft cells results in failure of graft function and rejection of the graft. In this review, a synthesis of our understanding of the process of corneal allograft rejection and the ways in which corneal allograft rejection can be circumvented will be considered.

The main points to be elaborated on are:

1. The normal cornea is immunologically privileged because it is not vascularized.
2. Trauma to the cornea as a result of surgery (corneal transplantation) or other penetrating injury, bacterial, viral, or fungal infection, or chemical burns—all of which induce inflammation and/or neovascularization—eliminate the immunologic privilege of this tissue.
3. Corneal allograft rejection is a typical cellular immune process involving T helper lymphocytes, T cytotoxic lymphocytes, and accessory leukocytes.
4. It is not known where corneal allograft antigens are initially recognized—*in situ*, external to the graft, or both.
5. Corneal allograft rejection is a localized immune response which does not engender measurable systemic humoral or cell-mediated immunity.
6. Antigens coded for by the major histocompatibility gene complex (MHC) are the targets of corneal allograft immune reactions.
7. Prevention of corneal allograft rejection in high risk situations will be achieved using a combination of locally applied mediators that inhibit neovascularization, inflammation, and T cell activation.

Animal Models of Corneal Allograft Rejection

Since the normal cornea is optically clear and is part of the exterior surface of the body, it is possible, with low power magnification, to observe changes in this tissue associated with rejection, such as infiltration of leukocytes, neovascularization, haze, and edema. Thus, it is feasible to record the appearance of a corneal allograft from the time of transplantation to the endpoint of rejection (Fig. 1).

The histopathology of corneal allograft rejection has been described *(1–8)* and mimics

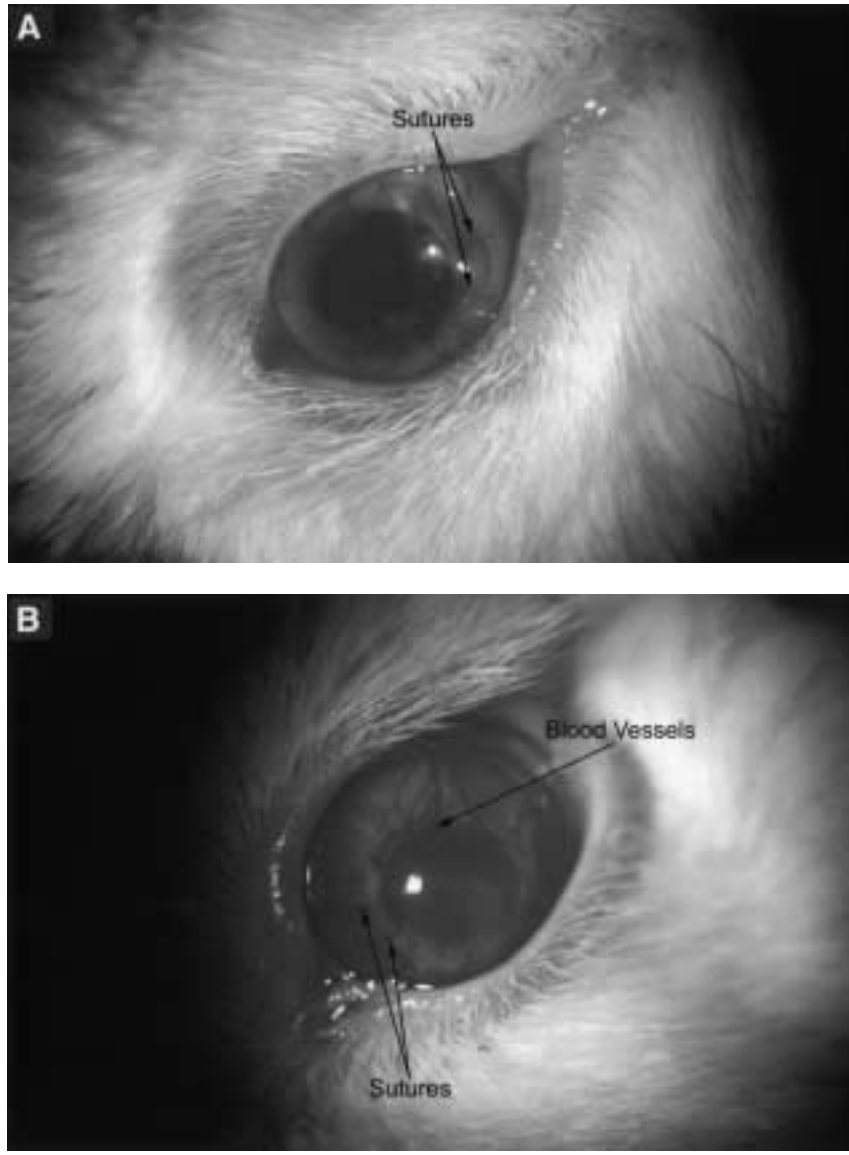

Fig. 1. Slit lamp photographs of rabbit corneal allografts. (A) Photograph of a graft five days after transplantation. Two of the sutures in the periphery of the graft are marked with arrows. At this early time the graft is optically clear and not vascularized. (B) Photograph of a rejected corneal allograft 19 d after transplantation. The graft is hazy, edematous, inflamed, and vascularized. Blood vessels growing into the graft are readily apparent. Grafts in this condition are end-stage and not salvageable by immunosuppression.

other forms of cell-mediated allograft rejection such as occur in the kidney, heart, and other organ allografts. During rejection a corneal allograft becomes vascularized and is infiltrated by a mixed population of leukocytes including polymorphonuclear leukocytes, lymphocytes, and macrophages (Fig. 2). At the time of transplantation, a corneal allograft

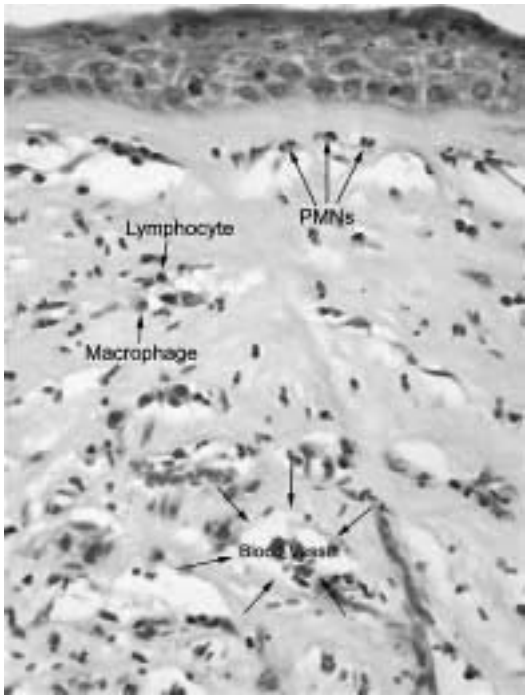

Fig. 2. Histopathology of corneal allograft rejection. In this photomicrograph of a corneal allograft undergoing rejection, numerous white blood cells of different morphologies can be seen in the stroma and epithelium of the graft. In particular, cells having the morphology of lymphocytes, macrophages, and polymorphonuclear leukocytes can be seen. Blood vessels are present in the corneal stroma (×100 magnification).

is avascular and consists of a multilayered epithelium, a collagenous stroma containing scattered keratocytes, and an inner monolayer of endothelial cells (Fig. 3). The rim of a corneal allograft is joined to the edge of the recipient's cornea and a watertight seal is established using a delicate nylon suture. Before a corneal allograft immune reaction can develop, the first cellular host event that must ensue is the infiltration of acute inflammatory cells including neutrophils and macrophages, into the wound site and around the nylon sutures that hold the graft in place. In patients, this acute inflammatory reaction is suppressed by topical steroids. The continued use of topical steroids as an immunosuppressive therapy for several months after transplantation is relatively effective in preventing cornea graft rejection in patients. Similarly, controlling the acute inflammatory reaction that occurs soon after corneal transplantation in animals can prevent early graft failure mediated by nonspecific damage of the graft by inflammatory cells and continued treatment can also prevent a subsequent antigen-specific immune response.

Rabbits, rats, and mice are commonly used in corneal allografting experiments. There are significant differences in the survival of corneal allografts in these three species and the technical difficulty of performing the surgery increases as the size of the animal and its cornea decreases. More than half of the corneal allografts placed into nonvascularized graft beds in rabbits survive indefinitely without the use of steroids or other immunosuppressive drugs *(9,10)*. However, when one is investigating the cellular and molecular components of the immune graft reaction it is necessary that a high percentage of recipients mount an immunologic attack on the corneal allografts. In order to predispose corneal allografts in rabbits to undergo graft rejection, we first induce vascularization of the recipient graft site with silk sutures. Using the technique reported by Hill and Maske *(11)*, three loops of silk suture are sewn into the anterior corneal stroma of the recipient rabbit approximately two weeks before corneal transplantation. The silk suture acts as an irritant, eliciting an inflammatory reaction and the ingrowth of blood vessels to and around the sutures (Fig. 4). Then the sutures are removed and the vascularized recipient cornea is replaced with a nonvascularized cornea allograft. In this way, recipient blood vessels are immediately adjacent to the corneal allograft when the transplant is in place. This

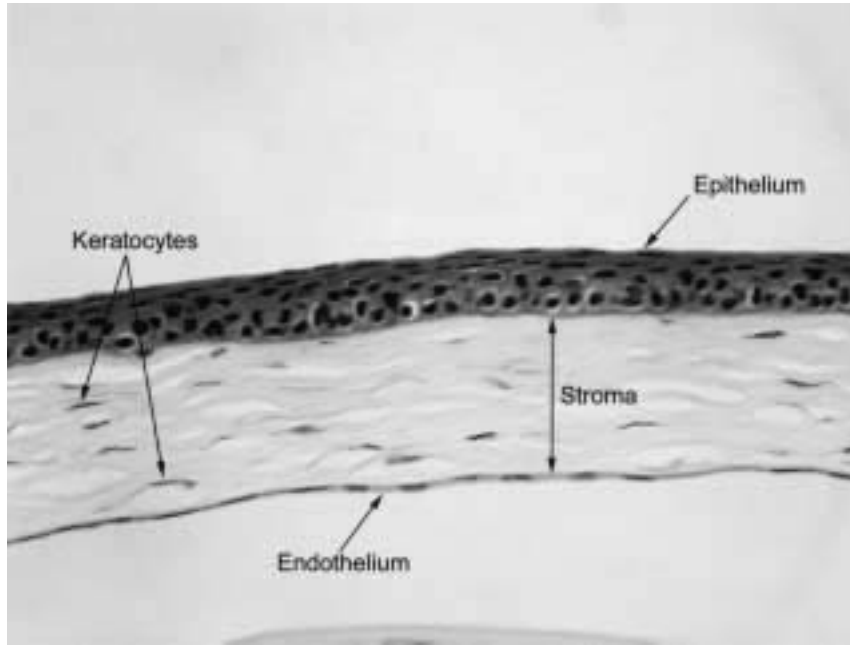

Fig. 3. Histology of the normal cornea. In this photomicrograph the structure of the normal cornea, consisting of an outer layer of epithelium, a middle layer of collagenous stroma with scattered keratocytes, and an inner layer of endothelial cells, is shown (×40 magnification).

juxtaposition of recipient blood vessels with the allograft facilitates vascular ingrowth into the allograft, promoting contact between donor alloantigens and the recipient's cellular immune apparatus. Using this approach, more than 80% of rabbit corneal allografts are subjected to immune reactions, providing a wealth of material for study *(10,11)*. Heat cautery or alkali burns also induce inflammation and vascularization of the cornea *(10,11)*.

When a rabbit corneal allograft placed into an avascular, immunologically privileged recipient graft site is compared with one placed into an inflamed and vascularized site which is no longer privileged, the differences between these two circumstances emerge. If a technically perfect and surgically uncomplicated corneal allograft is accomplished in a nonvascularized graft site the allograft is likely to survive indefinitely without the use of topical or systemic immunosuppression. Removal of sutures often can be achieved between 14 and 21 d; clear, healthy corneal allografts will survive for the lifetime of the animal. Placing the graft in a recipient cornea that has been inflamed and vascularized by the insertion of silk sutures as described above dramatically alters the immunologic privilege of the recipient cornea and the subsequent fate of the graft. Even if the surgical procedure is technically uncomplicated and there are no postsurgical sequelae, the majority of grafts placed in vascularized sites are subjected to an acute immune rejection response. In rabbits, rejection of corneal allografts placed in vascularized graft beds occurs between 12 and 25 d; the median survival time is 18 d (Fig. 5). Observation of corneal allografts in vascularized

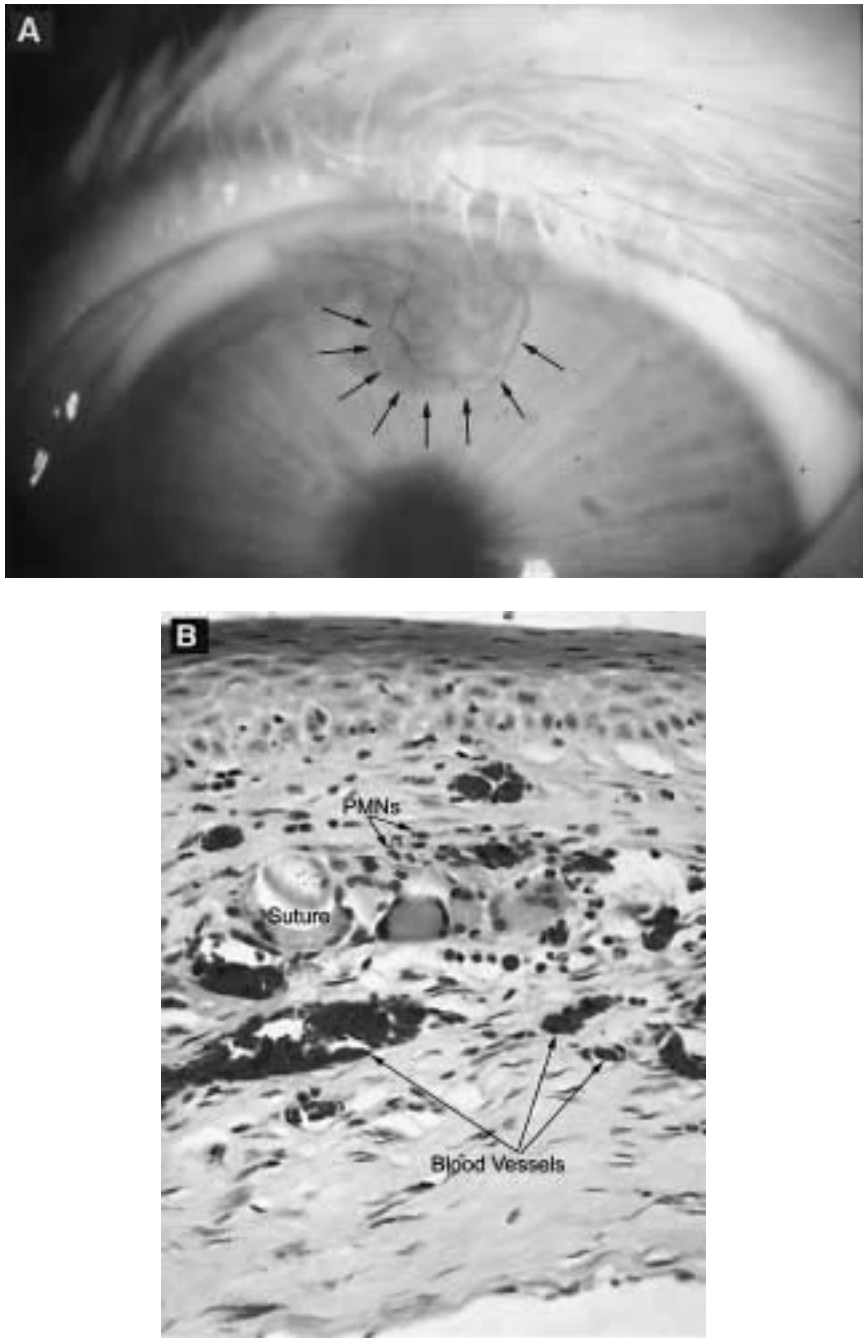

Fig. 4. Neovascularization of the rabbit cornea with silk sutures. (A) Slit lamp photograph of a vascularized cornea shortly after removal of the silk sutures. The dense neovascularization of the cornea in the area where the sutures were placed can be seen (arrows). (B) Photomicrograph showing the histopathology of corneal neovascularization induced by a silk suture. The presence of blood vessels and inflammatory cells in the stroma and disruption of the stromal matrix induced by the suture can be seen (×100 magnification).

grafts sites reveals that recipient vessels grow across the graft-host junction, beginning the process of neovascularization of the graft, during the first week after transplantation. Histological analysis of such grafts during the first week reveals a vigorous, acute inflammatory response at the graft-host junction very similar in cellular character to that seen in response to the silk suture (Fig. 4B). Sprouting vessels from the recipient cross into the corneal allograft and migrate centrally during the ensuing days. Even if suture removal is completed within 14 d after transplantation, eliminating the inflammatory and angiogenic stimulus, the grafts are usually subjected to a cellular immune response; the graft becomes edematous, opaque, and vascularized. The variability in the time course in which corneal allografts are rejected in rabbits is, in part, due to the varying degrees of histoincompatibility between donors and recipients in this random bred species.

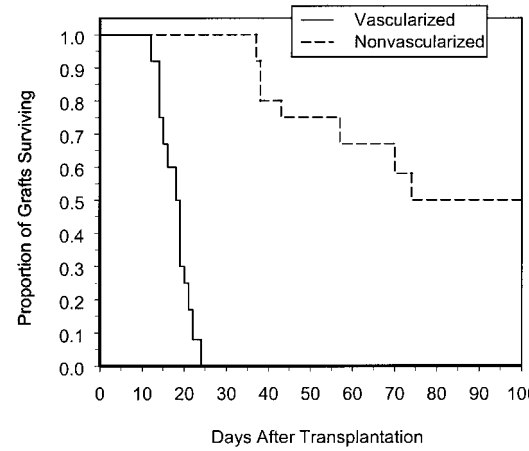

Fig. 5. Kaplan-Meier survival curves showing the difference in survival between corneal allografts placed into vascularized and nonvascularized graft sites in the rabbit eye. Typically 50% or more of corneal allografts placed into nonvascularized graft beds survive for a 100 d observation period, whereas 10% or fewer of corneal allografts placed into vascularized graft sites survive.

Experimental studies in corneal allografting date from the 1940s and 1950s. Investigations carried out by Medawar *(12)*, Maumenee *(9)*, and Billingham and Boswell *(13)* were performed in random bred rabbits. These studies established the principle of immunologic privilege of avascular tissues such as the cornea and showed that under certain circumstances allografts placed into this immunologically privileged site could be subjected to immunologic rejection particularly when inflammation and vascularization of the graft occurred *(14,15)* or when the recipient had been presensitized to donor alloantigens *(9,16)*. For many years, the rabbit was favored by vision researchers and in particular used in studies of corneal transplantation and the testing of immunosuppressive drugs to prevent graft rejection *(17–22)*.

Beginning in the 1970s and continuing to the present, investigators have used inbred strains of rats as donors and recipients of corneal allografts *(23–38)*. These investigations clarified the role of class I and class II MHC molecules in eliciting corneal allograft immune reactions *(25,27,28,30,34)* and led to studies of the cellular and molecular events in corneal allograft rejection *(29,31,36–38)*. As in the rabbit, rats receiving corneal allografts may or may not reject these grafts depending upon the intensity of the postgrafting inflammation and vascularization *(24,29)*. Approximately half of the allografts exchanged between MHC mismatched rats undergo immune rejection, whereas the remaining 50% survive even in the absence of immunosuppressive therapy *(29,31,36)*. Analysis of the cellular infiltrate in rat corneal allografts reveals that T lymphocytes of the CD4+ and CD8+ subsets migrate into corneal allografts *(39,40)* by 3 d after transplantation. In grafts destined to undergo a destructive immune

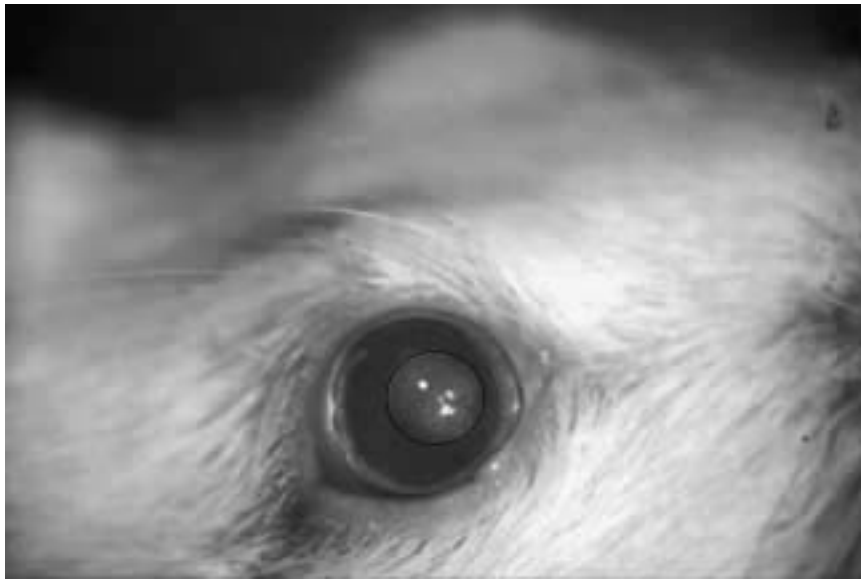

Fig. 6. Slit lamp photograph of a rejected corneal allograft in a BALB/c mouse. Fourteen days after transplantation the graft from a C57BL/6 donor is opaque and vascularized. The superimposed circle marks the circumference of the graft.

response, the accumulation of these cells continues with the migration of lymphocytes, macrophages, and Langerhans cells centripetally into the allograft. Recent studies have shown that a cytokine cascade accompanies this cellular infiltrate, and mediators such as interleukin-1 and interleukin-2, interferon gamma, tumor necrosis factor alpha, and a variety of chemokines are produced by cells infiltrating corneal allografts *(37,38,41)*.

In the past ten years a number of investigators have used inbred strains of mice as donors and recipients of corneal allografts *(5,42–48)*. The inbred mouse has many virtues as an experimental animal in which to study immunological phenomena. In contrast to the large percentage of corneal allografts that survive without immunosuppression in rabbits and rats, most, if not all, grafts in mice are rejected *(5,43,48–57)*. Our experience is that even though the mouse cornea is avascular, exchange of corneas between strains of mice mismatched at the MHC genes such as C57BL/6 and BALB/c invariably leads to graft rejection; unless immunosuppression is used, all corneal allografts are rejected (unpublished data). The mouse cornea is approx 3.5 mm in diameter and commonly 2 mm allografts are performed. It is necessary to hold grafts in place with at least 8, 10, or even 12 interrupted sutures. The trauma of performing corneal transplantation into the eye of a mouse and the presence of multiple sutures in such a small piece of tissue invariably leads to an intense, acute inflammatory response, which is followed by neovascularization and culminates in immune graft failure (*55*, Fig. 6). Of more than 200 allografts performed, we have not had a single mouse corneal allograft survive beyond 20 d without the use of immunosuppression (unpublished data). Thus, we conclude that the immunologic privilege which exists in the mouse cornea prior to transplantation is lost as a result of the surgical trauma, and corneal allografts in mice are at high risk of being rejected.

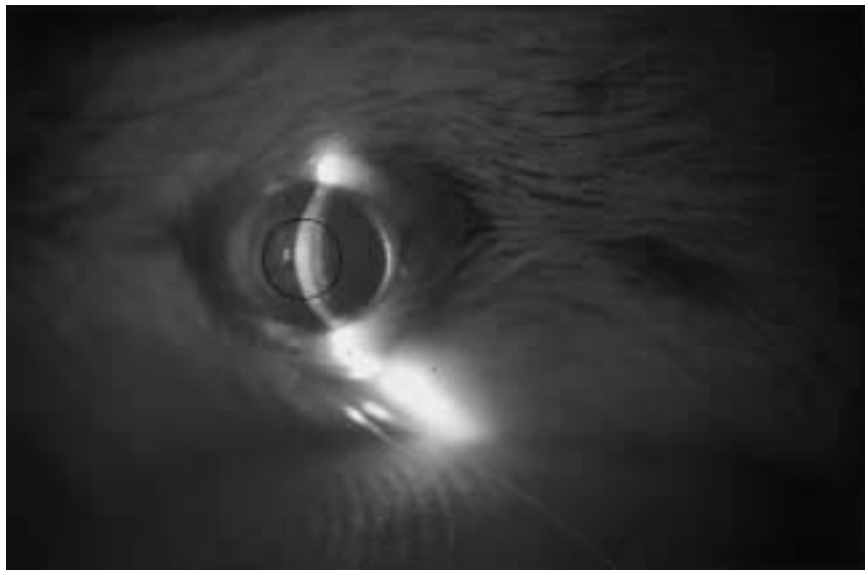

Fig. 7. Slit lamp photograph of a surviving corneal graft exchanged between two members of the same inbred strain. Shown here at 35 d, the syngraft is clear, making it difficult to discern the graft-host margins. In this photograph the light beam was narrowed in order to reveal the normal thickness of the graft. A black circle is superimposed just inside the graft-host junction. Such grafts survive for the life of the recipient.

Autografts and grafts exchanged between mice of the same inbred strain are subjected to a transient inflammatory response which rapidly wanes as the sutures are removed; these grafts are not rejected (Fig. 7).

Some investigators report that in order to ensure rejection of MHC mismatched grafts in mice, the graft bed must be altered by an irritating agent. Peeler, et al. *(42)* and Niederkorn, et al. *(58,59)* injected latex beads into the recipient graft site as a means of inducing immigration of Langerhans cells, whereas Sano, et al. *(60–62)* used alkali cautery or silk sutures as a means of inducing recipient graft bed inflammation and vascularization. These investigators believe that in order to ensure that the recipient will mount an immune attack on a corneal allograft and in order to eliminate immunologic privilege in the recipient cornea, it is necessary to first induce the migration of Langerhans cells into the recipient cornea, or to induce inflammation and vascularization. This is contrary to the results of others *(5,43,48–57)* indicating that corneal allografts exchanged between MHC-mismatched donor and recipient mice are invariably subjected to intense inflammatory responses, immune responses, and ultimately rejection. We conclude that corneal allografting in mice is inherently a high risk situation despite the fact that prior to transplantation the mouse cornea is an avascular tissue. By virtue of the small size of the cornea and consequently the even smaller size of the mouse corneal allograft and the postsurgical inflammation and vascularization which occurs, corneal allografts in this species are invariably predisposed to a terminal immune rejection response.

This is not to say that the mouse is not a useful species in which to study corneal allograft immune rejection. Many aspects of graft rejection can be studied in the mouse and some

of these will be discussed below (pages 11–13 and 17–18).

Antigenicity of the Cornea and the Expression of Major Histocompatibility Complex Antigens by Cornea Cells

The cornea consists of three main types of cells (Fig. 3). The outer surface consists of a layer of epithelial cells 5–6 cells deep. The corneal stroma, made up of collagen, is populated by a scattering of cells called keratocytes which have many properties in common with fibroblasts. The inner layer of the cornea consists of a monolayer of endothelial cells. In the periphery of the cornea where it adjoins the conjunctiva, there is a small population of Langerhans cells in the epithelium *(36,62)*.

The antigenicity of the intact cornea and of the individual cell layers of the cornea has been investigated *(63–67)*. The conclusion from these studies is that in aggregate the cornea is a weakly antigenic tissue. Collectively and individually, the cells of the cornea express low levels of class I MHC molecules and few or no class II MHC molecules. In fact, most investigators have had to resort to sensitive methodologies in order to demonstrate the presence of MHC on corneal epithelial cells, keratocytes, and endothelial cells.

Guymer and Mandel *(65)* found that the cornea, in comparison to the pancreas and skin, was by far the least antigenic and expressed the lowest level of class II MHC antigens. These investigators also noted that cornea cells did not respond to cytokines such as gamma interferon and interleukin-2 which stimulate the expression of class II MHC molecules in some other cell types. Langerhans cells, which express class II MHC molecules, are found in the corneal epithelium at the limbus, and presumably have the same origin and function as the Langerhans cells in the skin. The number of these cells in the central cornea is so low as to be negligible in terms of contributing to the antigenicity of this tissue *(65)*.

Gebhardt and Seto investigated the immunogenicity of tissue-cultured corneal stromal keratocytes *(66)*. They found that on a per cell basis corneal keratocytes were significantly less antigenic than blood mononuclear cells. Furthermore, it was noted that the immune response elicited by keratocytes was directed against class I MHC antigens; no detectable response to class II MHC antigens was recorded. At least 30×10^6 keratocytes were required to elicit a humoral or cellular immune response whereas individual corneas contained only 0.05×10^6 keratocytes. The conclusion was that although a corneal allograft may elicit a local immune response this response did not confer systemic humoral or cellular immunity on the graft recipient *(66)*.

In subsequent studies, Gebhardt *(67)* investigated the alloantigenicity of corneal tissue and the effect of the route of immunization with allogeneic corneal tissue on the generation of an immune response. Variable amounts of allogeneic corneal tissue were placed orthotopically, subcutaneously, or intraperitoneally in allogeneic recipients and the cellular and humoral immune response was measured at weekly intervals. It was noted that a corneal allograft placed in the orthotopic site in a vascularized graft bed was rejected but failed to elicit a systemic immune response as measured by the absence of a serum antibody response or a cellular cytotoxic response in the spleen and lymph nodes. Similarly, one cornea placed subcutaneously or intraperitoneally failed to elicit a cellular or humoral alloantigenic response. A minimum of four corneal allografts placed intraperitoneally was necessary to elicit a systemic alloimmune response; fewer than four corneas failed to induce systemic immunity *(67)*. Subsequent

studies confirmed that the alloimmune response engendered by four or more allogeneic corneas was directed against donor origin class I MHC antigens, not class II MHC antigens.

Thus, the weight of the evidence indicates that the cornea is a very weakly alloantigenic tissue and that when grafted orthotopically a corneal allograft elicits a local immune response that is not reflected in a systemic immune response measurable as serum alloantibody or cytotoxic effector cells in the lymphoid tissue.

It should be mentioned here that the results of recent studies using inbred mice and rats and some analyses in MHC-matched patients suggest the possibility that the principal antigenic targets on corneal allografts are minor histocompatibility antigens *(32,43,44,50, 61,68–73)*. Why corneal cells should present minor histocompatibility antigens in such a way as to elicit acute graft rejection when skin and other tissue grafts exchanged between donors and recipients that differ only in minor histocompatibility antigens elicit only weak, chronic rejection reactions remains unclear. The vast majority of corneal allografts performed in patients are MHC and minor histocompatibility antigen mismatches. The same is true for experimental corneal allografts in rabbits, rats, mice, and other species in which this procedure has been performed. The conundrum presented by the observations that suggest that minor histocompatibility antigens are dominant over MHC antigens in corneal allograft rejection is unsolved. It is well documented that skin grafts exchanged between mice differing only in minor histocompatibility genes result in graft survival times of months to years. Such grafts are subjected to a slow, chronic, and weak rejection response *(74)*. The results of studies in which corneal grafts are exchanged between mice or rats differing only in minor histocompatibility genes yielded graft rejection times of two to three weeks *(32,68,70)*. Explanation of why a vascularized skin graft should survive for extended periods of time while a nonvascularized corneal graft is abruptly rejected has not been forthcoming. Given that the cornea is a weakly antigenic tissue (see pages 10–11) in terms of MHC expression, it may be reasonably concluded that the antigenic load represented by the minor histocompatibility antigens in this site is vanishingly small and therefore not a significant immunogenic stimulus to the immune system. We believe that the recent reports regarding the role of minor histocompatibility antigens in corneal allograft rejection in patients suggest that the MHC matching was not complete. Regarding the observations in inbred rodents, we feel that further study will reveal that most, if not all, of the minor histocompatibility antigen-mediated corneal graft "rejection" is due to either technical failure of the grafts and/or the existence of mismatched MHC loci in the animals used in these studies. Additional investigation of the possibility that the rejection of corneal allografts is based on minor histocompatibility antigen differences between donor and recipient is required.

All organ allografts, by virtue of their vascularity, contain passenger leukocytes and these cells have been shown to contribute significantly to the antigenicity of allografts. The cornea, in its avascular condition, is essentially devoid of such passenger leukocytes and thus the alloantigenicity of this tissue is limited to the small amount of class I major histocompatibility complex molecules expressed on epithelial cells, keratocytes, and endothelial cells. The small population of Langerhans cells in the corneal epithelium *(75–84)* does not contribute to the antigenicity of a donor cornea under most circumstances because it

is only the central portion of the donor cornea that is transplanted to the recipient. Thus, very few, if any, donor origin Langerhans cells are transplanted when a corneal allograft is performed.

Centripetal migration of Langerhans cells can be induced by a variety of stimuli *(42,58, 60,85–87)*. Thus, corneal limbal Langerhans cells migrate into the central cornea in response to infections, physical damage to the epithelium, cautery, and acid and alkali burns. In such circumstances, a cornea not only becomes inflamed and vascularized, but also contains an increased number of Langerhans cells centrally. Clinically, corneas that are scarred, inflamed, and contain many Langerhans cells are not used as corneal allografts because they are opaque and cannot provide visual rehabilitation.

Corneal Immunologic Privilege

Medawar *(12)* concluded over 50 yr ago that foreign tissues placed in locations that are not vascularized survive without undergoing immunologic attack. The cornea is avascular and the preponderance of evidence dating back over the past half century confirms that this anatomic condition determines the immunologic privilege of this tissue.

There are at least two other properties of the cornea and the anterior chamber of the eye that have been proposed to explain the immunological privilege of this tissue. Griffith, et al. *(88)* and Yamagami *(53)* reported that Fas ligand (FasL) is expressed on cells lining the anterior chamber of the eye. It was concluded by both groups of investigators that interaction of FasL with Fas expressed on T lymphocytes in a corneal allograft induced apoptosis, eliminating the immune effector cells and thereby protecting the graft from rejection. In other studies it was found that CD95 ligand (CD95L) is expressed on corneal endothelial cells and it was concluded that immune privilege was conferred by virtue of apoptosis of T effector cells interacting with this ligand on the endothelium *(89,90)*. Unexplained by these studies is the fact that numerous T lymphocytes infiltrate corneal allografts and mediate rejection. The fact that these cells remain alive to mediate the immune graft reaction and the fact that viable T lymphocytes have been isolated and cloned from corneas during rejection *(91)* suggests that FasL-Fas interaction is not adequate to confer immunologic privilege on corneal allografts. It has also been reported that FasL expression by islet of Langerhans cells does not render them immunologically privileged *(92)*. Thus, the functional significance of FasL-Fas in suppressing a corneal allograft immune response or the response to other types of allografts is not apparent.

Another phenomenon that has been claimed to have an important role in modulating immune reactions in the cornea is termed anterior chamber-associated immune deviation (ACAID). Kaplan and Streilein *(93)* and others reported *(94–99)* that antigens introduced into the anterior chamber of the eye resulted in a failure of the injected animal to develop systemic cell-mediated immunity although a humoral response was elicited. For over 25 yr, a variety of cells and mediators have been implicated in the induction of ACAID. At the cellular level it has been reported that T cells *(100–103)*, macrophages *(104,105)*, natural killer cells *(106)*, B lymphocytes *(107)*, Langerhans cells *(108)*, CD8+ T cells *(109–112)*, and CD4+ T cells *(113–115)* mediate ACAID. Soluble mediators implicated in mediating ACAID include transforming growth factor beta *(116–123)*, interleukin-1 *(124,125)*, interleukin-2 *(126)*, interleukin-6 *(123)*, interleukin-10 *(127)*, interferon gamma

(128,129), tumor necrosis factor alpha *(130)*, the neuropeptides substance P and vasoactive intestinal peptide *(131)*, endogenous glucocorticoids *(132)*, a natural killer inhibitor *(133)*, and undefined blood and serum factors *(134–137)*. Regarding the immune privilege afforded a corneal allograft, it has been noted that corneal cells may produce substances which mediate ACAID *(53,88–90,138–140)*. Similarly, it is felt that the aqueous humor of the eye which bathes the inner surface of the cornea also may contain some factors which tend to suppress cellular immune reactions *(88,90,116–123,141)*. ACAID is thought by some investigators to be the most important response mediating corneal allograft privilege *(142–147)*. Paradoxically, however, it has also been stated that ACAID is no longer operative in corneas which are inflamed and vascularized *(46,60,146,148)*. Since virtually all corneal allografts undergo an initial acute inflammatory response subsequent to transplantation and since corneal allograft neovascularization occurs to some extent in virtually all grafts, at least at the graft periphery, thus eliminating ACAID, it is not clear how ACAID could influence the immunologic privilege of the surgically manipulated cornea. It is said that for corneal allograft immune privilege to be in effect none of the components of the immune reflex arc must be altered *(146)*. Thus, inflammatory cells and corneal Langerhans cells must not enter the graft, corneal alloantigens must not be shed from the graft and transported to the lymphoid tissues for presentation to T cells, vascularization of the graft must not occur, and T cells entering the graft must undergo apoptosis following encounter with FasL. However, one or more of these events occurs in all corneal allografts thereby compromising the immunologic privilege of this tissue. Immunologic privilege of the cornea only holds under circumstances in which an antigen is placed into the cornea in such a way as to avoid inflammation, minimize antigen processing and presentation by Langerhans cells, or avoid vascularization such that antigen is not transported to the lymphoid tissue, processed, and presented to T cells. Investigators who feel that ACAID mediates corneal immune privilege recognize that immune privilege relies upon the failure of corneal allograft antigens to stimulate an immune response *(142–147)*. The only way that this can be achieved is if the corneal alloantigens are sequestered within the graft such that the immune system is not signaled to respond; this situation applies only in the normal, noninflamed and nonvascularized cornea.

In patients it is clear that there are several risk factors that contribute to the likelihood that a corneal allograft will come under immune attack. Prior vascularization of the graft site is one of the most important risk factors. Other risk factors include prior rejection of a corneal allograft, large diameter grafts, and sensitization to donor alloantigens by blood transfusions *(149)*.

Immune Mechanisms of Corneal Allograft Rejection

It is not known if corneal alloantigens are recognized *in situ* in the graft or in the lymphoid tissues distant from the graft. One possibility that can be considered is that MHC antigens from a corneal allograft exit the graft passively by one of several routes, enter the circulation, and reach lymphoid tissues for processing and presentation to T lymphocytes (*150*, Fig. 8). Thus, alloantigenic molecules might be shed from the surface of the cornea into the tear film following transplantation. In all likelihood, however, antigens in the tears would be drained away in the tear ducts and

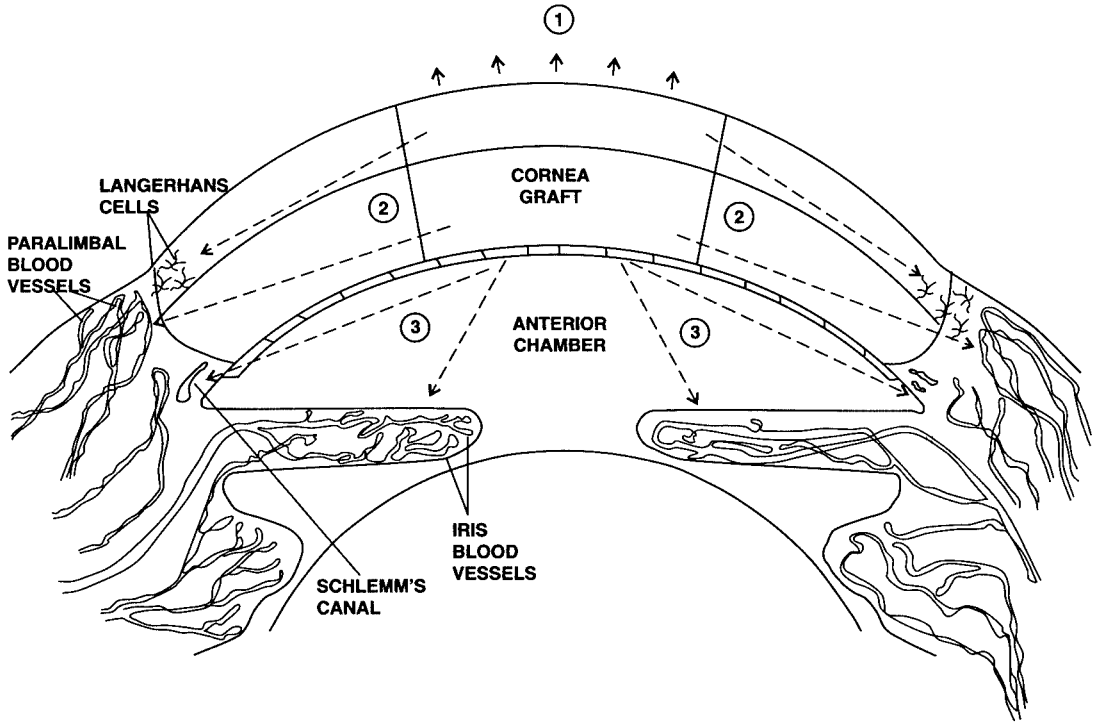

Fig. 8. The possible pathways of passive movement of corneal allograft antigens out of the graft and toward the recipient's immune system is shown schematically. (1) Alloantigens exiting the graft into the tears would drain into the tear ducts and be metabolized away without making contact with the immune system. (2) Alloantigens diffusing out of the graft laterally through the recipient cornea would reach the paralimbal blood vessels in very small amounts. If these antigens gained access to the blood they would be further diluted in the blood and the amount of alloantigen reaching the lymphoid tissues would be too low to stimulate a response. Similarly, alloantigens reaching the limbus might be taken up by Langerhans cells, but whether or not these cells migrate to the lymphoid tissues and present the antigens is not known. (3) Alloantigens diffusing into the anterior chamber might gain entry to the iris blood vessels or, more likely, might exit the eye in the drainage system consisting of the trabecular meshwork and Schlemm's canal. Antigens achieving this route of exit may reach the blood vessels and the lymphoid tissues, but in concentrations too low to stimulate active immunity in the recipient.

eliminated from the body. Lateral, passive movement of MHC antigens from the graft through the recipient cornea to the paralimbal blood vessels and transport to the spleen and lymphoid tissues is a second possibility. Since the amount of alloantigen in a corneal allograft is small and the amount that is spontaneously shed from the graft must be considerably smaller, the dilution of antigens reaching the circulation would be so great that the concentration of alloantigens reaching distant lymphoid tissues would be insufficient to activate T cells. Similarly, MHC antigens diffusing into the anterior chamber of the eye might leave the eye and enter the circulation by way of iris blood vessels or drainage through the ocular outflow system known as the canal of Schlemm. Here too, the dilution factor would be very large and the amount of alloantigen leaving the eye would be infinitesimally

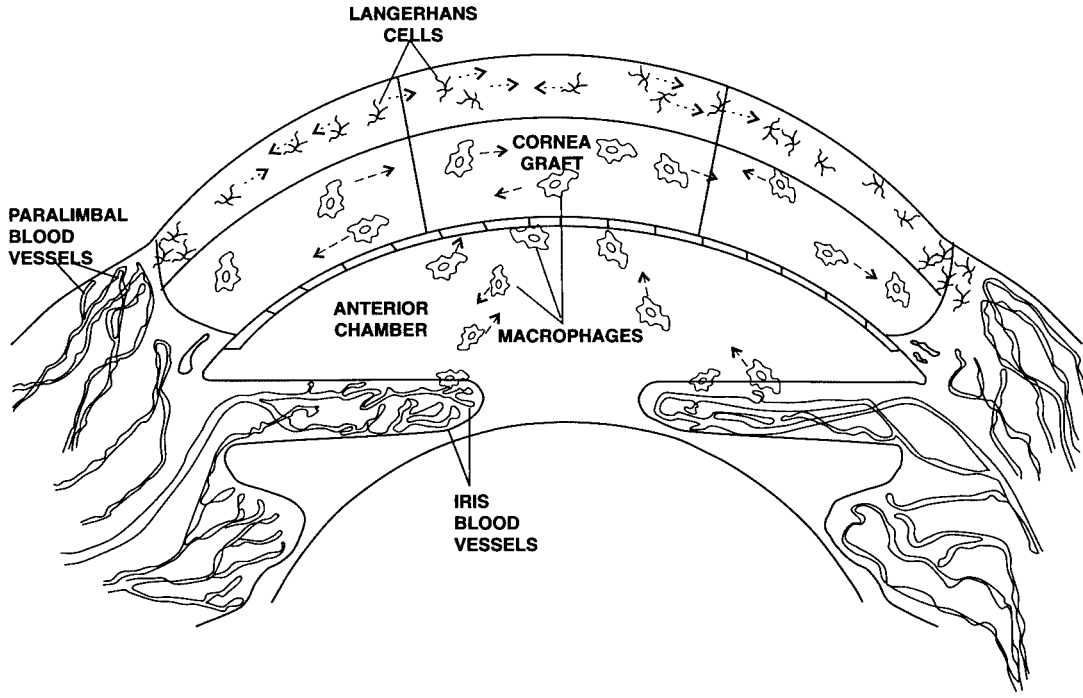

Fig. 9. The process of active transport of corneal allograft antigens from the graft to the recipient's immune system is shown schematically. Langerhans cells in the recipient's epithelium migrate into the graft epithelium and collect donor MHC antigens which are then transported to the recipient's immune lymphoid tissue such as the lymph nodes and spleen. Similarly, macrophages from the paralimbal blood vessels migrate into the graft, phagocytose graft alloantigens, and return to the blood and lymphoid tissues for presentation of antigen to T lymphocytes. A third possibility is shown whereby macrophages might enter the eye from iris blood vessels into the anterior chamber, phagocytose and process graft alloantigens in the anterior chamber or on the corneal graft endothelium, and return with the processed antigens to the iris vessels and the recipient's immune system.

small. Thus, we regard the possibility of passive diffusion of MHC antigens from corneal grafts to distant lymphoid tissues as an unlikely scenario by which corneal allograft immunity is initiated.

Another possibility that should be considered regarding the initiation of antigraft immunity outside a corneal allograft is shown in Fig. 9. In this case, Langerhans cells residing in the limbal region of the graft recipient and macrophages from the blood migrate into the corneal allograft and phagocytose graft alloantigens. These Langerhans cells and macrophages then migrate out of the allograft to distant lymphoid tissues such as the spleen and lymph nodes where the graft alloantigens are processed and presented to T lymphocytes. If antigen presentation occurs in the lymphoid tissues, the activated T lymphocytes would migrate to the graft as a result of chemotactic signals provided by accessory immune cells including polymorphonuclear leukocytes, Langerhans cells, and macrophages in the graft. This scenario involving active transport of graft alloantigens to the lymphoid tissues for presentation to T lymphocytes is one route whereby the allograft immune process can be initiated. The possibility of antigen presenta-

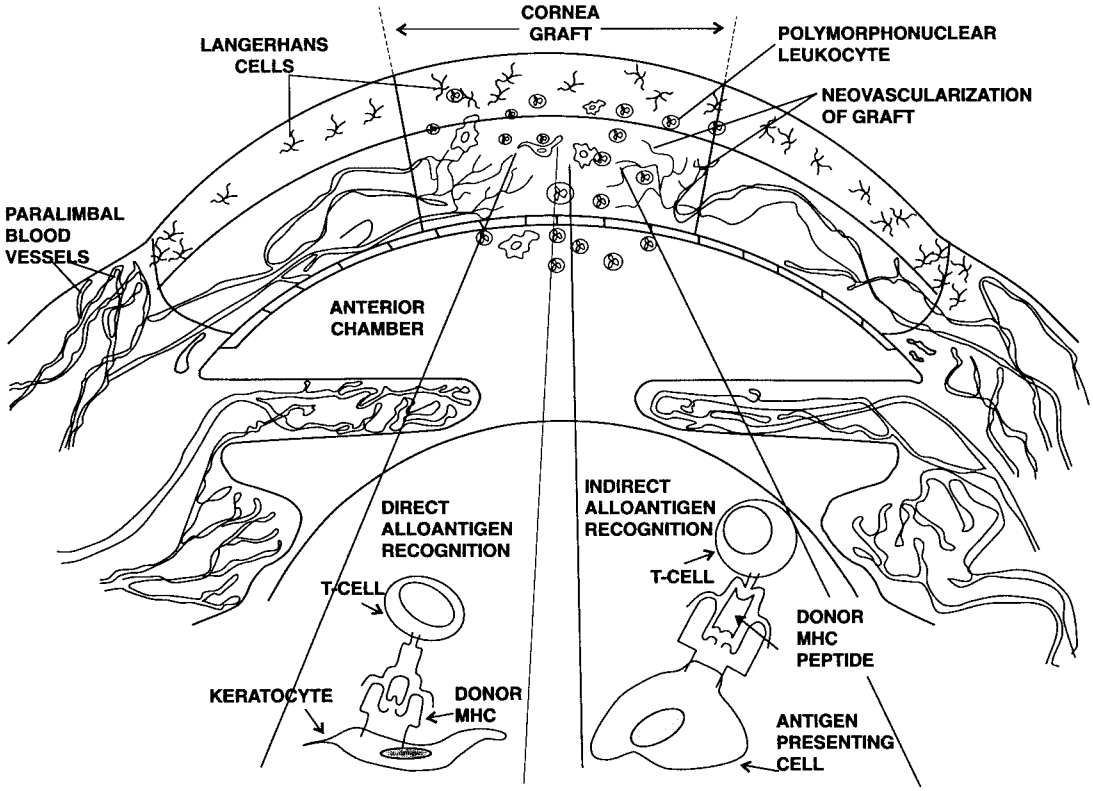

Fig. 10. The complexity of corneal alloantigen recognition and graft rejection is shown schematically. As depicted, direct alloantigen recognition could occur if corneal allograft cells express foreign MHC molecules which are recognized by T lymphocytes migrating into the graft. These T cells would then become activated and direct further immune attack on the graft. Alternatively, in the indirect recognition of alloantigen, antigen-presenting cells migrating into the allograft might process donor MHC antigens and present these antigens to lymphocytes entering the graft. In this instance, the recipient T cells would recognize self MHC and a donor MHC peptide. Direct or indirect alloantigen recognition may also occur outside of the graft in the lymphoid tissues. During rejection additional cells present in the graft include Langerhans cells, polymorphonuclear leukocytes, and macrophages. The neovascularization of the corneal allograft facilitates the entry of these cells into the graft.

tion *in situ* in the graft has not been ruled out however.

The rejection of a corneal allograft in rabbits and in other species in which this procedure has been performed has the features of acute, cell-mediated graft rejection. Corneal allograft rejection is a cell-mediated immune response involving T lymphocytes, macrophages, other inflammatory cells, and cytokines. There are no compelling data that demonstrate that antibodies alone can mediate corneal allograft rejection. Following corneal allografting into a vascularized graft site, histopathological analysis reveals acute inflammatory cells at the graft/host margins followed by the entry of both CD4+ and CD8+ T lymphocytes into the allograft stroma *(2,6,8)*. Numerous Langerhans cells, macro-

phages, neutrophils, and a few cells expressing B lymphocyte markers are also found in corneal allografts undergoing an acute rejection response. Thus, the cellular immune apparatus involved in primary corneal allograft rejection is identical to that which occurs in vascularized organ allografts undergoing a primary acute rejection episode.

In order to initiate the process of corneal allograft rejection, T lymphocytes must recognize graft alloantigens by either the direct or indirect alloantigen recognition pathways, or both *(63,151,152)* and respond to these antigens just as they do in the recognition of antigens in organ allografts (Fig. 10). As indicated, the direct or indirect recognition may occur outside the graft in the lymphoid tissues or within the graft. CD4+ T lymphocytes undergo lymphoblast transformation, proliferate, and produce a variety of cytokines, some of which mediate the maturation and proliferation of CD8 T cells and others that act as chemoattractants for neutrophils, macrophages, and Langerhans cells, which are necessary to complete the immune graft reaction process *(2,6,8)*. The CD8+ T lymphocytes recognize corneal alloantigens and in the presence of cytokines such as interleukin-2 and others made by the CD4+ T cells, these cells mature into cytotoxic effector cells. The CD8+ killer lymphocytes then are presumed to attack and kill corneal allograft cells as part of the rejection process. The role of inflammatory cells such as neutrophils, macrophages, and Langerhans cells which migrate into corneal allografts is to serve as accessory cells, producing the additional cytokines and chemokines needed to amplify the immune graft reaction. In addition, the neutrophils and macrophages phagocytose dead graft cells and other debris resulting from the immune rejection process. A role for natural killer cells and B lymphocytes, which are present in small numbers in corneal allografts during rejection, has not been delineated.

The mechanism(s) of immunologically mediated cell killing in corneal allograft rejection are not known. For example, the process of corneal cell alloantigen recognition may occur by either the direct or indirect pathways *(62)*. However, it is not known whether alloantigen recognition occurs in a corneal allograft or if corneal allograft antigens must be transported by antigen-presenting cells to regional lymph nodes and spleen (Fig. 9). It is possible that both local processing and presentation of corneal alloantigens and also distant processing and presentation in the lymphoid tissues occur. A second major gap in our knowledge regarding corneal allograft rejection is the actual mechanism of graft cell killing. It is abundantly clear that corneal allografts undergoing immune attack are populated by CD4+ and CD8+ T lymphocytes, as well as accessory cells such as macrophages, Langerhans cells, and neutrophils *(91)*. Both CD4+ and CD8+ T lymphocytes expressing activation markers are found in grafts during rejection indicating that both T helper and T cytotoxic cells are involved in the process. However, the precise immune mechanisms whereby these cells destroy corneal allograft cells is not known.

Attempts to implicate individual immune cell subsets in corneal allograft rejection have led some investigators to conclude that macrophages play a major role in graft cell destruction *(153–156)*. There is also evidence that CD4 lymphocytes are key mediators of corneal allograft rejection *(48,157–163)*. In other studies a role for CD8+ T lymphocytes was suggested *(7,31,91)*. Given this seemingly conflicting evidence and the cellular complexity of corneal allograft immune reactions, we conclude that the process is a multicellular one that involves cytokine-producing

T lymphocytes of the CD4 subset, cytotoxic CD8 lymphocytes, Langerhans cells, activated macrophages, and neutrophils all of which contribute to the immune and inflammatory aspects of the graft rejection process. Thus, the evidence points to cornea allograft rejection as a cellular immunopathologic process much the same as occurs in organ allograft rejection mediated by antigen-specific lymphocytes and cytokine-activated accessory cells.

Inhibition of Corneal Allograft Rejection: Drugs, Immunomodulators, and Manipulation of the Immune Response

Topically applied corticosteroids are used clinically to inhibit inflammation of corneal allografts in the days immediately following transplantation and to prevent acute graft rejection during the weeks and months following transplantation *(149)*; this approach has been the standard of therapy for use in corneal transplantation for over 40 yr. Within the past 10 yr, cyclosporine has been formulated for topical use as an alternative or adjunct to corticosteroids for preventing corneal allograft rejection in patients *(164,165)*. Notwithstanding refinements in the use of topical immunosuppressive drugs, corneal transplants performed in high risk situations in patients are likely to be subjected to immune attack, if not immune destruction *(158,166, 167)*. Some clinicians resort to the use of both topical and systemic immunosuppressive drugs in high risk patients but even with this aggressive therapy many grafts are rejected *(168–171)*. There is a clear need for alternative approaches and therapies to prevent corneal allograft rejection in high risk situations.

In experimental animals, many alternative therapies and attempts to manipulate the immune response have been tested. Examples in which immunomodulators have been used to alter the response to a corneal allograft are discussed below.

There is a wealth of information dating back to the 1960s on the phenomenon of immunological enhancement and the possibility of protecting cell and tissue allografts by "enhancing" an allograft *(172,173)*. Years of experimentation led to the conclusion that immunological enhancement is mediated by antibodies directed against graft antigens although the mechanism of enhancement still eludes us *(174)*. Twenty-five years ago Chandler, et al. and Binder, et al. *(177)* investigated the use of antibody-mediated enhancement as a means of protecting corneal allografts from acute rejection. Serum from guinea pigs immunized with rabbit blood leukocytes was purified and the immune globulin was shown to contain antibodies which would bind to both rabbit leukocytes and cornea cells. Next, rabbit corneas were incubated in the guinea pig immune or nonimmune globulins and then the corneas were transplanted into allogeneic rabbit hosts. The immune globulin preparation was found to significantly prolong the survival of corneal allografts placed in allogeneic recipients whereas nonimmune globulin had no effect *(175–177)*. Recently we produced a goat anti-rabbit immune globulin preparation which was enzymatically digested to remove the Fc portion of the globulin so as to prevent it from fixing complement. It was demonstrated that the $F(ab')_2$ portions of the immune globulin would bind to rabbit corneal cells in an immunologically specific way *(178)*. Nonimmune goat globulin or nonimmune $F(ab')_2$ fragments did not bind to rabbit corneal cells. Using radiolabeled immune and nonimmune $F(ab')_2$ fractions, it was shown that the binding of the immune globulin $F(ab')_2$ fragments occurred at both 4°C and 32°C. However, the half-life of association of the $F(ab')_2$ fragments with a rabbit cornea was approx 10 times longer at 4°C than at 32°C. In vivo, the immune

globulin $F(ab')_2$ fraction prolonged the survival of corneal allografts significantly beyond the time at which the radiolabeled $F(ab')_2$ molecules were found to be associated with the corneal allograft. This work was performed in a high risk situation in which the recipient graft bed had been vascularized prior to transplantation, and thus prolongation of corneal allograft survival by the enhancing $F(ab')_2$ molecules was concluded to be a powerful modulator of the antigraft response *(178)*.

We have also studied a costimulatory molecule blocking agent as a means of prolonging corneal allograft survival in rabbits *(179)* and mice (unpublished data). CTLA4 is a homolog of the CD28 molecule, both of which are expressed by T lymphocytes. These two homologs interact with the B71 and B72 coreceptors expressed on antigen-presenting cells. The binding affinity of CTLA4 for the B7 molecules is higher than that of CD28 *(180,181)*. Isolation and cloning of the cDNA for CTLA4 enabled investigators to create a soluble CTLA4 molecule which blocked T lymphocyte costimulation by antigen-presenting cells *(180,181)*. Linsley, et al. genetically engineered the CTLA4 gene so that a soluble fusion protein consisting of the CTLA4 moiety and an immunoglobulin Fc fragment was produced *(182)*. CTLA4-Fc, or CTLA4-Ig as it is often called, has been shown to prevent experimental organ allograft rejection in a variety of animals and organ and tissue transplantation experiments *(183–192)*. We have recently shown that incubation of rabbit corneal allografts in CTLA4-Ig followed by transplantation into vascularized recipient graft beds results in significantly prolonged survival of the grafts compared to control allografts *(179)*. We have also tested CTLA4-Ig in the mouse. C57BL/6 corneas were incubated in CTLA4-Ig for 18 h, transplanted into MHC mismatched BALB/c recipients, and the grafts observed daily for six weeks. As indicated above, all corneal allografts exchanged between C57BL/6 and BALB/c mice fail. When the C57BL/6 grafts were incubated in CTLA4-Ig the grafts survived indefinitely (unpublished data). Thus approaches involving enhancing antibodies and CTLA4-Ig may afford great promise for the future of corneal transplantation in patients.

Other approaches have been tested in experimental animals to attempt to prevent corneal allograft rejection. For example, an interleukin-2-PE-40 fusion protein consisting of T cell growth factor and the *Pseudomonas* exotoxin was given intraperitoneally to rats receiving corneal allografts *(193)*. Prolongation of corneal allograft survival was achieved. Whether or not this approach will have clinical application remains to be determined.

Recently it has been shown that the elimination of macrophages in the conjunctiva of the eye results in prolonged corneal allograft survival in rats *(153–156)*. Dichloromethylene diphosphate-containing liposomes were injected into the subconjunctiva of the eye. Phagocytosis of the liposomes by macrophages kills the cells and prolongation of corneal allograft survival was reported *(153,155)*.

It has also been reported that oral feeding of alloantigens alters alloantigen presentation such that the animals become at least transiently immunologically tolerant to the alloantigen and accept the corneal allografts for extended periods of time *(194–197)*. The approach of oral presentation of alloantigens and autoantigens may have a promising future if the dose, route, and timing of administration can be clarified. There are also studies indicating that treatment of corneal allografts with hyperbaric oxygen or ultraviolet light in vitro prior to transplantation promotes the survival of allografts *(198–204)*, possibly as a result of the elimination of Langerhans cells in the graft. Paradoxically, the number of these cells in a central corneal graft is vanishingly

small and therefore it is not clear why these treatments have a significant effect.

Summary and Conclusion

More than 40,000 corneal transplants are performed annually in the United States. Although many of these corneal transplants restore vision to a previously blind eye, the number that fail due to immunologic rejection warrants continued study of corneal transplantation in experimental animals. Since we now appreciate that the immunologic privilege of the cornea is tenuous and limited to the avascular and uninflamed cornea, it will be possible to focus on the development of effective, safe, and practical therapies which incorporate topical medications that block neovascularization *(205)* and T-lymphocyte activation *(180)* to prevent corneal allograft rejection. By studying experimental corneal transplantation in animals we will be able to refine and perfect methods for preventing rejection.

Acknowledgment

Supported in part by: NIH/NEI CORE Grant EY02377 and an unrestricted departmental grant from Research to Prevent Blindness, Inc., New York, NY.

References

1 Polack FM: Histopathological and histochemical alterations in the early stages of corneal graft rejection. J Exp Med 1962;116:709–717.
2 Kanai A, Polack FM: Ultramicroscopic changes in the corneal graft stroma during early rejection. Invest Ophthalmol 1971;10:415–423.
3 Polack FM, Kanai A: Electron microscopic studies of graft endothelium in corneal graft rejection. Am J Ophthalmol 1972;73: 711–717.
4 Gronemeyer U, Pulhorn G, Muller-Ruchholtz W: Allogeneic corneal grafting in inbred strains of rats. Albrecht v Graefes Arch Klin Exp Ophthalmol 1978;208:247–262.
5 She S-C, Steahly LP, Moticka EJ: A method for performing full-thickness, orthotopic, penetrating keratoplasty in the mouse. Ophthalmic Surg 1990;21:781–785.
6 Holland EJ, Chan C-C, Wetzig RP, Palestine AG, Nussenblatt RB: Clinical and immunohistologic studies of corneal rejection in the rat penetrating keratoplasty model. Cornea 1991;10:374–380.
7 Holland EJ, Olsen TW, Chan C-C, Bergstrom L, Palestine AG, Nussenblatt RB: Kinetics of corneal transplant rejection in the rat penetrating keratoplasty model. Cornea 1994;13:317–323.
8 Larkin DFP, Calder VL, Lightman SL: Identification and characterization of cells infiltrating the graft and aqueous humour in rat corneal allograft rejection. Clin Exp Immunol 1997;107:381–391.
9 Maumenee AE: The influence of donor-recipient sensitization on corneal grafts. Am J Ophthalmol 1951;34:142–152.
10 Chen YF, Gebhardt BM, Reidy JJ, Kaufman HE: Cyclosporine-containing collagen shields suppress corneal allograft rejection. Am J Ophthalmol 1990;109:132–137.
11 Hill JC, Maske R: An animal model for corneal graft rejection in high-risk keratoplasty. Transplantation 1988;46:26–30.
12 Medawar PB: Immunity to homologous grafted skin. III. The fate of skin homografts transplanted to the brain, to subcutaneous tissue, and to the anterior chamber of the eye. Br J Exp Pathol 1948;29:58–69.
13 Billingham RE, Boswell T: Studies on the problem of corneal homografts. Proc R Soc B 1953;141: 392–406.
14 Polack FM: The effect of ocular inflammation on corneal grafts. Am J Ophthalmol 1965;60: 259–269.
15 Inomata H, Smelser GK, Polack FM: Corneal vascularization in experimental uveitis and graft rejection. An electron microscopic study. Invest Ophthalmol 1971;10: 840–850.
16 Khodadoust AA, Silverstein AM: Studies on the nature of the privilege enjoyed by corneal allografts. Invest Ophthalmol 1972;11: 137–148.
17 Khodadoust AA: Penetrating keratoplasty in the rabbit. Am J Ophthalmol 1968;66:899–905.
18 Khodadoust AA, Silverstein AM: The survival and rejection of epithelium in experimental corneal transplants. Invest Ophthalmol 1969;8:169–179.
19 Salisbury JD, Gebhardt BM: Suppression of corneal allograft rejection by cyclosporin A. Arch Ophthalmol 1981;99:1640–1643.
20 Newton C, Gebhardt BM, Kaufman HE: Topically applied cyclosporine in Azone prolongs corneal allograft survival. Invest Ophthalmol Vis Sci 1988;29:208–215.
21 Guo A, Ohia E, Xu J, Bhattacherjee P, Kulkarni P: Effects of anti-inflammatory and immuno-

suppressive drugs on the heterolamellar corneal transplantation in rabbits. Curr Eye Res 1990;9: 749–757.
22 Gebhardt BM, Varnell ED, Kaufman HE: Cyclosporine in collagen particles: corneal penetration and suppression of allograft rejection. J Ocul Pharmacol Thera 1995; 11:509–517.
23 Lang RF, Riekhof FT, Steinmuller D: Interlamellar corneal grafts in rats. Effect of histocompatibility. Arch Ophthalmol 1975;93: 349–353.
24 Gebhardt BM: Factors affecting corneal allograft rejection in inbred rats. Transplant Proc XIII:1981; 1091–1093.
25 Treseler PA, Treseler CB, Foulks GN, Sanfilippo F: Allospecific cellular and humoral immunity to rat corneal transplants. Transplant Proc 1985;17:789–791.
26 Treseler PA, Sanfilippo F: Humoral immunity to heterotopic corneal allografts in the rat. Transplantation 1985;39:193–1201.
27 Treseler PA, Sanfilippo F: The expression of major histocompatibility complex and leukocyte antigens by cells in the rat cornea. Transplantation 1985;41:248–252.
28 Treseler PA, Sanfilippo F: Relative contribution of major histocompatibility complex antigens to the immunogenicity of corneal allografts. Transplantation 1985;41: 508–514.
29 Williams KA, Coster DJ: Penetrating corneal transplantation in the inbred rat: A new model. Invest Ophthalmol Vis Sci 1985;26: 23–30.
30 Treseler PA, Foulks GN, Sanfilippo F: The relative immunogenicity of corneal epithelium, stroma, and endothelium. Transplantation 1986;41:229–234.
31 Callanan D, Peeler J, Niederkorn JY: Characteristics of rejection of orthotopic corneal allografts in the rat. Transplantation 1988;45: 437–443.
32 Katami M, Madden PW, White DJ, Watson PG, Kamada N: The extent of immunological privilege of orthotopic corneal grafts in the inbred rat. Transplantation 1989; 48:371–376.
33 Katami M, Lim SML, Kamada N, Davies HFFS, Butcher GW, White DJG, Watson PG, Calne RY: A pure class II MHC disparity does not induce rejection of cornea or heart grafts in the rat. Transplant Proc 1990;22:2200–2201.
34 Ross J, Callanan D, Kunz H, Niederkorn J: Evidence that the fate of class II-disparate corneal grafts is determined by the timing of class II expression. Transplantation 1991;51:532–536.
35 Katami M, Graudenz MS, White DJG, Watson PG: The role of antigen-presenting cells in rat corneal graft rejection. Transplant Proc 1991;23:93–95.
36 Ross J, He Y-G, Pidherney M, Mellon J, Niederkorn JY: The differential effects of donor versus host Langerhans cells in the rejection of MHC-matched corneal allografts. Transplantation 1991;52: 857–861.
37 Torres PF, De Vos AF, Van Der Gaag R, Martins B, Kijlstra A: Cytokine mRNA expression during experimental corneal allograft rejection. Exp Eye Res 1996; 63:453–461.
38 King WJ, Comer RM, Hudde T, Larkin DFP, George AJT: Cytokine and chemokine expression kinetics after corneal transplantation. 2000;70:1225–1233.
39 Ross JR, Sanfilippo FP, Howell DN: Histopathologic features of rejecting orthotopic corneal xenografts. Curr Eye Res 1994;13: 725–730.
40 Takano T, Williams KA: Mechanism of corneal endothelial destruction in rejecting rat corneal allografts and xenografts: a role for CD4+ cells. Transplant Proc 1995; 27:260–261.
41 Torres PF, Slegers TPAM, Peek R, van Rooijen N, van der Gaag R, Kijlstra A, de Vos AF: Changes in cytokine mRNA levels in experimental corneal allografts after local clodronate-liposome treatment. Invest Ophthalmol Vis Sci 1999;40:3194–3201.
42 Peeler JS, Callanan DG, Luckenbach MW, Niederkorn JY: Presentation of the H-Y antigen on Langerhans' cell-negative corneal grafts downregulates the cytotoxic T cell response and converts responder strain mice into phenotypic nonresponders. J Exp Med 1988;168:1749–1766.
43 Joo C-K, Pepose JS, Stuart PM: T-cell mediated responses in a murine model of orthotopic corneal transplantation. Invest Ophthalmol Vis Sci 1995;36:1530–1540.
44 Sonoda Y, Sano Y, Ksander B, Streilein JW: Characterization of cell-mediated immune responses elicited by orthotopic corneal allografts in mice. Invest Ophthalmol Vis Sci 1995;36:427–434.
45 Holan V, Haskova Z, Filipec M: Transplantation immunity and tolerance in the eye. Rejection and acceptance of orthotopic corneal allografts in mice. Transplantation 1996;62:1050–1054.
46 Yamada J, Kurimoto I, Streilein JW: Role of CD4+ cells in immunobiology of orthotopic corneal transplants in mice. Invest Ophthalmol Vis Sci 1999;40: 2614–2621.
47 Yamagami S, Tsuru T: Increase in orthotopic murine corneal transplantation rejection rate with anterior synechiae. Invest Ophthalmol Vis Sci 1999;40:2422–2426.
48 Haskova Z, Usiu N, Pepose JS, Ferguson TA, Stuart PM: CD4+ T cells are critical for corneal, but not skin, allograft rejection. Transplantation 2000;69:483–487.
49 Yamagami S, Obata H, Tsuru T, Isobe M: Suppression of corneal allograft rejection after penetrating keratoplasty by antibodies to ICAM-1 and LFA-1 in mice. Transplant Proc 1995;27:1899–1900.
50 Haskova Z, Filipec M, Holan V: The role of major and minor histocompatibility antigens in orthotopic corneal transplantation in mice. Folia Biologica 1996;42:105–111.
51 Yamagami S, Isobe M, Yamagami H, Hori J, Tsuru T: Mechanism of concordant corneal xenograft rejection in mice. Transplantation 1997;64:42–48.
52 Hori J, Isobe M, Yamagami S, Mizuochi T, Tsuru T: Specific immunosuppression of corneal allograft rejection by combination of anti-VLA-4 and anti-LFA-1 monoclonal antibodies in mice. Exp Eye Res 1997;65:89–98.

53 Yamagami S, Kawashima H, Tsuru T, Yamagami H, Kayagaki N, Yagita H, Okumura K, Gregerson DS: Role of Fas-Fas ligand interactions in the immunorejection of allogeneic mouse corneal transplants. Transplantation 1997;64:1107–1111.
54 Yamagami S, Kawashima H, Endo H, Tsuru T, Shibui H, Kagawa Y, Hori J, Yamagami H, Isobe M: Cytokine profiles of aqueous humor and graft in orthotopic mouse corneal transplantation. Transplantation 1998;66:1504–1510.
55 Lau CH, Nicholls SM, Easty DL: A murine model of interlamellar corneal transplantation. Br J Ophthalmol 1998;82:294–299.
56 Kamiya K, Hori J, Obata H, Yamagami S, Tsuru T: Incidence of allograft rejection after corneal transplantation using Optisol-preserved corneas in mice. Transplant Proc 1999;31:2673–2674.
57 Kamiya K, Hori J, Kagaya F, Usui T, Amano S, Oshika T, Mizouchi T, Tsuru T, Yamagami S: Preservation of donor cornea prevents corneal allograft rejection by inhibiting induction of alloimmunity. Exp Eye Res 2000;70:737–743.
58 Niederkorn JY, Callanan D, Ross JR: Prevention of the induction of allospecific cytotoxic T lymphocyte and delayed-type hypersensitivity responses by ultraviolet irradiation of corneal allografts. Transplantation 1990;50:281–286.
59 Niederkorn JY, Mellon J: Anterior chamber-associated immune deviation promotes corneal allograft survival. Invest Ophthalmol Vis Sci 1996;37:2700–2707.
60 Sano Y, Ksander BR, Streilein JW: Fate of orthotopic corneal allografts in eyes that cannot support anterior chamber-associated immune deviation induction. Invest Ophthalmol Vis Sci 1995; 36:2176–2185.
61 Sano Y, Ksander BR, Streilein JW: Murine orthotopic corneal transplantation in high-risk eyes. Rejection is dictated primarily by weak rather than strong alloantigens. Invest Ophthalmol Vis Sci 1997; 38:1130–1138.
62 Sano Y, Ksander BR, Streilein JW: Langerhans cells, orthotopic corneal allografts, and direct and indirect pathways of T-cell allorecognition. Invest Ophthalmol Vis Sci 2000;41:1422–1431.
63 Khodadoust AA, Silverstein AM: Transplantation and rejection of individual cell layers of the cornea. Invest Ophthalmol 1969;8: 180–195.
64 Malinowski K, Tsai D, Manski W: The relative capacity of corneal, heart, kidney and skin cells to stimulate allogeneic lymphocytes. Immunol Commun 1979;8: 303–311.
65 Guymer RH, Mandel TE: A comparison of corneal, pancreas, and skin grafts in mice. Transplantation 1994;57:1251–1262.
66 Gebhardt BM, Seto SK: The immunogenicity of corneal stromal keratocytes. Transplantation 1988; 46:444–448.
67 Gebhardt BM: The effect of tissue dose and site of grafting on immunity to corneal allografts. Invest Ophthalmol Vis Sci 1988;29: 1663–1670.
68 Nicholls SM, Bradley BB, Easty DL: Effect of mismatches for major histocompatibility complex and minor antigens on corneal graft rejection. Invest Ophthalmol Vis Sci 1991;32:2729–2734.
69 Sonoda Y, Streilein JW: Orthotopic corneal transplantation in mice—evidence that the immunogenetic rules of rejection do not apply. Transplantation 1992;54:694–704.
70 Nicholls SM, Bradley BA, Easty DL: Apparent resistance to immunosuppression of MHC-matched corneal transplants. Transplantation 1995;59:325–329.
71 Bradley BA, Vail A, Gore SM, Rogers CA, Armitage WJ, Nicholls S, Easty DL: Negative effect of HLA-DR matching on corneal transplant rejection. Transplant Proc 1995;27:1392–1394.
72 Gore SM, Vail A, Bradley BA, Rogers CA, Easty DL, Armitage WJ: HLA-DR matching in corneal transplantation. Transplantation 1995;60:1033–1039.
73 Forrester D: Non-HLA antigens and HLA-DR matching in corneal transplantation. Br J Ophthalmol 1996;80:780–783.
74 Graff RJ: Minor histocompatibility genes and their antigens. In: Origins of Inbred Mice, Morse HC III, editor. Academic Press, 1978, pp. 371–389.
75 Brown J, Soderstrom CW, Winkelmann RK: Langerhans' cells in guinea pig cornea: response to chemical injury. Invest Ophthalmol 1968;7:668–671.
76 Streilein JW, Toews GB, Bergstresser PR: Corneal allografts fail to express Ia antigens. Nature 1979;282:326–327.
77 Bergstresser PR, Fletcher CR, Streilein JW: Surface densities of Langerhans cells in relation to rodent epidermal sites with special immunologic properties. J Invest Dermatol 1980;74:77–80.
78 Streilein JW, Toews GB, Bergstresser PR: Langerhans cells: functional aspects revealed by *in vivo* grafting studies. J Invest Dermatol 1980;75:17–21.
79 Gillette TE, Chandler JW: Immunofluorescence and histochemistry of corneal epithelial flat mounts: use of EDTA. Curr Eye Res 1981;1: 249–253.
80 Gillette TE, Chandler JW, Greiner JV: Langerhans cells of the ocular surface. Ophthalmology 1982;89: 700–711.
81 Hazlett LD, Grevengood C, Berk RS: Change with age in limbal conjunctival Langerhans cells. Curr Eye Res 1982;2:423–425.
82 Braude LS, Chandler JW: Corneal allograft rejection. The role of the major histocompatibility complex. Surv Ophthalmol 1983;27: 290–305.
83 Rodrigues MM, Rowden G, Hackett J, Bakos I: Langerhans cells in the normal conjunctiva and peripheral cornea of selected species. Invest Ophthalmol Vis Sci 1981; 21:759–765.
84 Choudhury A, Pakalnis VA, Bowers WE: Function and cell surface phenotype of dendritic cells from rat cornea. Invest Ophthalmol Vis Sci 1995;36:2602–2613.
85 Rubsamen PE, McCulley J, Bergstresser PR, Streilein JW: On the Ia immunogenicity of mouse corneal allografts infiltrated with Langerhans cells. Invest Ophthalmol Vis Sci 1984;25:513–518.
86 Ray-Keil L, Chandler LW: Rejection of murine heterotopic corneal

transplants. Transplantation 1985; 39:473–477.
87 Peeler JS, Niederkorn JY: Effect of Langerhans' cells on cytotoxic T lymphocyte responses to major and minor alloantigens expressed on heterotopic corneal allografts. Transplant Proc 1987; 19 (part 1): 316–319.
88 Griffith TS, Brunner T, Fletcher SM, Green DR, Ferguson TA: Fas ligand-induced apoptosis as a mechanism of immune privilege. Science 1995;270:1189–1192.
89 Hori J, Joyce N, Streilein JW: Epithelium-deficient corneal allografts display immune privilege beneath the kidney capsule. Invest Ophthalmol Vis Sci 2000;41: 443–451.
90 Hori J, Joyce NC, Streilein JW: Immune privilege and immunogenicity reside among different layers on the mouse cornea. Invest Ophthalmol Vis Sci 2000;41: 3032–3042.
91 Wackenheim-Urlacher A, Kantelip B, Falkenrodt A, Piqot X, Tongio M-M, Montard M, Delbose B: T-cell repertoire of normal, rejected, and pathological corneas: phenotype and function. Cornea 1995;14:450–456.
92 Kang S-M, Schneider DB, Lin Z, Hanahan D, Dichek DA, Stock PG, Baekkeskov S: Fas ligand expression in islets of Langerhans does not confer immune privilege and instead targets them for rapid destruction. Nature Med 1997;3: 738–743.
93 Kaplan HJ, Streilein JW: Immune response to immunization via the anterior chamber of the eye. II. An analysis of F1 lymphocyte-induced immune deviation. J Immunol 1978;120:689–693.
94 Niederkorn J, Streilein JW, Shadduck JA: Deviant immune responses to allogeneic tumors injected intracamerally and subcutaneously in mice. Invest Ophthalmol Vis Sci 1981;20: 355–363.
95 Niederkorn JY, Streilein JW: Analysis of antibody production induced by allogeneic tumor cells inoculated into the anterior chamber of the eye. Transplantation 1982;33:573–577.
96 Whittum JA, Niederkorn JY, McCulley JP, Streilein JW: Intracameral inoculation of herpes simplex virus type I induces anterior chamber associated immune deviation. Curr Eye Res 1983;2: 691–697.
97 Niederkorn JY, Streilein JW: Alloantigens placed into the anterior chamber of the eye induce specific suppression of delayed-type hypersensitivity but normal cytotoxic T lymphocyte and helper T lymphocyte responses. J Immunol 1983;131:2670–2674.
98 Mizuno K, Clark AF, Streilein JW: Induction of anterior chamber associated immune deviation in rats receiving intracameral injections of retinal S antigen. Curr Eye Res 1988;7:627–632.
99 Benson JL, Niederkorn JY: Immune privilege in the anterior chamber of the eye: alloantigens and tumour-specific antigens presented into the anterior chamber simultaneously induce suppression and activation of delayed hypersensitivity to the respective antigens. Immunology 1992;77:189–195.
100 Whittum JA, Niederkorn JY, McCulley JP, Streilein JW: Role of suppressor T cells in herpes simplex virus-induced immune deviation. J Virol 1984;51:556–558.
101 Niederkorn JY, Waltenbaugh C, Streilein JW: Abrogation of anterior-chamber-induced suppression of delayed-type hypersensitivity responses by monoclonal anti-*I-J* antibodies. Transplantation 1984;37:623–625.
102 Williamson JSP, Streilein JW: Impaired induction of delayed hypersensitivity following anterior chamber inoculation of alloantigens. Regional Immunol 1988;1: 15–23.
103 Wilbanks GA, Streilein JW: Characterization of suppressor cells in anterior chamber-associated immune deviation (ACAID) induced by soluble antigen. Evidence of two functionally and phenotypically distinct T-suppressor cell populations. Immunology 1990;71:383–389.
104 Williamson JSP, Bradley D, Streilein JW: Immunoregulatory properties of bone marrow-derived cells in the iris and ciliary body. Immunology 1989;67:1–8.
105 Okamoto S, Hara Y, Streilein JW: Induction of anterior chamber-associated immune deviation with lymphoreticular allogeneic cells. Transplantation 1995;59:377–381.
106 Apte RS, Sinha D, Mayhew E, Wistow GJ, Niederkorn JY: Cutting edge: role of macrophage migration inhibitory factor in inhibiting NK cell activity and preserving immune privilege. J Immunol 1998;160:5693–5696.
107 Niederkorn JY, Mayhew E: Role of splenic B cells in the immune privilege of the anterior chamber of the eye. Eur J Immunol 1995; 25:2783–2787.
108 Williamson JSP, Streilein JW: Induction of delayed hypersensitivity to alloantigens coinjected with Langerhans cells into the anterior chamber of the eye. Transplantation 1989;47:519–524.
109 Ksander BR, Bando Y, Acevedo J, Streilein JW: Infiltration and accumulation of precursor cytotoxic T-cells increased with time in progressively growing ocular tumors. Cancer Res 1991;51:3153–3158.
110 Benson JL, Niederkorn JY: *In situ* suppression of delayed-type hypersensitivity: another mechanism for sustaining the immune privilege of the anterior chamber. Immunology 1991;74:153–159.
111 Kosiewicz MM, Streilein JW: Intraocular injection of class II-restricted peptide induces an unexpected population of CD8 regulatory cells. J Immunol 1996;157:1905–1912.
112 Kezuka T, Streilein JW: In vitro generation of regulatory $CD8^+$ T cells similar to those found in mice with anterior chamber-associated immune deviation. Invest Ophthalmol Vis Sci 2000; 41:1803–1811.
113 Bando Y, Ksander BR, Streilein JW: Characterization of specific T helper cell activity in mice bearing alloantigenic tumors in the anterior chamber of the eye. Eur J Immunol 1991;21:1923–1931.
114 Li X-Y, D'Orazio T, Niederkorn JY: Role of Th1 and Th2 in anterior chamber-associated immune deviation. Immunology 1996;89: 34–40.

115 Takahashi M, Ishimaru N, Yanagi K, Saegusa K, Haneji N, Shiota H, Hayashi Y: Requirement for splenic CD4+ T cells in the immune privilege of the anterior chamber of the eye. Clin Exp Immunol 1999;116:231–237.
116 Cousins SW, McCabe MM, Danielpour D, Streilein JW: Identification of transforming growth factor-beta as an immunosuppressive factor in aqueous humor. Invest Ophthalmol Vis Sci 1991; 32:2201–2211.
117 Wilbanks GA, Streilein JW: Fluids of immune privileged sites endow macrophages with the capacity to induce antigen-specific immune deviation via a mechanism involving transforming growth factor-β. Eur J Immunol 1992;22: 1031–1036.
118 Taylor AW, Alarad P, Ye DG, Streilein JW: Aqueous humor induces transforming growth factor-β (TGF-β)-producing regulatory T-cells. Curr Eye Res 1997; 16:900–908.
119 Takeuchi M, Alard P, Streilein JW: TGF-β promotes immune deviation by altering accessory signals of antigen-presenting cells. J Immunol 1998;160:1589–1597.
120 Kosiewicz MM, Alard P, Streilein JW: Alterations in cytokine production following intraocular injection of soluble protein antigen: impairment in IFN-γ and induction of TGF-β and IL-4 production. J Immunol 1998;161: 5382–5390.
121 Ohta K, Wiggert B, Yamagami S, Taylor AW, Streilein JW: Analysis of immunomodulatory activities of aqueous humor from eyes of mice with experimental autoimmune uveitis. J Immunol 2000;164: 1185–1192.
122 Kezuka T, Streilein JW: Analysis of in vivo regulatory properties of T cells activated in vitro by TGFβ2-treated antigen presenting cells. Invest Ophthalmol Vis Sci 2000; 41:1410–1421.
123 Ohta K, Yamagami S, Taylor AW, Streilein JW: IL-6 antagonizes TGF-β and abolishes immune privilege in eyes with endotoxin-induced uveitis. Invest Ophthalmol Vis Sci 2000;41:2591–2599.
124 Benson JL, Niederkorn JY: Interleukin-1 abrogates anterior chamber-associated immune deviation. Invest Ophthalmol Vis Sci 1990; 31:2123–2128.
125 Dana MR, Dai R, Zhu S, Yamada J, Streilein JW: Interleukin-1 receptor antagonist suppresses Langerhans cell activity and promotes ocular immune privilege. Invest Ophthalmol Vis Sci 1998; 39:70–77.
126 Niederkorn JY: Exogenous recombinant interleukin-2 abrogates anterior-chamber-associated immune deviation. Transplantation 1987;43:523–528.
127 D'Orazio TJ, Niederkorn JY: A novel role for TGF-β and IL-10 in the induction of immune privilege. J Immunol 1998;160:2089–2098.
128 Streilein JW, Cousins S, Bradley D: Effect of intraocular gamma-interferon on immunoregulatory properties of iris and ciliary body cells. Invest Ophthalmol Vis Sci 1992;33:2304–2315.
129 Geiger K, Sarvetnick N: Local production of IFN-γ abrogates the intraocular immune privilege in transgenic mice and prevents the induction of ACAID. J Immuol 1994;153:5239–5246.
130 Ferguson TA, Herndon JM, Dube P: The immune response and the eye: a role for TNFα in anterior chamber-associated immune deviation. Invest Ophthalmol Vis Sci 1994;35:2643–2651.
131 Ferguson TA, Fletcher S, Herndon J, Griffith TS: Neuropeptides modulate immune deviation induced via the anterior chamber of the eye. J Immunol 1995;155:1746–1756.
132 Knisely TL, Hosoi J, Nazareno R, Granstein RD: The presence of biologically significant concentrations of glucocorticoids but little or no cortisol binding globulin within aqueous humor: relevance to immune privilege in the anterior chamber of the eye. Invest Ophthalmol Vis Sci 1994;35: 3711–3723.
133 Apte RS, Niederkorn JY: Isolation and characterization of a unique natural killer cell inhibitory factor present in the anterior chamber of the eye. J Immunol 1996;156: 2667–2673.
134 Ferguson TA, Hayashi JD, Kaplan HJ: The immune response and the eye. III. Anterior chamber-associated immune deviation can be adoptively transferred by serum. J Immunol 1989;143:821–826.
135 Wilbanks GA, Streilein JW: Studies on the induction of anterior chamber-associated immune deviation (ACAID). 1. Evidence that an antigen-specific, ACAID-inducing, cell-associated signal exists in the peripheral blood. J Immunol 1991;146:2610–2617.
136 Hadjikouti CA, Wang Y, O'Rourke J, Cone RE: Intracameral injection of antigen potentiates the production of antigen-specific T cell proteins in serum after the induction of delayed-type hypersensitivity. Invest Ophthalmol Vis Sci 1995; 36:1470–1476.
137 Streilein JW, Okamoto S, Hara Y, Kosiewicz M, Ksander B: Blood-borne signals that induce anterior chamber-associated immune deviation after intracameral injection of antigen. Invest Ophthalmol Vis Sci 1997;38:2245–2254.
138 Yamada J, Streilein JW: Induction of anterior chamber-associated immune deviation by corneal allografts placed in the anterior chamber. Invest Ophthalmol Vis Sci 1997;38:2833–2843.
139 Yamada J, Yoshida M, Taylor AW, Streilein JW: Mice with Th2-biased immune systems accept orthotopic corneal allografts placed in "high risk" eyes. J Immunol 1999;162:5247–5255.
140 Tanaka K, Yamada J, Joyce N, Streilein JW: Immunobiology of xenogenic cornea grafts in mouse eyes. Transplantation 2000;69: 610–616.
141 Ferguson TA, Griffith TS: A vision of cell death: insights into immune privilege. Immunol Rev 1997; 156:167–184.
142 Streilein JW: Anterior chamber associated immune deviation: the privilege of immunity in the eye. Surv Ophthalmol 1990;35:67–73.
143 Streilein JW, Wilbanks GA, Cousins SW: Immunoregulatory mechanisms of the eye. J Neuroimmunol 1992;39:185–200.
144 Streilein JW: Ocular immune privilege and the Faustian dilemma. The

Proctor Lecture. Invest Ophthalmol Vis Sci 1996;37:1940–1950.
145 Streilein JW: Immunoregulatory mechanisms of the eye. Prog Retin Eye Res 1999;18: 357–370.
146 Niederkorn JY: The immune privilege of corneal allografts. Transplantation 1999;67:1503–1508.
147 Streilein JW: Immunologic privilege of the eye. Springer Semin Immunopathol 1999;21:95–111.
148 Sonoda Y, Streilein JW: Impaired cell-mediated immunity in mice bearing healthy orthotopic corneal allografts. J Immunol 1993;150: 1727–1734.
149 Hodkin MJ: Complications of penetrating keratoplasty. In: The Cornea, Kaufman HE, Barron BA, McDonald MB, editors. Butterworth-Heinemann, Boston, 1998, pp 847–878.
150 Jones BR: Summing up. Present knowledge and problems of corneal graft failure. In: Corneal Graft Failure, Ciba Foundation Syposium. Elsevier, Amsterdam, 1973, pp 349–354.
151 Liu L, Sun YK, Xi YP, Maffei A, Reed E, Harris P, Suciu-Foca N: Contribution of direct and indirect recognition pathways to T cell alloreactivity. J Exp Med 1993; 177:1643–1650.
152 Shoskes DA, Wood KJ: Indirect presentation of MHC antigens in transplantation. Immunol Today 1994;15:32–38.
153 Van Der Veen G, Broersma L, Dijkstra CK, Van Rooijen N, Van Rij G, Van Der Gaag R: Prevention of corneal allograft rejection in rats treated with subconjunctival injections of liposomes containing dichloromethylene diphosphonate. Invest Ophthalmol Vis Sci 1994; 35:3505–3515.
154 Van der Veen G, Broersma L, Van Rooijen N, Van Rij G, Van der Gaag R: Cytotoxic T lymphocytes and antibodies after orthotropic penetrating keratoplasty in rats treated with dichloromethylene diphosphonate encapsulated liposomes. Curr Eye Res 1998;17: 1018–1026.
155 Slegers TPAM, van Rooijen N, van Rij G, van der Gaag R: Delayed graft rejection in pre-vascularized corneas after subconjunctival injection of clodronate liposomes. Curr Eye Res 2000;20: 322–324.
156 Slegers TPAM, Torres PF, Broersma L, van Rooijen N, van Rij G, van der Gaag R: Effect of macrophage depletion on immune effector mechanisms during corneal allograft rejection in rats. Invest Ophthalmol Vis Sci 2000; 41:2239–2247.
157 Duguid IGM, Koulmanda M, Mandel TE: Prolongation of heterotopic human corneal graft survival in mice treated with an anti-CD4 monoclonal antibody. Transplant Proc 1990;22: 2107–2108.
158 He YG, Ross J, Niederkorn JY: Promotion of murine orthotopic corneal allograft survival by systemic administration of anti-CD4 monoclonal antibody. Invest Ophthalmol Vis Sci 1991;32: 2723–2728.
159 Ayliffe W, Alam Y, Bell EB, McLeod D, Hutchinson IV: Prolongation of rat corneal graft survival by treatment with anti-CD4 monoclonal antibody. Br J Ophthalmol 1992;76:602–606.
160 Williams KA, Coster DJ: Monoclonal antibodies in corneal transplantation. Br J Ophthalmol 1992;76:577.
161 Takano T, Williams KA: Mechanism of corneal endothelial destruction in rejecting rat corneal allografts and xenografts: a role of CD4+ cells. Transplant Proc 1995; 27:260–261.
162 Pleyer U, Milani JK, Dukes A, Chou J, Lutz S, Ruckert D, Thiel H-J, Mondino BJ: Effect of topically applied anti-CD4 monoclonal antibodies on orthotopic corneal allografts in a rat model. Invest Ophthalmol Vis Sci 1996;35:52–61.
163 Tanaka K, Yamada J, Streilein JW: Xenoreactive CD4+ T cells and acute rejection of orthotopic guinea pig corneas in mice. Invest Ophthalmol Vis Sci 2000;41: 1827–1832.
164 Belin MW, Bouchard CS, Frantz S, Chmielinska J: Topical cyclosporine in high-risk corneal transplants. Ophthalmology 1989;96: 1144–1150.
165 Hill JC: The use of cyclosporine in high-risk keratoplasty. Am J Ophthalmol 1989;107:506–510.
166 Waldock A, Cook SD: Corneal transplantation: how successful are we? Br J Ophthalmol 2000;84: 813–815.
167 Dana MR, Qian Y, Hamrah P: Twenty-five-year panorama of corneal immunology. Emerging concepts in the immunopathogenesis of microbial keratitis, peripheral ulcerative keratitis, and corneal transplant rejection. Cornea 2000;19:625–643.
168 Hill JC, Maske R, Watson P: Corticosteroids in corneal graft rejection. Oral versus single pulse therapy. Ophthalmology 1991;98: 329–333.
169 Williams KA, Rodner D, Esterman A, Muehlberg SM, Coster DJ: Factors predictive of corneal graft survival. Report from the Australian Corneal Graft Registry. Ophthalmology 1992;99:403–414.
170 Bouchard CS, Cavanagh HD: The high-risk keratoplasty patient—quo vadis? Cornea 1994;13:1–3.
171 Reinhard T, Sundmacher R, Heering P: Systemic ciclosporin A in high-risk keratoplasties. Graefes Arch Clin Exp Ophthalmol 1996; 234:S115–S121.
172 Kaliss N: Immunological enhancement. Int Rev Exp Pathol 1969;8: 241–276.
173 Carpenter CB, d'Apice AJF, Abbas AK: The role of antibodies in the rejection and enhancement of organ allografts. Adv Immunol 22: 1-65, 1976.
174 Batchelor JR: The riddle of kidney graft enhancement. Transplantation 1978;26:139–141.
175 Chandler JW, Gebhardt BM, Kaufman HE: Immunologic protection of rabbit corneal allografts: preparation and in vitro testing of heterologous "blocking" antibody. Invest Ophthalmol 1973;12: 646–653.
176 Chandler JW, Gebhardt BM, Sugar J, Kaufman HE: Immunologic protection of rabbit corneal allografts. Transplantation 1974;17:146–148.
177 Binder PS, Gebhardt BM, Chandler JW, Kaufman HE: Immunologic protection of rat corneal antibodies with heterologous

blocking antibody. Am J Ophthalmol 1975;79:949–954.
178 Gebhardt BM, Varnell ED, Kaufman HE: Prolonged survival of corneal allografts incubated in alloantibody fragments. Transplantation 1999;67:594–599.
179 Gebhardt BM, Hodkin M, Varnell ED, Kaufman HE: Protection of corneal allografts by CTLA4-Ig. Cornea 1999;18:314–320.
180 Greenfield EA, Nguyen KA, Kuchroo VK: CD28/B7 costimulation: a review. Crit Rev Immunol 1998;18:389–418.
181 McCoy KD, Le Gros G: The role of CTLA-4 in the regulation of T cell immune responses. Immunol Cell Biol 1999;77:1–10.
182 Linsley PS, Brady W, Urnes M, Grosmaire LS, Damle NK, Ledbetter JA: CTLA-4 is a second receptor for the B cell activation antigen B7. J Exp Med 1991; 174:561–569.
183 Lenschow DJ, Zeng Y, Thistlewaite JR, Montag A, Brady W, Gibson MG, Linsley PS, Bluestone JA: Long-term survival of xenogenic pancreatic islet grafts induced by CTLA4Ig. Science 1992;257: 789–792.
184 Bolling SF, Lin H, Wei R-Q, Linsley P, Turka LA: The effect of combination cyclosporine and CTLA4-Ig therapy on cardiac allograft survival. J Surg Res 1994;57: 60–64.
185 Lin H, Wei R-Q, Gordon D, Linsley P, Turka LA, Bolling SF: Review of CTLA4Ig use for allograft immunosuppression. Transplant Proc 1994;26:3200–3201.
186 Steurer W, Nickerson PW, Steele AW, Steiger J, Zheng XX, Strom TB: Ex vivo coating of islet cell allografts with murine CTLA4/Fc promotes graft tolerance. J Immunol 1995;155:1165–1174.
187 Yin D, Fathman CG: Induction of tolerance to heart allografts in high responder rats by combining anti-CD4 with CTLA4Ig. J Immunol 1995;155:1655–1659.
188 Kirk AD, Harlan DM, Armstrong NN, Davis TA, Dong Y, Gray GS, Hong X, Thomas D, Fechner JH Jr, Knechtle SJ: CTLA4-Ig and anti-CD40 ligand prevent renal allograft rejection in primates. Proc Natl Acad Sci USA 1997;94: 8789–8794.
189 Onodera K, Chandraker A, Schaub M, Stadlbauer THW, Korom S, Peach R, Linsley PS, Sayegh MH, Kupiec-Weglinski JW: CD28-B7 T cell costimulatory blockade by CTLA4Ig in sensitized rat recipients. J Immunol 1997;159: 1711–1717.
190 Rey-Chaudhury P, Nickerson PW, Manfro RC, Zheng XX, Steiger J, Li YS, Strom TB: CTLA4Ig attenuates accelerated rejection (presensitization) in the mouse islet allograft model. Transplantation 1997;64:172–175.
191 Levisetti MG, Padrid PA, Szot GL, Mittal N, Meehan SM, Wardrip CL, Gray GS, Bruce DS, Thistlethwait JR Jr, Bluestone JA: Immunosuppressive effects of human CTLA4Ig in a non-human primate model of allogeneic pancreatic islet transplantation. J Immunol 1997;159:5187–5191.
192 Judge TA, Wu Z, Zheng X-G, Sharpe AH, Sayegh MH, Turka LA: The role of CD80, CD86, and CTLA4 in alloimmune responses and the induction of long-term allograft survival. J Immunol 1999; 162:1947–1951.
193 Herbort CP, de Smet MD, Roberge FG, Nussenblatt RB, FitzGerald D, Lorberboum-Galski H, Pastan I: Treatment of corneal allograft rejection with the cytotoxin IL-2-PE40. Transplantation 1991;52: 470–474.
194 He Y-G, Mellon J, Niederkorn JY: The effect of oral immunization on corneal allograft survival. Transplantation 1996;61: 920–926.
195 MA D, Mellon J, Niederkorn JY: Oral immunisation as a strategy for enhancing corneal allograft survival. Br J Ophthalmol 1997;81: 778–784.
196 Ma D, Mellon J, Niederkorn JY: Conditions affecting corneal allograft survival by oral immunization. Invest Ophthalmol Vis Sci 1998;39:1835–1846.
197 Ma D, Li X-Y, Mellon J, Niederkorn JY: Immunologic phenotype of hosts orally immunized with corneal alloantigens. Invest Ophthalmol Vis Sci 1998;39: 744–753.
198 Chandler JW, Ray-Keil L, Gillette TE: Experimental corneal allograft rejection: description of murine model and a new hypothesis of immunopathogenesis. Curr Eye Res 1983;2:387–397.
199 Ray-Keil L, Chandler JW: Reduction in the incidence of rejection of heterotopic murine corneal transplants by pretreatment with ultraviolet radiation. Transplantation 1986;42:403–406.
200 Guymer RH, Mandel TE: UV-B irradiation of donor skin and cornea prior to allotransplantation in mice. Transplant Proc 1989;21: 3771–3772.
201 Dana MR, Olkowski ST, Ahmandian H, Stark WJ, Young EM: Low-dose ultraviolet-B irradiation of donor corneal endothelial and graft survival. Invest Ophthalmol Vis Sci 1990;31:2261–2268.
202 Katami M, White DJG, Watson PG: Site-specific immunosuppression for corneal graft survival in the rat. Transplant Proc 1992; 24:1406–1408.
203 Hill JC, Sarvan J, Maske R, Els WJ: Evidence that UV-B irradiation decreases corneal Langerhans cells and improves corneal graft survival in the rabbit. Transplantation 1994;57:1281–1283.
204 He Y-G, Niederkorn JY: Depletion of donor-derived Langerhans cells promotes corneal allograft survival. Cornea 1996;15: 82–89.
205 Yatoh S, Kawakami Y, Imai M, Kozawa T, Segawa T, Suzuki H, Yamashita K, Okuda Y: Effect of a topically applied neutralizing antibody against vascular endothelial growth factor on corneal allograft rejection of rat. Transplantation 1998;66:1519–1524.