Essentials in Ophthalmology

Cornea and External Eye Disease

T. Reinhard F. Larkin
Editors

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Cornea and External Eye Disease

Editors Thomas Reinhard
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Cornea and External Eye Disease

Corneal Allotransplantation, Allergic Disease and Trachoma

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Foreword

The Essentials in Ophthalmology series represents an unique updating publication on the progress in all subspecialties of ophthalmology.

In a quarterly rhythm, eight issues are published covering clinically relevant achievements in the whole field of ophthalmology. This timely transfer of advancements for the best possible care of our eye patients has proven to be effective. The initial working hypothesis of providing new knowledge immediately following publication in the peer-reviewed journal and not waiting for the textbook appears to be highly workable.

We are now in the third cycle of the Essentials in Ophthalmology series, having been encouraged by readership acceptance of the first two series, each of eight volumes. This is a success that was made possible predominantly by the numerous opinion-leading authors and the outstanding section editors, as well as with the constructive support of the publisher. There are many good reasons to continue and still improve the dissemination of this didactic and clinically relevant information.

G.K. Krieglstein
R.N. Weinreb
Series Editors

Preface

This third *Cornea and External Eye Disease* volume comprises eleven reviews of moving points in corneal biology, disease pathogenesis and management.

In this volume we have gathered a number of chapters on and around the topic of cornea and limbus transplantation. Jerry Niederkorn reviews our increasing understanding of the components of immune privilege enjoyed by corneal transplants, a privilege unrivalled in the field of transplantation. This privilege is relative and is neither universal nor immutable. Rejection remains the major threat for corneal transplants, in the settings of conventional penetrating keratoplasty, of newer lamellar surgical techniques and of course especially in patients at high rejection risk.

Strategy on how to prevent immune rejection is controversial; differing analyses being described in the chapters by Douglas Coster and Alex Reis. Some benefit of HLA matching has been found in high rejection risk corneal transplantation, but transplantation antigen matching is undertaken only in European centres. Is use of systemic immunosuppressive drugs justified in corneal graft recipients, among whom are some in whom blindness would result from loss of donor corneal transparency? Risks of drug adverse effects vs. benefits of maintaining a functioning transplant should be considered in any candidate for a corneal transplant at high rejection risk. It is noteworthy that quality of life in blind patients is significantly more compromised than in those renal failure patients requiring dialysis [1, 2]. We hope you enjoy reading this volume.

Thomas Reinhard
Frank Larkin

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Chapter 1

Immune Privilege of Corneal Allografts 1

Jerry Y. Niederkorn

Core Messages

- Multiple anatomical, physiological, and immunoregulatory factors contribute to the immune privilege of corneal allografts. These factors conspire to prevent the induction and expression of immune responses to the histocompatibility antigens on the corneal allograft.
- Corneal allografts also elicit a dynamic immunoregulatory process that deviates the immune response from a destructive pathway to one of tolerance. Together, these conditions create an immune privileged environment and promote corneal allograft survival.
- Corneal allografts enjoy immune privilege that is unrivaled in the field of transplantation. However, this immune privilege is neither universal nor immutable. This has led some to dismiss immune privilege of corneal allografts out of hand. Moreover, the success of renal, cardiac, and liver transplants has improved over the past 3 decades and has reached levels similar to corneal allografts – an observation that has further fueled protests that corneal allografts are no different than other organ allografts, and that immune privilege is a misnomer. However, comparing survival rates among these categories of allografts is a bit like comparing an apple to an orange. For the comparisons to be valid, we must either compare the survival of corneal allografts in patients treated with the same intense systemic immunosuppressive agents that are used in renal, cardiac, or liver transplant patients, or compare all four categories of patients when the only treatment is topical corticosteroids (i.e., the standard prophylactic therapy in keratoplasty patients). The latter proposition, of course, is absurd, but does emphasize the importance of including all of the parameters when making comparisons relating to immune privilege.
- Prospective studies in animal models have unequivocally shown that in the absence of antirejection drugs, corneal allografts have dramatically higher acceptance and long-term survival rates than other categories of allografts such as skin transplants.

1.1 History of Corneal Transplantation and Immune Privilege

The notion that corneal tissues could be successfully transplanted was proposed three centuries ago by Erasmus Darwin, the grandfather of Charles Darwin. The first reported attempt at experimental corneal transplantation was performed in 1835 by Bigger, who transplanted a corneal allograft to a pet gazelle [1]. In 1838, Kissam attempted the first corneal transplant in a human subject and grafted a pig cornea onto a patient's eye using four interrupted sutures and without the use of anesthesia [2]! Almost half a century later corneal transplantation was once again attempted on humans when May transplanted rabbit corneal xenografts to humans and noted that the 24 attempts failed due to "imperfect technique and the inability to keep the eyes properly bandaged" [3]. It took almost seven decades before the first successful corneal transplant was grafted from a human donor to a human recipient [4, 5]. Since then, corneal transplantation has emerged as the most common form of solid tissue transplantation in the United States and the United Kingdom [6, 7].

The concept that the cornea and the anterior segment of the eye might be endowed with unusual immunological properties can be traced to Sir Peter Medawar, who noted the remarkable survival of orthotopic corneal allografts transplanted to the ocular surface and heterotopic skin allografts placed into the anterior chamber

Table 1.1 Immune rejection of corneal allografts and skin allografts in rats

Histocompatibility barrier	Incidence of rejection	
	Skin allograft (%)	Corneal allograft (%)
MHC + multiple minor histocompatibility antigens	100	38 to >90
MHC class I only	100	18–35
MHC class II only	100	0–10

(AC) of the rabbit eye. Medawar recognized the significance of the unusual properties of the corneal transplant and the anterior chamber over which it was transplanted, and coined the term "immune privilege" [8]. Clinical observations in human keratoplasty patients and results from experimental animal studies support the notion that corneal allografts enjoy immune privilege [5, 6, 9]. In routine human keratoplasty, no HLA matching is performed and topical corticosteroids are the only immunosuppressive agents administered. This is in sharp contrast to all other forms of solid tissue transplantation. Animal studies have provided perhaps the most compelling evidence for the immune privilege of orthotopic corneal allografts [5]. In rodent models of penetrating keratoplasty, the incidence of immune rejection of corneal allografts differing from the hosts at all known histocompatibility gene loci (i.e., MHC plus minor histocompatibility loci) can be as low as 38%, with the average being approximately 50%, even though immunosuppressive drugs are not used [5]. Corneal allograft survival is even more impressive when histocompatibility matching is applied. Corneal allografts mismatched with the host only at MHC class I loci enjoy long-term survival in 65 and 72% of the rat and mouse hosts, respectively [5]. Corneal allograft survival in rodents mismatched with the corneal allograft donor only at MHC class II loci display the most pronounced example of immune privilege, with graft rejection occurring in less than 10% of the hosts. In contrast, skin allografts in each of these categories are invariably rejected (Table 1.1). These remarkable findings have led to the misconception that the immune privilege of corneal transplants is universal and immutable.

1.2 How Successful Is Corneal Transplantation?

Although it is commonly stated that corneal allografts enjoy a first-year survival rate as high as 90%, the long-term survival rate is considerably lower and drops to 74% at 5 years and 62% at 10 years [7]. Moreover, graft survival is even worse in patients who are considered "high-risk" based on the presence of preexisting corneal neovascularization, ongoing ocular inflammation, or a history of previous corneal graft rejection. In these conditions, 10-year graft survival plummets to 35% [10]. In recent years, the success rate for renal, cardiac, and liver transplants has improved and has reached a level similar to corneal transplants, with approximately 75% of the grafts surviving at 5 years [7]. Unlike other categories of solid organ transplants, which have demonstrated improved survival over the past 10–15 years, the long-term survival of corneal transplants has not changed [7]. The improved survival of other organ transplants is largely a result of improved immunosuppressive drugs. In contrast, topical steroids continue to be the only immunosuppressive agents routinely used for preventing corneal allograft rejection and have been the mainstay among prophylactic immunosuppressive agents for decades. Unlike the rejection of cardiac, renal, and hepatic transplants, which pose a risk for survival and justify more aggressive immunosuppressive therapy, rejection of corneal allografts has far less serious consequences, which explains the ophthalmologist's reluctance to use systemic immunosuppressive drugs, which carry serious side effects and can significantly affect the patient's quality of life.

Summary for the Clinician: Success of Corneal Allografts

- In the absence of risk factors, such as inflammation and neovascularization of the graft bed, corneal allografts enjoy immune privilege.
- Corneal allografts survive in the absence of HLA matching and without the use of systemic immunosuppressive drugs, which is further evidence of their immune privilege.
- Immune privilege of corneal allografts is not universal or immutable. Factors associated with corneal inflammation and neovascularization rob the cornea of its immune privilege and increase the risk for rejection.
- Topical application of corticosteroids is the mainstay prophylactic antirejection treatment. Risk to benefit ratio for keratoplasty patients precludes the use of more aggressive immunosuppressive protocols that have led to a steady improvement in the survival rates for kidney, liver, and heart transplants. In contrast, the success of corneal allografts has not improved over the past 3 decades.

1.3 Immune Rejection of Corneal Allografts

The beneficial effects of MHC matching in promoting the acceptance of other categories of allografts has been demonstrated, but remains controversial in corneal transplantation [7, 11]. One study has shown no benefit from MHC class I and class II matching on corneal allograft survival [6], while another study has reported a modest, albeit significant benefit of MHC class I matching, but an increased risk of rejection with MHC class II matching [12]. Studies in both humans and animals have clearly demonstrated that MHC class I antigens are expressed on all three layers of the cornea, while MHC class II antigens are conspicuously absent under nonpathological conditions. Minor histocompatibility antigens are also expressed in the cornea and can provoke corneal graft rejection [4, 5]. In fact, studies in both rats and mice suggest that minor histocompatibility antigens pose a greater barrier than MHC antigens for corneal allograft survival [13–15]. It has been estimated that 90% of the MHC antigens are expressed on the corneal epithelium, leading some to propose that removal of this layer might reduce the immunogenicity of corneal allografts and promote their survival. However, removal of donor epithelium prior to corneal transplantation did not enhance corneal allograft survival in 228 keratoplasty patients in one study [16]. Moreover, investigations in mice suggest that the corneal epithelium plays an active role in dampening inflammation, and that the removal of the corneal epithelium jeopardizes corneal allograft survival [9, 17].

Studies on the mechanisms of corneal graft rejection in patients have been largely inferential, as they have relied on in situ immunohistochemical phenotyping of cell surface markers on immune cells and the identification of cytokines in rejected corneal allografts. Animal studies, especially those in rodents, have provided the most useful insights into the mechanisms of immune rejection of corneal allografts. Maumenee was the first to unequivocally demonstrate that corneal allograft rejection was immune-mediated [18]. Using a rabbit model of penetrating keratoplasty, Maumenee demonstrated that rabbits that received skin grafts 2 weeks prior to the application of orthotopic corneal allografts from the same donor, rejected their corneal allografts at an accelerated tempo, thereby demonstrating immunological memory, and establishing the immunologic basis for corneal allograft rejection. In the late 1960s and mid 1970s, Khodadoust and Silverstein demonstrated that corneal allograft rejection was a cell-mediated process that could be adoptively transferred with lymphocytes that had been specifically sensitized to the corneal allograft donor's histocompatibility antigens [19, 20].

1.3.1 Role of CD4+ T Lymphocytes in Corneal Allograft Rejection

The development of the rat and, subsequently, the mouse model of penetrating keratoplasty paved the way for a series of studies exploring the immune mechanisms of corneal allograft rejection. Using these models, investigators have established that T cells, especially CD4+ T helper cells, were capable of mediating corneal allograft rejection [4]. Depletion of CD4+ T cells by in vivo antibody treatment or by gene deletion results in a steep reduction in the rejection of corneal allografts in rats and mice [4]. Likewise, there is a close correlation between corneal allograft survival and the down-regulation of CD4+ T cell immune responses [5]. CD4+ T cells can contribute to corneal allograft rejection in a number of ways. Delayed-type hypersensitivity (DTH) responses to alloantigens are mediated by CD4+ T cells and are closely correlated with corneal allograft rejection in mice. CD4+ T cells, especially the Th1 population, produce interferon- γ (IFN-γ) and tumor necrosis factor-α (TNF-α), which are known to induce apoptosis of corneal cells [4]. CD4+ T cells can also produce cell-contact-dependent apoptosis of corneal cells [21]. Although CD4+ T cells have been widely proclaimed as the sole mediators of corneal allograft rejection, it is noteworthy that depletion of CD4+ T cells by in vivo treatment with antibody or by deletion of the CD4 gene in mice does not abolish corneal allograft rejection; in fact, approximately 50% of the CD4+ T cell-deficient mice and rats go on to reject their corneal allografts [22–24]. In contrast, T cell-deficient mice do not reject corneal allografts, indicating that in addition to CD4+ T cells, one or more other T cell subsets can contribute to corneal allograft rejection.

1.3.2 Role of CD8+ T Lymphocytes in Corneal Allograft Rejection

CD8+ T cells are the other major subset of T lymphocytes that has been implicated in organ graft rejection. The notion that CD8+ cytotoxic T lymphocytes (CTL) mediate graft rejection has been embraced by numerous investigators. CD8+ CTL can kill allogeneic cells in vitro, including corneal cells. Moreover, CD8+ lymphocytes are among the mononuclear cells that are detected in rejected corneal allografts. However, rodent studies have shown that donor-specific CTL are not detected in hosts that have rejected corneal allografts. Moreover, corneal allograft rejection occurs unabatedly in CD8 knockout (KO) mice, perforin KO mice, or mice treated

with anti-CD8 monoclonal antibody [4]. Unlike the condition with other allografts, corneal allograft rejection does not culminate in the development of donor-specific CTL. However, hosts with prevascularized corneal graft beds have a dramatically increased incidence and tempo of corneal allograft rejection. In these hosts, corneal allograft rejection elicits robust donor-specific CTL responses [25]. Moreover, CD8+ CTL collected from "high-risk" hosts that have rejected corneal allografts can induce corneal allograft rejection when adoptively transferred to severe combined immune deficient (SCID) mice, indicating that under certain conditions, CD8+ T cells can mediate corneal allograft rejection [26].

1.3.3 Role of Antibodies in Corneal Allograft Rejection

Although the weight of evidence suggests that corneal allograft rejection is T cell-mediated, there are reports suggesting a role for cytotoxic antibody. Antibodies specific for the donor's histocompatibility antigens can be detected in the serum of keratoplasty patients. An interesting correlation between ABO incompatibility and corneal allograft rejection in high-risk patients has been reported [6]. The incidence of rejection in patients with ABO-incompatible corneal allografts was twice that found in recipients who received ABO-compatible corneal grafts. ABO hemagglutinins are IgM antibodies, which are excellent complement-fixing immunoglobulins with potent cytolytic activity. ABO blood group antigens are expressed on human corneal epithelial and endothelial cells [27], and in vitro studies have shown that corneal endothelial cells are highly susceptible to cytolysis by complement-fixing antibodies [4, 28]. This is consistent with the notion that under certain conditions, antibody might contribute to corneal allograft rejection. Results from experiments in mice lend further support for this hypothesis. Donor-specific alloantibodies have been detected in the serum of mice at the time of corneal allograft rejection [28]. Passive transfer of alloantibodies to T cell-deficient mice, which normally do not reject corneal allografts, results in corneal allograft rejection [29, 30]. In contrast, corneal allograft rejection occurs in both B cell-deficient mice and complement-deficient mice, indicating that complement-fixing antibodies are not required for corneal allograft rejection, and that other immune effector mechanisms can also mediate graft failure in the absence of alloantibody [28, 31].

1.3.4 Role of Macrophages and NK Cells in Corneal Allograft Rejection

The immune system is composed of two distinctly different components: the adaptive and the innate immune systems. The adaptive immune system is characterized by exquisite antigen specificity and the participation of an intact T cell repertoire. Adaptive immune responses require several days to develop, but display long-term memory, which is manifested by swift responses to subsequent encounters with the original antigen. T lymphocytes, B lymphocytes, and antibodies are the primary elements of the adaptive immune system. The innate immune system is comprised of granulocytes, macrophages, natural killer (NK) cells, and the alternate pathway of the complement system. In contrast to the adaptive immune system, the innate immune system is characterized by its rapid activation by pathogens via recognition of toll-like receptors and pathogen-associated molecular patterns (PAMP) that are expressed on various microorganisms. Although the innate immune responses are swift, the responding cells lack antigen specificity and do not display memory.

Animal studies have provided compelling evidence that elements of the innate immune system indirectly contribute to corneal allograft rejection. DTH reactivity to donor histocompatibility antigens is closely correlated with corneal allograft rejection [4]. Macrophages are a major cell population in DTH lesions and are present in rejected corneal allografts. Studies in both the mouse and rat models of penetrating keratoplasty have shown that elimination of periocular macrophages by subconjunctival injection of liposomes containing the macrophagicidal drug clodronate prevents corneal allograft rejection [4]. However, further analysis has revealed that macrophages do not act as effector cells by damaging the donor corneal graft, but appear to be crucial antigen presenting cells (APC) that activate CD4+ T cells, which enter the graft and function as the end stage effector cells that deliver the lethal hit to the corneal allograft [4]. Neutrophils are also present in the inflammatory infiltrate of rejected corneal allografts, but there is little evidence to support an important role for them in corneal allograft rejection.

NK cells act as "first responders" to viral infections, and are believed to play an important role in the immune surveillance of neoplasms. Recent studies in the rat model of penetrating keratoplasty suggest that NK cells might also participate in corneal allograft rejection [32, 33]. Cells with surface markers that are characteristic of NK cells have been detected in the corneal stroma and the aqueous humor of hosts with rejected corneal allografts.