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Update on the Management of High-Risk Penetrating Keratoplasty

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Abstract

Purpose of Review In this article, we review the indications and latest management of high-risk penetrating keratoplasty.
Recent Findings Despite the immune privilege status of the cornea, immune-mediated graft rejection still remains the leading cause of corneal graft failure. This is particularly a problem in the high-risk graft recipients, namely patients with previous graft failure due to rejection and those with inflamed and vascularized corneal beds. A number of strategies including both local and systemic immunosuppression are currently used to increase the success rate of high-risk corneal grafts. Moreover, in cases of limbal stem cell deficiency, limbal stem cell transplantation is employed.
Summary Corticosteroids are still the top medication for prevention and treatment in cases of corneal graft rejection. Single and combined administration of immunosuppressive agents, e.g., tacrolimus, cyclosporine, and mycophenolate are promising adjunctive therapies for prolonging graft survival. In the future, cellular and molecular therapies should allow us to achieve immunologic tolerance even in high-risk grafts.

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Introduction

Corneal transplantation is the most prevalent type of human organ transplantation. Primary corneal grafts with no complications that are placed in avascular "low risk" beds have extremely high success rates. However, in cases of vascularized corneas and/or history of multiple grafts, as "high risk" beds, there is up to 70% rejection rate despite intense local immunosuppressive therapy [1–3]. Although lamellar keratoplasty has augmented graft survival rate, immunological reasons are still the most common cause of graft failure [4, 5]. A number of factors including inflammation, corneal neovascularization, and niche disturbance lead to breakdown of the immune privilege of cornea followed by graft rejection [6••]. Immunosuppressive therapy is currently the main strategy available for preventing graft rejection in immunologically high-risk graft recipients.

Immune Status of the Cornea

The crucial factor that determines the success and failure of corneal transplantation in different cases is the immune privilege status of the cornea. Several factors are responsible for this immune privilege. Immune cells are restricted in reaching the cornea owing to deficiency of vascular system. Lacking corneal lymphatic system avoids high transfer of antigens and antigen-presenting cells (APCs) to T cell pools like lymph nodes (LNs). Moreover, the cornea expresses low levels of major histocompatibility complex (MHC) antigens, which results in fewer targets for immune cells. T cell and complement

activation are also suppressed by production of a special series of immunomodulatory factors and neuropeptides, e.g., α-melanocyte-stimulating hormone (α-MSH) and transforming growth factor (TGF)-β [7], and stimulated FAS⁺ T cells are induced to undergo apoptosis by CD95 (Fas) ligand. Although, the cornea is endowed with resident dendritic cells (DCs), they are universally MHC class II negative; however, they are capable of expressing class II antigens after inflammation or transplantation [8, 9]. Finally, the anterior chamber contains antigens that are responsible for selective and adoptive transferable inhibition of the systemic immune reaction, called "anterior chamber-associated immune deviation (ACAID)" [7].

High-Risk Cornea

The characteristics of a high-risk cornea include vascularization of cornea, the creation of lymphatic drainage to cervical LNs, corneal migration of LCs, maturation of local epithelial LCs and stromal DCs [10], and up-regulation of pro-inflammatory cytokines such as interleukin (IL)-1 and tumor necrosis factor (TNF)-α [8–10]. It has been shown that IL-1 and TNF-α inhibit immunomodulatory pathways such as ACAID, increase expression of MHCs and maturation of DCs, and also augment the expression of adhesion and chemotactic factors [11, 12].

Risk Factors

Corneal Vascularization

Vascularized corneas have a much higher risk of graft rejection compared to avascular corneas. According to collaborative corneal transplantation studies (CCTs), corneas with deep stromal vascularization in two or more quadrants are considered "high risk." The onset and severity of rejection are determined by degree and depth of preoperative corneal vascularization. Furthermore, once corneal rejection occurs, the likelihood of reversal also depends on the degree of corneal vascularization [13]. Figure 1 showed complete corneal vascularization and conjunctivalization (limbal stem cell deficiency—LSCD) after a severe alkaline chemical burn.

Prior Graft Rejection and Failure

Prior corneal graft failure is a significant risk factor for subsequent failure, especially if it is a result of an allograft rejection [14]. Immune mediators released following previous graft rejection may predispose to more efficient recognition and rejection of corneal graft. Moreover, presensitization may occur when antigens of donor and recipient are shared such that the afferent blockade is circumvented. It is believed that a
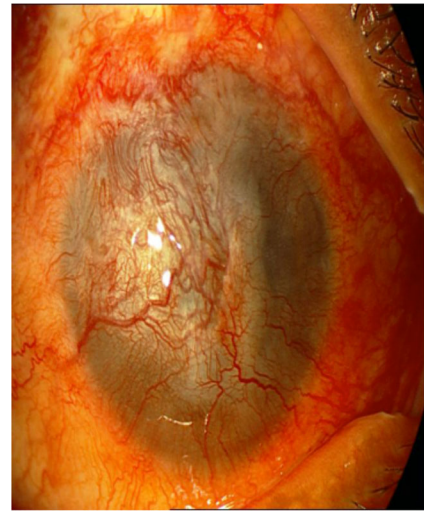
Fig. 1 Total corneal conjunctivalization and vascularization after a severe alkaline chemical burn

sympathetic reaction following corneal surgery results in everlasting omission of immune privilege for future corneal allografts, even for transplantations in contralateral eye or using allograft with different expression of foreign histocompatibility antigens from previous graft [15••]. The residua of previous surgery including corneal neovascularization and peripheral anterior synechiae may increase the chance of subsequent graft failure. The Cornea Donor Study (CDS) has shown an increased risk of graft failure in those with a history of a definite graft rejection episode [16]. The results of CCTS indicated that the quantity of prior corneal grafts is a major risk factor for next graft failure, which the probability of rejection increases approximately 1.2-folds with each extra graft [3]. Graft rejection occurs earlier and follows a much more fulminant course in regrafts than in first grafts.

Past Intraocular Surgery

CCTS results showed an association of prior intraocular surgery with graft survival [13]. The recognized risk factors for graft failure include lensectomy, vitrectomy, and any procedure for lowering the intraocular pressure (IOP) [13]. It has been claimed that concurrent vitrectomy with keratoplasty results in rise in risk of immunologic rejection [17••, 18].

Herpes Simplex and Herpes Zoster Keratitis

It has been shown that herpetic keratitis predisposes an eye to immunologic graft failure. Graft failure following rejection episodes is significantly more frequent in corneal transplantations due to herpetic keratitis than keratoconus [19]. The use of oral antivirals for prophylaxis has significantly reduced the recurrence rate of herpes simplex; [20] nonetheless, these grafts remain at higher risk of failure from immunologic

rejection. Herpes simplex cases treated with systemic acyclovir and immunosuppression with cyclosporin A (CsA) or mycophenolate mofetil showed a rate of graft survival comparable with normal-risk keratoplasty [21]. Favorable outcomes with penetrating keratoplasty (PKP) in patients with herpes zoster ophthalmicus have been reported [22].

Past History of Anterior Segment Inflammatory Disorder and/or Anterior Synechia

A major risk factor of corneal graft failure is the presence of inflammation. Each inflammatory reaction in the anterior segment may accelerate the afferent and efferent arms of allograft response. Autoimmune diseases that have higher risk of rejection include uveitis, ocular mucous membrane pemphigoid (MMP), eye-involved collagen vascular disorders, Steven-Johnson syndrome (SJS), and atopic keratoconjunctivitis.

Anterior synechiae, which can develop following inflammatory events, can likewise impair ocular immune privilege and increase the risk of graft rejection in the presence of three or four quadrants of iris synechiae [13]. In the CCTS, the failure rate from any cause doubled if the eye had three or four quadrants of anterior synechiae [13]. Eyes with anterior synechiae have an increased incidence of glaucoma [14]. Both glaucoma and traction induced by synechiae on the corneal endothelium may lead to endothelial cell loss and graft failure [14]. Figure 2 shows high-risk PKP in a patient with extensive peripheral anterior synechiae formation.

Ocular Surface Disease

Chemical injuries, SJS, MMP, and congenital aniridia, have been considered as risk factors for corneal graft failure, mostly due to surface failure. However, these patients are also at higher risk for immunologic rejection [13]. In general, PKP is not performed as a primary procedure in the presence of LSCD. The appropriate strategy is first to perform a limbal stem cell transplant followed by a later keratoplasty if still necessary. Figure 3 showed PKP in a patient after limbal stem cell transplantation.

Recipient Age

CCTS results indicate that the age of the host is a major risk factor for transplant rejection. Individuals less than 40 years old had the double risk of graft rejection and failure compared to older patients [13]. Furthermore, a considerable risk of transplant failure has been shown in pediatric population. The graft survival rate in children younger than 15 years was found to be approximately 60–70% at 2 years for first-time grafts [23]. Immunologic and several non-immunologic factors including inadequate follow-up and child's inability to report symptoms are responsible for graft failure. Interestingly, CDS showed
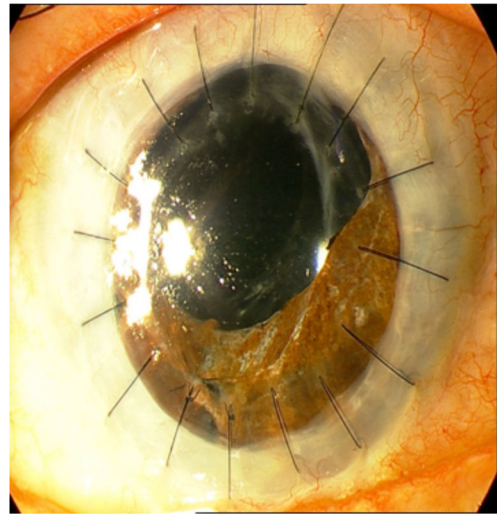
Fig. 2 High-risk penetrating keratoplasty in a patient with extensive peripheral anterior synechiae formation

trends toward a higher rate of graft failure in patients 70 years or older versus those younger than 60 years [17••].

Glaucoma

Although a higher rate of overall graft failure has been demonstrated in glaucoma patients, there is less evidence for a higher risk of immune rejection; nonetheless, some studies have suggested an association [24]. A report by the CDS found that glaucoma was a risk factor for endothelial rejection in univariate but not in multivariate analysis [25]. CDS group also found that previous glaucoma-filtering surgery and glaucoma medication use were risk factors for immune rejection. The highest risk was with both filtering surgery and glaucoma medication use, with a 10-year rejection incidence of 35 versus 14% in those with no glaucoma treatment history [13]. The overall high rate of graft failure in patients with previous glaucoma surgery (e.g., related to endothelial cell loss) may confound determination of the influence of pre-existing glaucoma on rejection.

Strategies to Reduce the Risk of Rejection in High-Risk Keratoplasty

Preoperative Considerations

Transplantation is delayed until ocular inflammation has been controlled for a minimum of 6 months. In severe cases including SJS, MMP, and Mooren's ulcer, any surgical manipulation may be delayed for at least 1 year (except for emergency tectonic grafts). Patients with atopic or vernal keratoconjunctivitis may benefit from treatment with topical ± systemic CsA or tacrolimus [26]. Preoperative (or intraoperative) treatment

Fig. 3 Penetrating keratoplasty in a patient after cultivated limbal epithelial transplantation (CLET). **a** Before CLET. **b** 6 months after CLET. **c** 3 months after PKP. **d** A year and a half after PKP
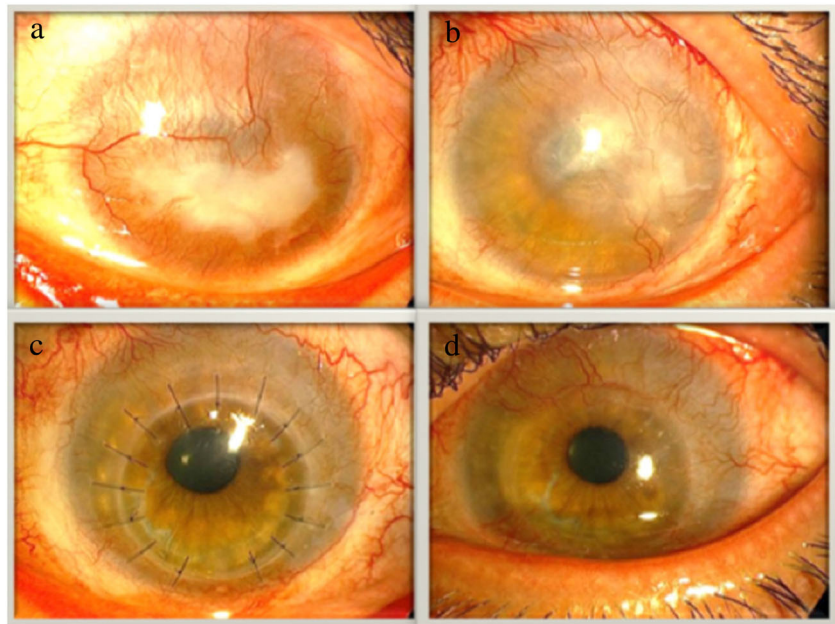
of vessels with an anti-vascular endothelial growth factor (VEGF) agent has also been reported but remains under study.

A healthy ocular surface is essential for the success of corneal transplantation. Tear film status should be optimized; lubricant gels/ointments and artificial tears may benefit the ocular surface. Blepharitis and meibomian gland dysfunction is treated; adnexal abnormalities including entropion, ectropion, and trichiasis/distichiasis are corrected. Punctal occlusion and tarsorrhaphy are likewise important adjunctive measures to improve the tear film. Partial LSCD needs conservative managements. Total LSCD needs surgical intervention. After limbal stem cell transplantation, subsequent PKP should be delayed for at least 3 months.

Tissue Matching

The CCTS was unable to detect any beneficial effect of tissue matching on the rate of immune reactions, graft failure, or the rate of failure caused by rejection [13]. However, the effect of HLA matching in high-risk cases may have been masked by using too much steroids, which can suppress HLA expression. On the other hand, the CCTS indicated a measurable increase risk of failure, owing to rejection or other causes, in ABO incompatible cases [27]. The estimated rate of failure was 31 and 41% in ABO compatible and ABO incompatible patients, respectively. These results corroborate other studies showing that non-MHC antigens may be crucial elements in corneal allograft rejection [27, 28].

Other studies, however, have found that matching of donors and recipients for class I histocompatibility antigens may reduce the likelihood of rejection in high-risk PKP [29]. The results of randomized, single-blinded Functional Antigen Matching in Corneal Transplantation (FANCY) trial started in 2009 will provide further information on the role of different class I histocompatibility antigens [30••]. The effect of class II matching remains controversial at the present time [31, 32]. After excluding CCTS data, a meta-analysis suggested a beneficial effect of MHC class II mismatching in high-risk eyes [33]. The Corneal Transplant Follow-up Study II (CTFS II) is evaluating the role of HLA DR-matching in high-risk keratoplasty cases [30••]. Moreover, the effect of gender on the corneal transplant failure or rejection was evaluated in a large sample study. It is indicated that male to female corneal transplants are more prone to graft rejection or failure [34].

Postoperative Management

The outcome of immunologically high-risk keratoplasty is largely dependent on the postoperative care. The primary aims of postoperative managements include prevention, early diagnosis, and proper treatment of rejection. To establish the early diagnosis, it is useful to educate every patient with rejection symptoms. Frequent follow-up visits allow the identification of early signs of inflammation. Postoperative graft vascularization increases the risk of allograft rejection [35]. In the case of corneal vascularization, interrupted sutures can be selectively removed.

Immunosuppressive Therapy

Local and systemic immunosuppressive therapies are often necessary for preventing transplant rejection in immunologically high-risk hosts. While corticosteroids are still the main

choice for both prevention and treatment of corneal transplant rejection; there are more targeted therapies that can modulate the immune response in high-risk patients.

Corticosteroids

In high-risk patients, topical steroids are used frequently in the postoperative period followed by long-term indefinite use when no contraindications exist. Typically, they are used every 2 to 4 h for the first few weeks with a gradual decrease over the next several months. Topical difluprednate has been used in the prevention and management of graft rejection in high-risk grafts [36••]. In high-risk cases particularly with concomitant systemic inflammatory diseases, systemic steroids are frequently used peri-operatively. Prednisone (1 mg/kg) is usually started around the time of surgery, which can then be tapered on an individualized schedule within 1–2 months. Because of their adverse effects, every attempt should be made to avoid long-term usage of systemic steroids.

Calcineurin Inhibitors

Cyclosporin A

Cyclosporine (CsA) has potent anti-inflammatory and non-myelotoxic immunosuppressive effects that are mediated by binding to two cytoplasmic proteins called cyclophilin A and cyclophilin D [37••], which are critical in T cell activation and proliferation. The beneficial effects of topical CsA in keratoplasty include attenuating the migration of T lymphocytes into the grafts, suppression of corneal neovascularization, optimizing the ocular surface by inhibiting lymphocyte infiltration into lacrimal/accessory glands and conjunctiva, and raising the numbers of goblet cells and also tear production [13].

Topical CsA has been shown in a number of clinical studies to be effective in high-risk keratoplasty [38, 39]. In a study by Inoue et al., cases with high-risk PKP treated with CsA 2% (86 eyes) were retrospectively compared with controls (97 eyes). The rate of rejection-free survival of transplants was 70% and 45% in CsA and control groups, respectively. However, it has been shown that the long-term survival was the same in two groups [40]. The additive effect of topical CsA and topical steroids has also been demonstrated [41].

In very high-risk cases, particularly in monocular patients, systemic CsA is effective in preventing corneal graft rejection [42]. Some have found systemic CsA to have only a moderate and short-term effect on reducing corneal graft rejection [43]. Even, one study found no benefit from systemic CsA [40]. There are a few points to consider when using systemic CsA.

A vital issue is that this strategy must be taken in long term. It seems that short-term administration of systemic CsA for 6 to 12 months cannot result in long-term prevention of graft

rejection. The optimal duration of treatment is best individualized based on the severity and incidence of rejection attacks.

A starting dose of 3–4 mg/kg/day has been recommended [44]. With appropriate monitoring, the risks of therapy can be offset by the potential restoration of vision in patients who would otherwise remain blind. Combination therapy with another immunosuppressive agent such as azathioprine or mycophenolate may be necessary in patients who continue to experience corneal graft rejection on CsA. In addition, the use of a sustained intracameral CsA delivery system has been reported in high-risk keratoplasty patients [45]. The results of this study showed long-term survival of corneal grafts in addition to no significant change in density of corneal endothelial cells after 6 months [45]. Although no complication by implanting the developed CsA drug delivery system was reported, more evaluations are required for replacing such strategies with current therapeutic methods.

The complications of topical CsA are generally related to the discomfort (e.g., burning) associated with its application. For systemic CsA, hypertension, nephrotoxicity, hepatotoxicity, hirsuitism, gingival hyperplasia, neurotoxicity, sensory abnormalities, and reactivation of latent tuberculosis have all been reported as a complication in high-risk keratoplasty [46, 47]. Many of these complications are uncommon if the patient is appropriately screened before and monitored during therapy [43].

Tacrolimus

Tacrolimus is a macrolide antibiotic with potent immunosuppressive activity. The mechanism of action is very similar to CsA, that is, it binds to calcineurin and inhibits the T cell receptor-mediated signal transduction required for transcription of interleukin-2 and other lymphokines. In recent years, tacrolimus has been used increasingly as an alternative to CsA in liver and renal transplantation [48].

Topical tacrolimus (0.03%, 2–4 times daily) has been used as a powerful second-line immunosuppressive agent after normal and high-risk PKP [49]. Topical tacrolimus might be more potent than topical CsA for the prevention of graft rejection [50]. Likewise, systemic tacrolimus in high-risk cases results in fewer graft rejection episodes and longer graft survival compared with CsA [51•, 52••]. In a study, oral tacrolimus was initiated at a dose of 2 mg/day (1 mg twice daily) on the day of surgery and trough levels are measured 12 h after the last dose of tacrolimus. The mean tacrolimus dosage was 2.5 mg/day per patient (range 2–8 mg/day) [53]. Additionally, in another study, the oral tacrolimus was started at 0.03 mg/kg per day in two divided doses and then adjusted based on clinical efficacy up to 0.08 mg/kg per day [54]. Like CsA, the duration of therapy should be individualized and it is best combined with other agents. The choice between systemic tacrolimus and CsA remains a matter of experience and personal preference; although, there is some recent evidence for superiority of tacrolimus. Its complications are

paraesthesia, tremors, headache, fatigue, increased blood pressure, reversible rise in serum creatinine (nephrotoxicity), and diabetes [53].

Agents with Anti-Proliferative Effects

Mycophenolate Mofetil

Mycophenolate mofetil blocks the proliferation of T and B cells by inhibiting guanosine synthesis. It has been shown to prolong the survival of high-risk keratoplasty [55]. In one study, the effects of mycophenolate (1 g twice daily) was comparable to CsA (adjusted to blood trough level of 120–150 ng/ml) in its efficacy [56]. In a retrospective evaluation by Birnbaum et al., 417 high-risk cases treated with systemic CsA or mycophenolate were enrolled [57]. The rejection-free survival rate was 75% at 1 year, and 60% at 3 years for CsA compared to the mycophenolate group which had 89 and 72% rejection-free survival rate after 1 and 3 years, respectively. In a different study, mycophenolate was administered at an initial dose of 2 × 1000 mg and was tapered to 2 × 500 mg 1 month after surgery. It was continued another 6 months at 2 × 250 mg then discontinued a year after surgery [58]. It has been used as a single agent or in combination with CsA, tacrolimus, or sirolimus [59].

Reversible adverse effects observed with mycophenolate included gastrointestinal toxicity, bone marrow suppression, arthralgia, and rarely infection [55]. Given its safety profile, it may be considered as first line agent for high-risk keratoplasty.

Rapamycin

Rapamycin is a bacterial macrolide with both immunosuppressive and antifungal effect. It inhibits proliferation and activation of T helper cells and expands T regulatory cells. Rapamycin can effectively prevent neovascular proliferation and immune rejection of organ transplants [60]. Its efficacy has been shown in ameliorating autoimmune uveoretinitis and increasing high-risk corneal graft survival [60, 61]. Rapamycin may be considered as an effective alternative for immunosuppression in high-risk patients, similar to mycophenolate, particularly for long-term maintenance. Patients on rapamycin experience various side effects ranging from hypercholesterolemia to gingivitis; however, most of these are reversible.

Azathioprine

Azathioprine is a purine analog with immunosuppressive activities at the level of DNA. It is no longer considered as a single agent in corneal transplantation. Nowadays, its role in high-risk corneal transplantation is limited to systemic therapy as an adjunct to CsA (or tacrolimus) in resistant cases.

Experimental Approaches

Blocking the Activation and Action of T Cells

Monoclonal Antibodies to T Cell Antigens

The primary advantage of monoclonal antibodies is their specificity toward the target antigen and their safety profile. By blocking the effect of IL-2, they inhibit the proliferation of T cells. Monoclonal antibodies against CD3, CD4, CD8, IL-12, and αβ T cell receptors have beneficial effects after parenteral administration in experimental animal models [62]. Daclizumab (Zenapax) and basiliximab (Simulect) are both directed against the alpha subunit (Tac/CD25) of the IL-2 receptor of activated T cells. When compared to systemic CsA, a lower efficacy with improved side-effect profile was found for basiliximab [63]. As experience accumulates, these biologically active agents are likely to play a more important role in immunosuppressive regimens in high-risk corneal transplant.

Blocking of Co-Stimulatory Signals

The activation of T cells is dependent on co-stimulatory signals. A main co-stimulatory signal is between T cell CD28 with APCs-B7 molecules. Inhibiting the CD28 and B7 interaction using a B7 high affinity-bonded recombinant fusion protein, CTLA4-Ig, has resulted in increasing the allograft survival [64]. Both topical administration of CTLA4-Ig in rats and systemic anti-CD28 monoclonal antibody have graft-surviving effects [65]. In addition, it has been shown that former incubation of rabbit corneal graft with CTLA4-Ig improves the graft survival in high-risk, but not low-risk, cases [66].

Regulating the Immune Reaction Using Cytokines and Peptides

Transferring both IL-4 and IL-10 genes into graft epithelial layer led to increased corneal graft survival. These cytokines inhibit the activation of Th1 cells and turn the T cells toward a Th2 phenotype [67–69]. Hamrah and colleagues reported that regional treatment of host mouse eyes with α-melanocyte-stimulating hormone (as an immunomodulatory peptide) resulted in prolonged corneal allograft survival [70].

Inhibition of the Antigen-Presenting Cells

An approach for inhibiting presentation of antigens is to decrease the amount of donor LCs and DCs in corneal grafts. This method, in animal models, has decreased the incidence of graft rejection [71]. The effects of donor graft epithelium removal in low-risk keratoplasty, which leads to eliminating donor LCs in addition to diminishing HLA introduction, were

examined in a randomized clinical trial but found to have no effect on the rate of immune rejection [72].

Inhibiting Immune Access to the Graft

Immune Cell Trafficking The host effector immune cells invade the transplant via cell adhesion molecules found at corneal inflammation sites [73]. Systemic administration of antibodies against intercellular adhesion molecule (ICAM)-1, leukocyte function antigen (LFA)-1, very late antigen (VLA)-1, and VLA-4 in mice has resulted in inhibition of graft rejection [74–76]. It will be interesting to see if an upcoming new topical therapy for dry eyes, (Lifitegrast), which similarly inhibits adhesion molecules [77], will have any effect on graft rejection.

Chemokines are similarly recognized as important mediators of immune cell trafficking in corneal graft rejection. In a study by Hamrah et al., the survival rates of corneal grafts were measured in various types of chemokine and chemokine receptor knockout mouse models. The results demonstrated that corneal allografts in CCR1 knockout mice survive better than grafts in wild-type recipients [12]. Recently, CCR7 (−/−) grafts led to fewer migrating donor antigen-presenting cells and IFN-gamma producing T cells in draining lymph nodes when compared to wild-type grafts, demonstrating the importance of CCR7 in host T cell priming [71].

Attenuating Corneal Neovascularization Hemangiogenesis and lymphangiogenesis of the cornea enhance the migration of immune cells and the development of rejection. Attenuating corneal neovascularization by argon laser photoablation, cryotherapy, and fine needle diathermy has resulted in decline in vessel formation, but re-treatment is often necessary owing to the short-term effects. In recent years, availability of anti-angiogenic agents has led to significant interest in treating corneal neovascularization with anti-VEGF locally (topical, subconjunctival, and intrastromal) [6••, 78–82, 83•]. In animal models, VEGF neutralization improves the survival of corneal allografts [84–85, 86••]. Clinically, bevacizumab, a monoclonal antibody inhibiting VEGF, has been used most frequently for the treatment of corneal neovascularization in the pre- and postoperative period [13, 78, 81, 83•, 87–88, 89••]. It can potentially reduce the inflammatory process and prevent corneal graft rejection [90•]. It has been shown that bevacizumab inhibits not only angiogenesis but also lymphangiogenesis in mice [91]. Some authors have described the use of bevacizumab for interface and/or stromal vessels after deep anterior lamellar keratoplasty (DALK) [92–94]. Anti-VEGF therapy can be combined with other anti-angiogenesis modalities including fine needle diathermy [81].

In one study, the efficacy of topical bevacizumab (5 mg/ml five times per day on average) was examined in 30 eyes of 27 patients with progressive corneal neovascularization owing to various corneal illnesses. All of these patients were not responding to routine anti-inflammatory treatments. On average, 61% of vascularization area and 24% of vessel diameter were decreased following topical bevacizumab therapy [95]. Furthermore, meta-analysis of human clinical studies revealed that the corneal neovascularization area attenuated by 32% after subconjunctival injection, 48% after topical treatment, and 36% overall following anti-VEGF administration [96•]. Thus, simultaneous administration of topical and subconjunctival bevacizumab might result in better corneal graft survival rate in most of high-risk patients [88]. In several animal studies, application of topical and subconjunctival ranibizumab caused regression of corneal neovascularization and prevented graft rejection [97–100]. While studies to determine the precise role of anti-VEGF agents in high-risk keratoplasty are still ongoing, safety is also the concern. There is a report of recipient bed melt and wound dehiscence after penetrating keratoplasty and subconjunctival bevacizumab injection [101].

Induction of the Allospecific Tolerance

Oral Immunization with Donor-Specific Alloantigens

Oral administration of antigen is an efficient way to diminish the immune reaction against several alloantigens. Feeding of animals with cells containing alloantigens led to tolerance induction. The mouse corneal epithelial and endothelial cells derived from donors were cultivated and orally administrated to recipients, which led to a 50% attenuation of the graft rejection rate [102].

Lymphadenectomy

The duration of corneal graft survival after high-risk corneal transplantation was doubled following investigational removal of mouse submandibular lymph nodes [103]. It was attributed to "immunologic ignorance," as prior sensitization abolished the effect of lymph node resection. Furthermore, migration of APCs to lymph nodes was inhibited by blocking the vascular endothelial growth factor receptor-3 (a procedure known as "molecular nonsurgical lymphadenectomy"), which led to uniform graft survival in all cases [104].

Gene Therapy

Genetic modulation of donor grafts using recombinant viral vectors has been shown to improve graft survival rates in animal models [105]. For instance, ex-vivo transfer of programmed cell death ligand 1 (PD-L1) gene using lentivirus into corneas significantly attenuated graft rejection via modulating immune cells that are infiltrating the graft. This outcome was associated with attenuation of cytotoxic CD8+ and

Table 1 Stepwise management of high-risk keratoplasty

1. Potent and frequent topical steroids—continued indefinitely on a tapering dose
2. Topical tacrolimus (or CsA) as adjunctive therapy—continued indefinitely
3. Systemic steroids peri-operatively
4. Systemic mycophenolate (azathioprine or rapamycin alternative)
5. Systemic tacrolimus (or cyclosporine) in addition to mycophenolate

natural killer T cells, as well as reduction of pro-inflammatory cytokine expression [106]. Therefore, gene transfer approaches may provide an opportunity to reduce the immunologic response to allograft without significantly compromising endothelial cell viability.

Conclusion

Immune-mediated graft rejection is the most frequent cause of corneal transplantation failure. The risk factors for graft failure are corneal vascularization, prior graft rejection and failure, young age, ocular surface disease, previous intraocular surgery, history of anterior segment inflammatory disease and synechia, history of herpes simplex or zoster keratitis, and glaucoma. The current recommended approach is summarized in Table 1. Preoperative MHC and non-MHC antigens matching are still controversial. New modalities including anti-VEGF therapy, modulating the immune response with biological agents, are exciting approaches that remain under investigation.

Compliance with Ethical Standards

Conflict of Interest Ali Djalilian, Sayena Jabbehdari, Alireza Baradaran-Rafii, Ghasem Yazdanpanah, Pedram Hamrah, and Edward Holland declare no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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•• Of major importance

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