Review Article

Management and prevention of corneal graft rejection

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The management of an episode of corneal graft rejection (CGR) is primarily by corticosteroids. Immunomodulators are useful for long-term immunosuppression and in dealing with cases of high-risk (HR) corneal grafts. The classical signs of CGR following penetrating keratoplasty (PKP) include rejection line, anterior chamber (AC) reaction, and graft edema. However, these signs may be absent or subtle in cases of endothelial keratoplasty (EK). Prevention of an episode of graft rejection is of utmost importance as it can reduce the need for donor cornea significantly. In our previous article (IJO_2866_22), we had discussed about the immunopathogenesis of CGR. In this review article, we aim to discuss the various clinical aspects and management of CGR.

Key words: Corneal graft rejection, endothelial rejection, graft failure, immune privilege, keratoplasty

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The incidence of CGR following PKP depends on several factors such as the indication for which it is performed, recipient age, and donor-related factors. The reported incidence varies widely; however, an analysis of studies, including a large cohort, suggests it ranges between 19% and 41%.[1-6] The incidence of rejection is usually high in the first 1–3 years post-transplantation,[3,6,7] however a rate of 23% has been reported at 15 years.[8]

Collaborative Corneal Transplantation Studies (CCTS) have evaluated the effect of donor-recipient major histocompatibility complex (MHC) matching and cross-matching on corneal transplant survival in high-risk (HR) keratoplasty.[9] It estimated that 30% of grafts fail due to rejection by three years in the ABO-incompatible group, whereas 16% fail in the ABO-compatible group.[4] According to the Australian corneal graft registry (ACGR), of the 20,336 grafts registered, 5,687 (28%) grafts have failed due to various reasons, out of which 1,594 (28%) are due to graft rejection.[5] In a study by Williams *et al.*,[6] the overall 10-year survival estimate was found to be 65%. The survival rate was best with keratoconus (95%), followed by corneal dystrophies (55%), infectious leukomas (49%), trauma (33%), and chemical burns (14%). A primary graft had a survival rate of 81%, and second and third grafts had survival rates of 33% and 16%, respectively.[6]

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Risk factors

Donor factors

The various factors implicated are donor age, MHC compatibility, ABO compatibility, and eye banking practices.[6,9,10] Donor age has no impact on graft rejection incidence except when the donor is too young (<10 years).

The role of human leukocyte antigen (HLA) matching in CGR is controversial. According to CCTS, HLA matching did not affect overall graft survival, the incidence of irreversible rejection, or rejection episodes. The feasibility of performing HLA matching before every corneal transplant is also a matter of concern. Besides, the major long-term studies on corneal transplants such as the ACGR and CDS did not explore the impact of HLA matching. Similarly, the role of matching for ABO compatibility is also controversial. Major studies published so far do not recommend ABO matching before corneal transplantation.[9,11-13] It is important to note here that the result of the studies on HLA typing and ABO compatibility must be interpreted carefully because these studies suffer from limitations of variability in the outcome measures analyzed (such as graft survivability rather than rejection-free rate), or inclusion of different cohort (normal risk vs. high-risk keratoplasty) and testing methods for HLA matching.[14]

Factors associated with eye banking such as preservation status, death enucleation time, the time between death to transplant, death to graft time, type of storage media used, and

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factors related to the processing of the tissues do not have any impact on the graft rejection rate.[4,15,16]

Host factors

In contrast to donor factors, host factors significantly impact the occurrence of CGR. Much understanding about these host factors comes from the ACGR, the Swedish Corneal Transplant Register (SCTR), the UK Transplant Registry (UKTR), and the CDS.[16-19] These factors include primary diagnosis, recipient age, sex, previous transplant, corneal neovascularization, recent surgery, glaucoma, anterior synechiae, and lens status.

Primary diagnosis is a significant factor for subsequent graft rejection. Corneal opacity due to infectious or traumatic causes can be associated with an increased risk of rejection.[5,20]

Corneal lymphangiogenesis and neovascularization will compromise the inherent corneal immune privilege, thereby increasing graft rejection risk. This is reflected in the decreased chances of graft survival with higher quadrants of vascularization in the ACGR study group. The graft survivability was 83% at four-year follow-up in the absence of neovascularization while it was 73%, 66%, 63%, and 50% in the presence of 1-, 2-, 3-, and 4-quadrant neovascularization, respectively.[19] Khodadoust and Karnema noted the rejection frequency to be 3.5% in avascular, 13.3% in slightly vascularized, 28% in fairly vascularized, and 65% in a very vascularized cornea. Risk of rejection increases with the number of quadrants of corneal neovascularization as well as with the total number of vessels crossing the graft host junction.[21]

Recipient age has been proposed to be a risk factor for graft rejection and several reports suggest young recipient age to be a risk factor for CGR.[22,23] CCTS found recipient age <40 years to be associated with an increased chance of rejection-associated graft failure.[24]

Corneal regraft puts the graft at a high risk of CGR as the host is allo-sensitized, and the previous graft has already breached the corneal immune privilege.

A previous history of glaucoma is a risk factor for CGR. Several studies have reported poor graft survival in cases with a history of glaucoma.[16,19,25]

Recent intraocular surgery could also increase the risk of graft rejection due to a breach in the blood-aqueous barrier. The associated inflammation leads to the migration of immune cells and inflammatory mediators to the graft bed which further compromises the immune privilege of the cornea and anterior segment.[24] The presence of anterior synechiae exposes the donor endothelium to blood vessels that may increase rejection risk.[24]

Surgery-related factors

Graft size, lens status, and anterior vitrectomy all have been predicted to be potential factors for graft rejection.[10,24,26,27] However, most of the studies have reported the impact of these factors on graft survival instead of graft rejection. Both large and small grafts could increase the chances of graft rejection.[24] A large graft or an eccentric graft increases the chances of rejection by increasing the donor cornea's proximity to the host limbal vasculature and lymphatics in the limbal marginal plexus of blood vessels leading to an increased transfer of mature donor antigen-presenting cells (APCs).[20]

Postoperative factors

Suture-related problems such as loose sutures/broken sutures/exposed suture knots could lead to constant irritation with subsequent vascularization and graft rejection.[15,28,29] Formation of anterior synechiae, especially at the graft host junction in the postoperative period, could elicit graft rejection. Subsequent surgical intervention or suture removal can also elicit graft rejection.

High-risk graft

The CCTS defined "high-risk cornea" as a cornea with vascularization of two or more quadrants extending at least 2 mm into the stroma or previous graft rejection in the affected eye.

Clinical presentation

The clinical presentation of CGR varies depending upon the type of rejection. The following discussion is about the clinically important endothelial rejection. Most episodes of CGR occur within the first six months of the surgery, though it can occur any time after surgery. The symptoms of CGR include redness, decreased vision, light sensitivity, or discomfort. Almost 30% of cases may be asymptomatic.[30]

The signs of acute CGR reflect the underlying pathological changes such as vascular dilation, vascular transudation, cellular infiltration, and tissue edema.[31] Circumciliary congestion is often the earliest manifestation of CGR.[32] Cellular infiltration can manifest in the form of a cellular reaction in the anterior chamber (AC) or the cornea itself. Discrete subepithelial infiltrates similar to those seen in adenoviral keratoconjunctivitis are seen in 10–15% of cases either in isolation or with associated signs of endothelial rejection. The cellular infiltrate in the form of endothelial keratic precipitates (KPs) can occur as scattered lesions or linear deposits. The linear form, also known as the Khodadoust line, consists of a linearly oriented wave of white blood cells starting from the peripheral cornea, extending towards the center, and is the pathognomonic sign of CGR. The Khodadoust line separates immunologically damaged endothelium associated with corneal edema from the unaffected endothelium [Fig. 1]. The cellular infiltrate into the AC manifests as cells in aqueous humor. The uveal tissue vessels are leaky owing to the vasodilation and result in leakage of proteins into the AC in the form of flare.[32] Tissue edema manifests in the form of arcuate stromal or segmental or differential corneal edema, which is another hallmark feature of CGR.[30,33] If left untreated, the edema can involve the entire cornea and could indicate early endothelial failure.[32] Apart from the classical signs, CGR can present with unusual manifestations like a rise in intraocular pressure (IOP) and a new onset epithelial defect. These unusual manifestations are often common in the pediatric age group.[34]

Types of graft rejection

CGR can be classified according to the anatomical layers affected, such as epithelial, stromal, and endothelial rejection. Epithelial and stromal rejections are usually self-resolving and respond to steroids. Their clinical significance lies in the fact that they could precede an episode of endothelial rejection. The various types of CGR have been summarized in Table 1.

Management

Immediate

The outcome of CGR depends on early diagnosis and immediate steroid initiation.

Systemic Steroids

In cases of early presentation with severe endothelial graft rejection, intravenous steroids are useful. The basis for pulse steroid therapy is that it leads to a profound drop in circulating lymphocytes. The lymphocyte drop is maximal at 4–6 h, with recovery by 48 h. Hill and coworkers found that pulse therapy with a single dose of the intravenous pulse

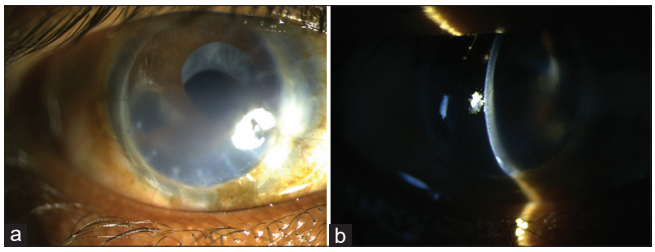

Figure 1: (a) & (b) - Combined stromal and endothelial graft rejection 1 year following penetrating keratoplasty showing differential graft edema with stromal haze in the inferior half of the graft

Table 1: Differentiating features among various types of corneal graft rejection

Type of rejection	Clinical features
Epithelial	Eye is often quiet, asymptomatic, or mildly inflamed and self-resolution occurs in most cases. Epithelial rejection line (elevated, undulating line that stains with fluorescein and Rose Bengal dye); starts in the periphery from vascularized margin of the graft, and gradually progresses to the center of the graft over a few days; consists of an intense leukocytic (predominantly polymorphonuclear) infiltration.
Chronic stromal or subepithelial infiltrates	Mimics lesions seen in epidemic adenoviral keratoconjunctivitis. Lesions are whitish, 0.2 to 0.5 mm in diameter, located in the anterior stroma, and restricted to the graft in absence of conjunctivitis. Disappear with topical corticosteroid treatment leaving faint subepithelial scars rarely.
Stromal	Usually seen following lamellar keratoplasty. Isolated peripheral stromal infiltrates and haze in a previously clear graft, adjacent to an area of vascularization.
Endothelial	Most common and most serious form. Characteristic triad of Khodadoust line, AC cells, and corneal edema.

of 500 mg methylprednisolone improves the reversibility of rejection to 80% compared to 39% with hourly topical steroid therapy alone.[35] In a subsequent study, the author compared single (n = 31) versus double (n = 30) versus three (n = 29) doses of intravenous injection of 500 mg methylprednisolone (IVMP) in 250 ml saline or dextrose over at least 30 min at 24 h interval, where symptoms were present for <3 weeks. All three treatment protocols had equal efficacy in reversing the rejection episode with rates of 74.2%, 79.3%, and 83.3%, respectively. Besides, there was no statistical difference in graft survival between the groups. The signs of reversal include improvement in clinical signs, return of graft clarity, reduction in corneal thickness, and improvement of visual acuity. Few authors have proposed intravenous dexamethasone as a cost-effective alternative to IVMP. In a retrospective, non-randomized, interventional case series, Tandon *et al*.[36] compared the outcome between 500 mg IVMP (n = 51) and 100 mg IV dexamethasone in 150 ml of 5% dextrose solution as a slow infusion over 1–2 h as a single-pulse therapy (n = 47). Dexamethasone had better outcomes than IVMP in terms of reversibility of graft rejection (72.3% vs. 45%), post-treatment best-corrected visual acuity (BCVA), and lower pachymetry values. The authors hypothesized that dexamethasone's better outcome could be due to a greater glucocorticoid activity and a comparatively longer biological half-life (24–72 h) compared to that of methylprednisolone (12–36 h).

Topical/intracameral/intravitreal

They are the mainstay of therapy for all forms of graft rejection. In cases of endothelial rejection, 1% prednisolone acetate is administered every 1–2 h for the first few weeks followed by gradual tapering over 2–3 months once signs of resolution appear. In high-risk cases or one-eyed patients, low-dose steroids can be continued lifelong. For cases other than endothelial rejection, topical steroids are given 4–6 times a day, followed by tapering over 6–8 weeks. In cases of therapy-resistant endothelial allograft rejection, intracameral injection of triamcinolone acetonide (4 mg in 0.1 ml) may be an additional treatment modality.[37] Birnbaum *et al*.[38] suggested the intracameral application of corticosteroids by means of an AC flush as an adjunctive measure. You *et al*.,[39] investigated the efficacy of intravitreal triamcinolone acetonide (IVTA) injection in the treatment of endothelial graft rejection. Intravitreal dexamethasone implant can be used in refractory cases of CGR, however, no randomized controlled trial (RCT) has been performed to assess the efficacy of this therapy.[40]

Supportive therapy

It includes the administration of prophylactic antibiotics, cycloplegics, topical lubricants, and anti-glaucoma medications depending upon the associated symptoms, signs, and IOP.

Long term

Corticosteroids

Long-term systemic steroids are not recommended due to the risk of serious complications. However, topical steroids can be continued for a long time.[41-43]

Steroid-sparing agents

(a) *Cyclosporine:* The role of topical cyclosporine in the treatment of CGR is limited. Different cyclosporine concentrations such as 2%, 0.5%, and 0.05% have been evaluated in the past. Javadi *et al*.[44] did not find any advantage of adding 2% topical cyclosporine to topical steroids in treating or preventing CGR in high-risk (history of rejection episodes) cases. Thus, it appears that cyclosporine A has a limited role in treating CGR, except for cases where the conventional treatment fails.

(b) Tacrolimus (FK506), Mycophenolate Mofetil (MMF): These are primarily to prevent an episode rather than treat an acute episode of CGR. Systemic tacrolimus (2–12 mg daily) has been shown to reduce graft rejection in HR corneal transplantation, with a graft survival of 65%.[45] MMF has been demonstrated to be effective and safe in kidney and heart transplantations at a dose of 3 g per day and for liver transplantation using a dose of 2 g per day.[46]

CGR following Anterior lamellar keratoplasty (ALK)

The incidence of CGR following anterior lamellar ALK depends on various factors such as duration of postoperative corticosteroid regimen, recipient age and race, type of trephination, vascularization, and timing of suture removal. The reported graft rejection rates following deep anterior

lamellar keratoplasty (DALK) range between 0% and 20%, with a mean follow-up ranging from 12 to 77 months.[24,47-57] The rejection episodes usually occur within the first 2 years of the transplant.[24,52,57-59] In patients with herpes simplex keratitis, the incidence of stromal rejection varies from 0% to 50%.[60-63] Borderie *et al*.[64] reported a 10% rate of stromal rejection at 3 years in 149 ALK eyes with a longer duration of corticosteroid regimen (7 months).

Risk factors

Recipient factors

Corneal neovascularisation of the recipient bed is the single most important risk factor for rejection.[24] The relative risk of rejection is reported to be almost 3-fold higher for black patients.[65] Younger age is associated with an increased risk of rejection owing to a more active immune response. Atopic patients are at a greater risk of ocular inflammation because of the release of inflammatory mediators.[66] Studies have shown that the long-term outcomes of DALK in patients with vernal keratoconjunctivitis (VKC) are comparable with non-VKC patients, however, the immediate postoperative course such as suture-related problems, early vascularization, and epithelial healing problems can be more in VKC patients who needs close monitoring.[67-69]

ACGR reports have suggested inflammation and a history of steroid use to be affecting graft survival significantly.[19] A history of PKP in the fellow eye might increase the risk of rejection.

Surgery-related factors

These are similar to those of PKP, however a 3-fold higher relative risk of graft rejection episode after DALK with manual trephinationh as been rep orted in literature compared to femtosecond laser-assisted DALK.[70]

Clinical presentation

The patients remain asymptomatic usually but can present with symptoms such as redness, pain, photophobia, and a decrease in visual acuity. They may appear as isolated peripheral stromal infiltrates and haze in a previously clear graft, adjacent to an area of vascularization [Fig. 2].

Management

Treatment of graft rejection depends on the type of rejection; however, in all cases, topical corticosteroids are the mainstay of treatment.

Following DALK, endothelial immune reactions cannot occur and the donor cornea may suffer epithelial, subepithelial, and stromal rejection only. Previous inflammation such as VKC and herpetic eye disease significantly increases the risk of stromal rejection. Steroids are the treatment of choice and several studies have shown a prolonged steroid therapy following DALK can prevent an episode of stromal rejection. An undetected episode of stromal rejection can lead to interface haze and interface vascularization. Hence, a close monitoring of such cases to detect early cases of graft rejection is recommended.[71,72]

CGR following endothelial keratoplasty (EK)

The risk of graft rejection and rejection-related graft failure is lower after EK compared to PKP. Several factors such as a lower antigenic load, absence of sutures, and a lack of exposure of the graft to immune mediators present on the ocular surface could be responsible for such a low rate. Price *et al*.[65] have reported Descemet stripping automated endothelial keratoplasty (DSAEK) rejection rates of 7.6% at 1 year and 12.0% at 2 years. Anshu *et al*.[73] compared the relative risk of immunologic rejection episodes in patients who underwent Descemet's membrane endothelial keratoplasty (DMEK) (n = 141), Descemet's stripping endothelial keratoplasty (DSEK) (n = 598), and PKP (n = 30), and at 2 years it was 1%, 12%, and 18%, for DMEK, DSEK, and PKP group, respectively.

Risk factors

The risk factors for graft rejection following EK are similar to those for PKP. ACGR 2018 has reported a significant difference across groups ($p < 0.001$), with grafts performed for Fuchs endothelial corneal dystrophy (FECD) having significantly better survival than those performed for failed graft, bullous keratopathy, and trauma-induced endothelial decompensation.[19] Postoperative cessation of steroids has been found to be a risk factor for the development of DSAEK rejection.[74]

Clinical presentation

Endothelial immune responses following DSEK/DSAEK/DMEK are clinically more subtle than after PKP. Price *et al*.[65] have reported 35% of the patients to be asymptomatic following rejection in DSEK and the immune reaction was only diagnosed during a routine check. Patients will present with visual disturbances and iritis symptoms such as pain, redness, photophobia, and watering but not as severe as following PKP. The signs are also not as prominent as PKP. The various signs reported in endothelial rejection following DSEK include isolated KPs, which might be focal or diffuse (60–70%), corneal edema (10–25%), and AC cells (25%) [Fig. 3]. Khodadoust lines are rare, but can occasionally occur.[75] These precipitates could also occur on peripheral corneal areas that were initially denuded by descemetorhexis but not covered by the donor graft. Most rejection episodes following DMEK are subtle. The various manifestation includes conjunctival injection, diffuse or sectoral corneal edema, fine KPs restricted to the donor endothelium, and mild AC inflammation. Monnereau *et al*.[76] have shown that in postoperative cases of DMEK, changes in endothelial cell morphology might be detected even before an allograft rejection occurs. Thus, serial specular microscopy in the postoperative period may help in the early detection of cases of graft rejection following DMEK.

Corneal endotheliitis caused by herpes simplex virus postoperatively often possess a challenge in diagnosis. Several points may help in differentiating an episode of viral endotheliitis from graft rejection such as history, focal distribution of KPs, presence of KPs on both host and donor endothelium, associated iritis, and IOP rise.

Management

The management of graft rejection is similar to that following PKP.

Prevention of CGR

Surveys done among corneal surgeons have shown variability in the approach to the prevention and treatment of allograft rejection. These practice patterns have been evolving with time due to the availability of newer topical and systemic agents.[77] Topical prednisolone was deemed a vital drug both for the prevention and treatment of graft rejection, in the cornea society survey of 1989.[78] But, a 2004 survey showed an inclination towards the use of topical cyclosporine and loteprednol.[79] A 2011 survey on the current practice patterns of the cornea society, showed very little change from previous practice.[77]

The regimen employed in the prevention of rejection depends on the surgeon, geographic location, and the risk

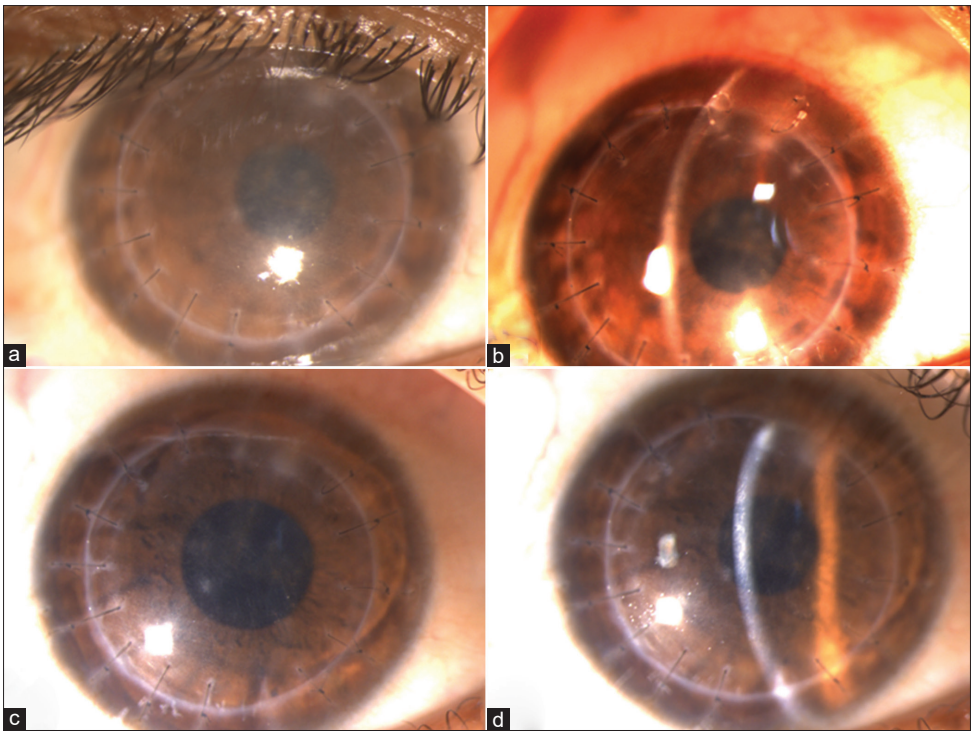

Figure 2: (a) & (b) - Stromal graft rejection following anterior lamellar keratoplasty showing graft edema with diffuse stromal haze. (Note the loose sutures at 12 and 1 o'clock, which was responsible for the rejection); (c) & (d) - Complete resolution of the graft edema after intensive therapy with topical steroids

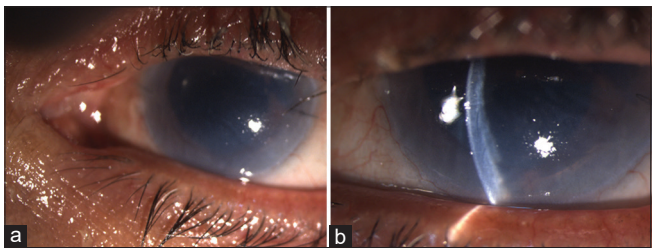

Figure 3: (a) & (b) - Endothelial rejection following DSAEK showing differential edema of the donor lenticule (inferior)

category of the transplant. Preventive therapy targets these basic pathophysiological processes [Fig. 4]:

Reduction of corneal neovascularization

Laser photocoagulation

Argon laser therapy reduces corneal vascularization[80] by obliterating efferent vessels which are thin and deep and have faster blood flow. Complications such as damage to the corneal endothelium, crystalline lens, suture lysis, corneal hemorrhage and thinning, crystalline deposits on the iris, iris atrophy, and pupillary peaking are associated.[69]

Photodynamic therapy[81] is minimally invasive that obliterates the neovascular network with no collateral damage but several sessions may be required.[82]

A recent study by Gerten *et al.*,[83] has shown a combination of bevacizumab and argon laser therapy to cause a marked decrease in corneal neovascularization. Argon laser therapy obliterates the mature pathological vessels while bevacizumab prevents further angiogenesis.

Fine needle diathermy

Fine needle diathermy, a cheaper alternative to laser, involves the use of a monopolar diathermy unit. With the lowest power setting, each vessel can be treated individually. This method can obliterate both afferent and efferent vessels at varying depths with equal efficacy.[84] Complications include intrastromal bleeding, crystalline deposits, transient opacification of the corneal stroma, and the development of striae.[85] The latter two effects are transient and resolve in 24 to 48 h.[86] This technique has been refined further, using an electrolysis needle that is much more flexible and precise. It may also stimulate further vascularization via the release of proangiogenic factors.[87] Angiography-guided fine needle diathermy can be used to selectively treat feeder vessels with minimal thermal energy.[88]

Anti-vascular endothelial growth factor (VEGF)

Anti-VEGF agents work by preventing new blood vessel formation or angiogenesis by inhibiting VEGF production.[89] This might not be effective in individuals with mature blood vessels as they are rich in pericytes and pro-angiogenic factors are minimal in these cases.[90] Table 2 summarizes various types of anti-VEGF and their mechanism of action.

Topical steroids

Topical steroids are the mainstay of treatment for actively proliferating corneal neovascularization. Effects of topical cortisone, dexamethasone, and prednisolone have been studied extensively.[94] When supplemented with heparin and cyclodextrins, their effects are amplified manyfold.[95] Steroids work best when administered directly after or before corneal surgery, by inhibiting pro-inflammatory cytokines, inhibiting vascular dilatation, and eliminating lymphocytes.[96] The side-effects associated with long-term steroid use cannot be overlooked which can be glaucoma, cataract, and increased risks of infections.[97]

Role of Immunosuppression

Although the cornea is immune privileged, CGR is the most common cause of graft failure. Therefore, prophylactic immunosuppression is routinely practiced post-keratoplasty, especially in high-risk cases.[98]

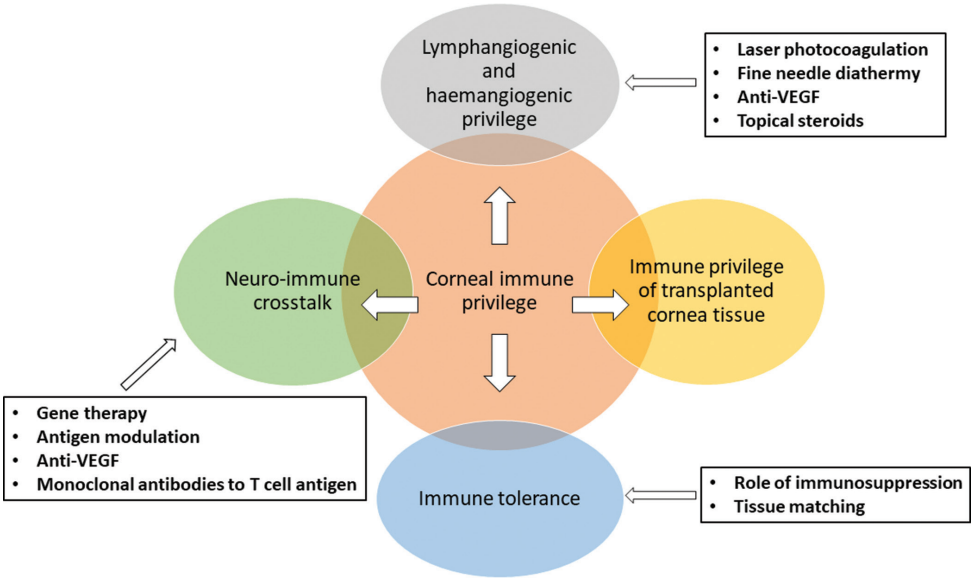

Figure 4: Flowchart showing preventive therapy targets of the basic pathophysiological processes involved in corneal graft rejection

Topical drugs

Corticosteroids: The practice of using topical corticosteroids for the first six postoperative months is universal. This is the primary cause for the low frequency of rejection in this period.[20] Prednisolone is the most commonly used drug regardless of the risk status or the lens status. Other commonly used preparations include difluprednate, loteprednol, and fluorometholone.[77]

Cyclosporine A (CsA): The beneficial effects of topical CsA in keratoplasty include reduction in migration of T lymphocytes, suppression of corneal neovascularization, and increasing the number of goblet cells and tear production.[99] However, the role of CsA in the prevention of graft rejection is controversial as several studies have conflicting results.[100]

Tacrolimus: Topical tacrolimus is available in two concentrations 0.1% and 0.03%. Dhaliwal *et al*.[101] in their study on long-term efficacy and side-effects of topical tacrolimus, 0.03% ointment, found it to be a promising second-line immunosuppressant in the management of high-risk grafts.

Systemic therapy

Systemic therapy is advocated as prophylaxis in patients with a high risk of rejection.[25]

a. *Corticosteroids:* Oral corticosteroids can be administered at any time during transplantation, which is in the pre-operative, intraoperative, or postoperative period. There is no consensus at present on the best time to initiate systemic steroids and their dosage is based on the body weight of the patient.[102] It can also be combined with other immunosuppressive agents to prevent graft rejection in high-risk cases.[79] The usual dose is 60–80 mg daily (depending on body weight); however, the duration of treatment is widely variable, ranging from 1 day to 12 months.[103]
b. *Cyclosporine A:* It acts by decreasing the synthesis and secretion of cytokines by inhibiting the interleukin (IL)-2 pathway.[104] In high-risk cases, systemic CsA is effective in preventing graft rejection.[105] Its eventual concentration in the aqueous humor is far lower than that in plasma. The initial dosage is usually 3–4 mg/kg/day, with subsequent titration depending on the severity and incidence of rejection attacks.[99] A study by Inoue *et al*.[106] did not find any effect of oral CsA in reducing the incidence of graft rejection on long-term use in high-risk keratoplasty patients. Liver and kidney toxicity, raised blood, hirsutism, gingival hyperplasia, neurotoxicity, and reactivation of latent tuberculosis have been described.[99]
c. *Tacrolimus:* Tacrolimus is a macrolide, which binds to the FK506 binding protein and inhibits the activated T cell by blocking the calcineurin pathway. Systemic tacrolimus has been reported to reduce graft rejection episodes in high-risk cases, with a graft survival rate of 65%.[107] Yamazoe *et al*.[108] found that a dosage of 10–20 ng/ml significantly reduces graft rejection episodes and prolongs graft survival. It causes better suppression of allo-immunity in comparison to cyclosporine but the adverse effect of renal dysfunction limits its usage. Complications that can occur with tacrolimus, besides nephrotoxicity include hypertension, hyperglycemia, paresthesia, tremors, headache, and fatigue.[99]
d. *MMF:* Its active substance mycophenolic acid (MPA) inhibits inosine monophosphate dehydrogenase, which inhibits the de novo synthesis of guanosine nucleotides, resulting in selective inhibition of T and B lymphocyte proliferation. In a study by Reinhard *et al*., MMF showed a rejection-free rate of 89% as opposed to 67% in the control group during the first year of surgery. No significant difference was found between the two groups with respect to graft failure.[109,110] However, a retrospective study by Birnbaum *et al*.[111] reported a significantly greater effect from MMF in comparison to cyclosporine to prevent graft rejection but no significant difference was found in terms of graft survival. Side-effects include gastrointestinal toxicity, bone marrow suppression, arthralgia, and infection.[110]
e. *Rapamycin:* Isolated from Streptomyces hygroscopicus, rapamycin forms a complex with the immunophilin FK binding protein and this complex then inhibits the mammalian target of rapamycin (mTOR). It acts by decreasing IL-2-mediated activation of T lymphocytes. Its efficacy is similar to that of MMF as shown by Birnbaum *et al*.[112] The combination therapy is effective but is not well tolerated.[113] Most of the complications of rapamycin are reversible and are varied from hypercholesterolemia to gingivitis.
f. *Azathioprine:* It is a purine analog with immunosuppressive

Table 2: Various types of anti-VEGF, their mechanism of action, and side-effects

Name of anti-VEGF	Description	Mechanism of action	Remarks
Bevacizumab	Full-length humanized murine monoclonal IgG1 antibody that recognizes all isoforms of VEGF-A	Reduces the mean vessel diameter and vascularized area by 24% and 61% respectively.[91]	Delayed wound healing and progression of stromal thinning in higher doses (>1%) and longer duration (>1 month).[92]
Ranibizumab	Recombinant humanized monovalent, monoclonal antibody fragment that inhibits all isoforms of VEGF-A	Shrinkage of stable corneal neovascularization, in terms of vessel diameter and its area.	Expensive and superior to bevacizumab in terms of its onset of action and degree of efficacy.[93]
Aflibercept	Recombinant fusion protein which binds to VEGF-A and VEGF-B	Interacts with PDGF and placental growth factor and reduces ligand-induced activation of receptors.	-

activities at the level of DNA. It is no longer used as a single agent in corneal transplant but is used as an adjunct to cyclosporine or tacrolimus in resistant cases.[99]

Tissue matching

It is not a universal practice except in some countries for recipients who have a high risk of HLA class I and class II rejection.

Novel approach for immunomodulation in corneal transplantation

Immune privilege is lost after a high-risk transplantation. Therefore, newer, and more effective modalities are under evaluation targeting immunomodulation in these cases.

Gene therapy

Gene therapy can be used to modify allografts ex vivo, as corneal tissue allows its storage in culture for 4 weeks without significant loss of function. Recombinant viral vectors like adenovirus, lentiviruses, and adeno-associated viruses are used as gene transfer vehicles.[114] The efficiency of gene transfer into corneal cells is very low.[115] Adeno-associated viruses and lentivirus have more long-term gene expression in target cells due to their low immunogenic profile and their integration into the cellular genome.[116] Lentivirus-mediated overexpression of PD-L1 in cultured corneas significantly prevents CGR by modulating graft infiltrating cells, associated with a reduction of natural killer T cells and cytotoxic CD8 + T cell infiltration and attenuation of pro-inflammatory cytokine expression.[117] IL-10 gene transfer and inhibition of pro-inflammatory cytokines cytokine IL-12 have shown varying results.[118] IL-10 gene transfer mediated through adenovirus in cultured corneas led to a significant prolongation of graft survival in outbred sheep corneal transplant model. Over-expression of adenovirus-associated neurotropic growth factor (AdNGF) in ex vivo cultured corneas significantly prolonged corneal allograft survival in a rat transplant model.[119] OXB-202 (previously known as EncorStat) is a donor cornea modified before transplant by ex-vivo genetic modification using a lentiviral vector. These genes encode angiostatic human proteins, endostatin and angiostatin, which prevent rejection by suppressing neovascularization.[120]

Antigen modulation

Corneal tissues contain a resident population of APCs. These APCs utilize new vessels infiltrating the graft bed to migrate to draining lymphoid tissue and activate host allo-reactive T cells, inciting a cascade that leads to rejection.[20] Treatment of donor corneas with interleukin-10 and transforming growth factor- ß1 altered the phenotype and function of APCs that reside in these tissues. These APCs enriched corneas on transplantation, reduce the frequencies of interferon-gamma and effector T cells, cause allo-sensitization in the hosts, and diminished graft infiltration of CD45+ and CD4+ cells, thereby increasing corneal allograft survival.[121]

Nanoparticles: In a recent study, Iriyama *et al*.[122] used micellar nanovectors, made of polyethylene glycol (PEG)-b-polycation copolymers containing ethylene diamine units in their side chains, as non-viral gene vectors. Compared to control mice, nanovector-treated mice showed a 45% decrease in experimentally induced vascularization of the cornea. Nano-micelle-modified rapamycin, when used in mouse models, prolonged the allograft survival by more than 2 months, with less inflammatory infiltration, decreased production of pro-inflammatory factors, and elevated recruitment of myeloid-derived suppressor cells (MDSCs). This then inhibits the proliferation of CD4 + T cells through increased expression of inducible nitric oxidase and arginase-1.[123] Recently, tacrolimus-loaded nanoparticles, methoxy poly (ethylene glycol-block-poly (D, L)-lactic-co-glycolic acid) were studied in rats for pharmacokinetics.[124]

Anti-VEGF

In addition to regulating angiogenesis, VEGF plays an important role as a modulator of the immune microenvironment. Targeting blood and lymphatic vessels which are trafficking potentially alloreactive immune cells into the graft, is a logical treatment strategy in a pre-vascularized high-risk corneal transplant.[20] VEGF-trap aflibercept, an anti-hemangiogenic drug, was found to be significantly more effective in long-term graft survival, as compared to anti-VEGF-C (VGX-100, anti-lymphangiogenic) and sVEGFR-3 (VGX-300, anti-lymphangiogenic), in a murine model of high-risk transplantation. In comparison with untreated controls, all approaches improved survival.[125] Local blockade of VEGF-A in the inflamed cornea of mice by treating them with VEGFR1/R2 trap increased the expression of pro-inflammatory as well as immune regulatory cytokines in the corneal microenvironment. It significantly inhibited the infiltration of CD11c + dendritic cells into the cornea and caused local upregulation of Foxp3 gene expression.[126]

Sorafenib, a potent inhibitor of RAS/RAF kinase and tyrosine kinases such as VEGFR-2, PDGFR-ß, and VEGFR-3 is in use as an anti-cancer drug that aims at tumor proliferation and neovascularization. Sorafenib was compared with dexamethasone, dimethyl sulfoxide, and phosphate-buffered saline, all administered subconjunctivally, in high-risk PKP done in a murine model. The sorafenib group showed reduced expression of VEGF-C, tumor necrosis factor-alpha, interleukin-6, VEGFR-2, and VEGFR-3.[127] Combined subconjunctival and topical bevacizumab treatment increased survival in high-risk corneal grafts, as studied in prospective, consecutive, interventional case series of 50 human eyes.[128]

Monoclonal antibodies to T cell antigen

Despite being safe and specific to the target antigen, the use of monoclonal antibodies in corneal transplantation has been limited.[99] Experimental studies in animal models using anti-CD4 monoclonal antibodies in the perioperative period resulted in prolonging graft survival and increasing transplantation tolerance.[129] Intraperitoneal injections of anti-CD4 monoclonal antibody, given four times perioperatively, reduced the rejection rate of MHC mismatched corneal grafts in adult animals.[130] Injection of anti-T lymphocyte antibodies intracamerally has been shown to reverse the ongoing corneal rejection in animal and human models. But this has also resulted in severe intraocular inflammation in one case, probably due to localized cytokine release syndrome or complement activation.[131] Subconjunctival injection of anti-CD25 MAb causes prolongation of corneal graft survival in rats.[132] Anti-CD80 and anti-CD86 antibodies subconjunctival injection has been shown to prevent CD4 T cell-mediated herpetic keratitis.[133] The use of basiliximab and alemtuzumab against CD25/T cells and CD52/T cells, respectively, have been tried during the induction phase in two case reports with preventive and therapeutic effects in CGR. Abatacept against CD80, CD86/APC has shown a weak effect in animal models during maintenance therapy.[134] Basiliximab showed lower efficacy but had a more favorable side effect profile in comparison to oral cyclosporine in high-risk keratoplasty cases.[135]

Prevention and management of high-risk corneal graft are challenging. The above discussion includes agents tried both clinically as well as experimentally. Corneal anti-VEGF, fine needle diathermy, and laser photocoagulation can be tried if they are available. Immunomodulator therapy preferably MMF should be started in such cases. Postoperatively prolonged steroid regimen along with the continuation of immunomodulators can improve the long-term outcome of corneal transplantation in high-risk cases.

Conclusion

CGR is the most common cause accounting for the majority of graft failure cases. The management of an episode of CGR is primarily by steroids. The quest to find a suitable steroid-sparing agent has provided some alternatives but not as efficacious as steroids. Management of high-risk grafts offers significant challenges. The advent of new oral immunosuppressive agents like MMF has improved the outcome of corneal grafts in such cases. The clinical spectrum of CGR following EK offers a challenge in early diagnosis. A careful slit lamp examination and pachymetry can provide a clue in such cases. The outcome of CGR is satisfactory if detected early. A meticulously planned follow-up and patient counseling are vital for the early detection of CGR.

Method of Literature Search

Articles related to CGR were searched using Medline, PubMed, Cochrane Library Database, EMBASE, and Scopus. The search was conducted using the following terms: CGR, endothelial rejection, epithelial rejection, stromal rejection, corneal immune privilege, high-risk keratoplasty, corneal neovascularization, penetrating keratoplasty, endothelial keratoplasty, and lamellar keratoplasty. The abstracts of all the articles were screened and relevant articles were included in this review. Reference lists from the selected articles were further checked to obtain further relevant articles not included in the electronic database. The emphasis was primarily to include randomized clinical trials and prospective studies; however, small case series and retrospective studies were included if found significant.

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Conflicts of interest

There are no conflicts of interest.

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