Novel concepts in the immunology and
treatment of vascularized high-risk corneal allotransplants

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ABSTRACT

Corneal allotransplantation has been the principal surgical treatment for vision loss caused by diseases of the cornea that result in scarring and opacification. Most authors define a “high-risk” cornea as that of a previously failed corneal graft or a cornea with vascularization in at least 2 quadrants. These high-risk corneal transplants have rejection rates approaching 70%, even with maximal local and systemic immune suppression. The management of high risk corneal transplants, which until recently was a slowly evolving field in ophthalmology, remains a highly controversial yet important topic.

INTRODUCTION

Corneal allotransplantation has been the principal surgical treatment for vision loss caused by diseases of the cornea that result in scarring and opacification. Penetrating keratoplasty is the oldest and most common form of human solid tissue transplantation. The success of penetrating keratoplasty is high, with 5 year survival rates as high as 90%. This is in part due to the fact that the cornea has long been recognized as “immune privileged”. Regularly these “normal risk” corneal transplants, where the donor cornea is grafted into an avascular, non-inflamed non-sensitized eye, need only local immunosuppression with medications such as topical corticosteroids. Although dosing is easy and systemic side effects are low, long-term use of corticosteroids carries multiple risks in the eye such as infection, poor wound healing, glaucoma and cataracts. On the other hand, “high-risk” corneal transplant prognosis is poor. These high-risk corneal grafts have rejection rates approaching 70% even with maximal local and systemic immune suppression. Strikingly, these rejection rates are even higher than those seen with living donor kidney transplantation, where five year survival rates are above 80%. The management of high-risk corneal transplants, which until recently was a slowly evolving field in ophthalmology, remains a highly controversial yet important topic.

BACKGROUND

Although not universally accepted, most authors define a “high-risk” cornea as that of corneas that have previously rejected a graft or a cornea with vascularization in at least 2 quadrants. Recent studies from multiple laboratories have shown that corneal neovascularization disrupts the immune privilege, otherwise enjoyed by naïve corneas. This “immune privilege,” first observed in the late 1800s by van Dooremaal and later extensively studied by Streilein is now known as anterior chamber associated immune deviation (ACAID). ACAID involves the eye, spleen, thymus and sympathetic nervous system. Multiple factors compose ACAID. One of these, transforming growth factor beta (TGF-Beta), is essential in the induction of ACAID. Antigen presenting cells exposed to TGF-B produce increased levels of IL-10, decreased levels of IL-12 and downregulate CD40 co-stimulatory molecules. Regulatory T cells (CD4+CD25+) have been shown to inhibit target cells by making direct contact. ACAID’s immunomodulatory role is essential for the maintenance of a clear cornea. It prevents excessive inflammation, scarring and, thus preserves corneal clarity. This occurs in the presence of foreign antigen from infection, or in transplantation, yet it permits a degree of immunologic protection against infection. The disruption of ACAID and its resultant neovascularization produces a host-mounted immune
response more similar to that of vascularized solid tissue transplants than to naïve avascular corneas. In these high-risk corneas, rejection rate and tempo are directly proportional to the amount of vascularization in the recipient bed. In vascularized grafts, the recruitment of allospecific T cells by chemokines is seen very early. These allospecific T cells have been shown to promote destruction of endostatin-producing cells, resulting in increased corneal neovascularization, massive infiltration of effector T cells and graft rejection.

Several factors have been known to confer a high-risk status to a corneal transplant. These include the proliferation of blood vessels and lymphatic channels, especially in the corneal deep stroma, increased MHC II expression, maturation of dendritic cells, chemokine production, and interruption of the blood-ocular barrier. It is well established that the risk of rejection is increased in regrafts. This is especially true when two or more grafts have been previously rejected. Although still controversial, it has been postulated that the increase in rejection rate is due to the neovascularization related to the rejection process of the previous cornea and not to allosensitization. Other host factors associated with a greater risk of rejection are uveitis, herpes simplex keratitis, atopic dermatitis and eczema. Active inflammation and/or infection during surgery greatly increase the probability of graft failure due to rejection. Other high-risk associations have been reported and include vitreous adhesions, multi-surgical approach, young recipient age, larger graft size, and eccentric grafts.

Glaucoma is an important risk factor for graft failure but not graft rejection. The rejection of high-risk vascularized corneal allografts is an immunological process mediated principally by allospecific T cells. The use of immunosuppressive agents in high-risk corneal transplantation is associated with a high rate of systemic complications. A better understanding of how allospecific T cells mediate high-risk corneal allograft rejection may lead to the development of new and safer forms of anti-rejection therapy. Several laboratories, including ours, are dedicated to the understanding of high-risk corneal transplant kinetics. We have previously shown that high-risk vascularized corneal allografts behave like vascularized solid organ transplants. In these transplants, the production of early inflammatory signals is extremely important in regulating the subsequent production of T-cell chemo-attractants responsible for T-cell recruitment, and the increased rejection rate observed in high-risk vascularized corneal allografts. The modulation of these cytokines by upregulation, downregulation, blockade or mimicry may play a role in the treatment and prevention of rejection.

**DETECTION OF ALLOGRAFT REJECTION**

Early detection of graft rejection is essential. Regular frequent follow-ups as well as patient compliance are of paramount importance. Patient symptoms, including red eye tearing, photophobia, hyperemia and loss of vision are strongly associated with graft rejection. Therefore, patients should be instructed to advise the physician of any acute changes. Although further studies need to be performed in humans, we have shown in mice that corneal thickness measurements using spectral domain OCT as early as postoperative day 15 are a good predictor for graft outcome. The quantification of corneal edema, combined with early detection of endothelial loss and assessment clinical acute changes of signs and symptoms can lead to early detection and prompt treatment of graft rejection.

Epithelial rejection is the earliest form of rejection to be seen. An epithelial rejection line (deposits of lymphocytes) usually stains with fluorescein or Rose Bengal. Kaye’s dots (epithelial infiltrates) appear at the suture lines. Epithelial rejection is followed by endothelial rejection, which is the most common form of graft rejection. Fine keratic precipitates or a Khodadoust line of precipitates may be seen. The rejection episode is irreversible once endothelial decompensation is established, thus immediate treatment is warranted.

**MEDICAL MANAGEMENT**

Since it is easier to prevent rejection than to stop rejection once it has started, prophylaxis is essential in the management of high-risk patients. Prophylaxis should be individualized according to the risk of rejection, and perhaps the primary cause of insult (Stevens-Johnson, caustic burn, failed graft, infection, etc). This is followed by topical steroids (1% prednisolone acetate) four times a day for the first 4 months and then slowly tapered off during a period of 2 more months. In the case of moderate to high-risk patients, a longer and more intense corticosteroid regimen is needed. Clinical practice varies widely. This is probably due to the fact that there is a scant amount of evidence-based clinical observations. We usually give 1% prednisolone acetate drops every 2 hours with a corticosteroid ointment at night. The dose is slowly tapered off over a period of 6 months and a low potency steroid is continued indefinitely. Based on evidence showing early inflammatory responses, we give patients with high-risk corneal grafts a one gram dose of methylprednisolone (Solu-medrol®, Pfizer) at the time of surgery and oral prednisone at 1mg/kg/day, with quick tapering over a month. In cases where adjunctive therapy is needed, either because of steroid side effects or ineffective treatment, topical cyclosporine A (0.5–2.0%) is added as our first line of adjunctive treatment.
Cyclosporine A is a powerful immunomodulator that inhibits the proliferation of activated and cytotoxic T-lymphocytes and spares the T-suppressor cell populations that have been shown to prevent rejection of high-risk corneal allografts. Several authors have found systemic immunosuppression with cyclosporine A partially helpful. This practice has become less widely accepted in the last few years due to the high rate of side-effects. On the other hand, topical cyclosporine A has a more significant effect than systemic cyclosporine, with fewer side effects. Tacrolimus and Mycophenolate Mofetil have also been found to be helpful. (Figure 1A and B) Once rejection is detected, aggressive treatment is warranted. The mainstay of treatment is still topical corticosteroids. Several treatment options proposed in the literature have been summarized below. (Table 1)

### FUTURE

Our knowledge of the immunology of corneal rejection has grown several folds in the last few decades. We are just beginning to understand the complex kinetics of cytokine production at the site of transplantation; this will enhance the possibility of creating localized immunosuppression favorable to the graft with little or no systemic side effects. Promising immunomodulating drugs that may help suppress T-effector or activate T-regulatory cells are currently being studied. Our growing knowledge in the function of regulatory T-cells and their interaction with corneal allotransplantation is also opening a new field of research for the treatment of rejection. Currently, most of our treatments are administered topically to the corneal epithelium. This requires multiple applications and patient compliance. Gene therapy could enable us to treat a specific molecular mechanism of disease in a single dose. This could decrease reliance on patient compliance and side effects, which should enhance treatment outcomes. The cornea is an excellent site for gene therapy. Its anatomy, immune privilege, and accessibility make it an optimal target tissue.55,56 On the other hand, the prevention of a vascularization, or its regression, has been a big field of research. Recent publications have shown that by blocking vascular endothelial growth factor (VEGF), postoperative hemangiogenesis and lymphangiogenesis may be attenuated.
inhibited. This in turns leads to a decrease in recruitment of antigen presenting cells and therefore an improvement in graft survival. Ultimately, antiangiogenesis in itself may prove inadequate in preventing rejection and combined antiangiogenesis and immune suppression will be more effective in maintaining corneal allograft survival.

The future of high-risk corneal transplantation is promising. Utilizing animal models, promising treatments are currently being developed. Our knowledge of the pathophysiological mechanisms of corneal disease has greatly increased in the last several years. Few of the current treatments reflect our newly acquired knowledge and are, therefore, non-specific for the actual mechanism of disease. The anterior segment is a perfect model for slow drug delivery systems that are currently being developed. These include sustained release polymers, nanoparticles and implants that can be placed both inside the eye or in the subconjunctival space. These novel delivery systems combined with recent advances in the field of angiogenesis (VEGF inhibitors), localized immunomodulation, gene therapy as well as better surgical techniques where lower antigen load is transplanted are making their way to the bedside.