Clinical course and therapy of corneal graft rejection

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Abstract
Despite the immune-privileged cornea and anterior segment of the eye, allograft rejection is the most common cause of corneal graft failure. Fortunately, most rejection episodes do not cause irreversible graft failure if recognised early and treated adequately. Immunosuppression, particularly with corticosteroids, is widely accepted to be effective in the prevention and treatment of rejection. However, there is no consensus on optimal methods of administration, dosage or treatment duration. In addition, HLA matching should be considered routinely in keratoplasty. It is the only way of improving corneal graft prognosis which is free of side effects and long lasting. Experimental approaches including the use of antibody-based and gene therapy are being developed but may be difficult to translate from the laboratory to the clinical practice.

Key words: Corneal graft, rejection, management, immunosuppression.

Introduction
Prevalence of allograft rejection
Corneal transplantation, first reported a century ago, in 1906⁵⁴, is the oldest and most frequent form of transplantation⁵³. At the same time it is considered as the most successful transplantation in humans, but several long term studies indicate that the opposite might be the case. Despite the clinical experience over a century, and notwithstanding the immune-privileged cornea and anterior segment of the eye, survival of human corneal allografts is not better in either the shorter or longer term than that of many other organ transplants. Corneal graft survival falls steadily from the time of surgery to 55% at 15 years¹³. Furthermore, the results have failed to improve over the past 15 years, a period in which outcomes for solid organ grafts have generally improved progressively. Whereas for conditions such as keratoconus and corneal dystrophies the results of keratoplasty are excellent, the prognosis of acquired corneal opacities is worse than for kidney and heart transplantation. In contrast to an overall survival rate of renal grafts with greater than 90%, the 5-year prognosis for penetrating keratoplasty in the presence of risk factors is estimated to

Table 1. Corneal graft rejection rate in clinical studies

<table>
<thead>
<tr>
<th>Author</th>
<th>Number</th>
<th>Incidence (%)</th>
<th>Irreversible (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alldredge &amp; Krachmer/2/, 1981</td>
<td>156</td>
<td>29</td>
<td>5</td>
</tr>
<tr>
<td>Pleyer et al./36/, 1990</td>
<td>740</td>
<td>4–37*</td>
<td>3–28*</td>
</tr>
<tr>
<td>Reinhard et al./42/, 1997</td>
<td>646</td>
<td>18</td>
<td>n.m.</td>
</tr>
<tr>
<td>Poon et al./38/, 2001</td>
<td>49</td>
<td>53</td>
<td>16</td>
</tr>
<tr>
<td>Bourne et al./28/, 2004</td>
<td>394</td>
<td>23</td>
<td>7</td>
</tr>
<tr>
<td>Birnbaum et al./5/, 2005</td>
<td>417</td>
<td>n.m.</td>
<td>28–40</td>
</tr>
<tr>
<td>Sangwas et al./46/, 2005</td>
<td>1927</td>
<td>53</td>
<td>36</td>
</tr>
</tbody>
</table>

n.m. = not mentioned
*dependent from risk factors
be approximately 50% ⁹. From a clinical point of view, the initially favorable prognosis of penetrating keratoplasty can be narrowed by immunological considerations. The effects of immune-mediated graft rejection on keratoplasty have been clearly shown in numerous studies. Interestingly, it seems that the rejection rate has not changed in the last 50 years (Table 1). The ever-increasing specificity and efficacy of systemic immunosuppression probably accounts for some of the improvements observed in solid organ graft survival in recent years. Unfortunately, systemic immunosuppression has only a limited place in corneal transplantation because of the associated morbidities. Therefore, more efficient preventive and therapeutic efforts are required to improve the prognosis after keratoplasty.

**Immunological features of cornea**
The cornea has been thought to lack immunogenicity because of its low antigen expression. HLA class I antigens are expressed particularly in the corneal epithelium and, much less densely, in the stroma and endothelium. HLA class II antigens have been found only scattered in the corneal epithelium, particularly at the limbus region and in the corneal stroma. However, the expression can change under certain conditions. An enhanced expression of HLA class I and II can be induced by inflammation due to infection, graft rejection, and even by the surgical process itself. In contrast, extending storage or low-dose UVB radiation decreased HLA class II antigens (DR) expression in human corneas.

**Immune privilege**
Compared to other forms of transplantation, keratoplasties performed in “low risk” patients such as dystrophic conditions have a relatively favorable prognosis even without tissue typing or systemic immunosuppressive therapy. Medawar (1948) was the first who realized that the anterior chamber of the eye has some unique immunological features and coined the term “immunological privilege” for the anterior chamber of the eye and brain. He explained the favourable prognosis of penetrating keratoplasty by the absence of sensitising histocompatibility antigens in the graft, rapid replacement of donor tissue by the recipient, and avascularity of the donor cornea. It later became clear that of these criteria only avascularity remained a significant factor. The immunological privilege of the anterior segment of the eye has been summarized in the term “anterior chamber–associated immune deviation” (ACAID). Of primary significance in its regulating, ACAID is an active system, which is linked to regulatory T cells.

Induction of the specific anterior chamber privilege can take place through a number of antigens, particularly histocompatibility antigens. Exposition of small amounts of antigens after penetrating keratoplasty, i.e. the orthotopic corneal allograft, leads to an antigen-specific down-regulation of Th1–immune responses, thus promoting graft success.

**Mechanisms of corneal allograft rejection**
The mechanisms involved in corneal allograft rejection are complex and still remain poorly understood. Experimental keratoplasty models have contributed in great detail to our understanding and indicate that both, local as well as systemic immune mechanisms contribute to transplant rejection. In a simplified form, graft rejection can be differentiated into three phases:
- The donor antigen has to be released, recognised and transported to the draining lymph tissue (afferent arm)
- Alloantigens have to be processed to induce a specific cellular immune response (central stage)
- Effector cells gain access to and attack the graft, eventually destroying it (efferent arm)

**Antigen Presentation**

**Antigen-presenting cells**
Langerhans cells (LCs), derived from bone marrow cells, are professional antigen-presenting cells (APCs). These dendritic cells play a dominant role in the processing and presentation of antigens. They carry MHC class II molecules and are important stimulators of both T and B cells.

![Figure 1. Direct and indirect antigen presentation](image)

1) Recipient Langerhans cells (LCs) migrate from the limbus centripetally into the corneal graft. There they internalise and process alloantigens. 2) The presentation of foreign peptides to host T cells leads indirectly to a donor–specific immune response. 3) Donor LC migrate centrifugally out of the allograft and prime directly T cells in the draining lymph nodes. Alloantigen specific T cells recognize either presented corneal antigens or allo­genic epitopes on the MHC molecules. 4) Activated T cells proliferate and infiltrate the graft tissue.
In contrast to LCs that have been extensively studied, the role of macrophages is less clear. Macrophages have phagocytic capability, secrete a variety of inflammatory cytokines and express low levels of MHC class II antigens and costimulatory molecules.

**Antigen presentation: Direct/Indirect Pathway**

Two pathways of antigen presentation, either “direct” or “indirect” (Fig. 1), have been proposed in alloantigen recognition. Both pathways are involved in the corneal alloresponse. However, the exact contribution still remains elusive. In experimental models, the indirect pathway is the driving force in both normal-risk and high-risk keratoplasty, while the direct pathway may play a role in the high-risk setting.

**T cell activation**

Antigen-specific activation of T lymphocytes is dependent on a series of signal transduction between T cells and APCs. For full activation and proliferation of T cells, at least two signals are required: signal 1 is transmitted by the interaction of T cell receptor/CD3 complex with the MHC present on APCs, and signal 2, the costimulatory signal, is transmitted by interaction of the surface molecules on the T cells (e.g. CD28 and CD40 ligand) and the APCs (B7 and CD40). If T cells are not triggered efficiently via these two pathways they will become unresponsive. Thus, selective blockade of each of the costimulatory signal pathways can prolong the allografts survival.

It is well established that corneal allograft rejection occurs via a T cell–mediated immune response. Based on the cytokines that they produce, CD4+ T cells are divided into two distinct subsets: Th1 and Th2 cells. Th1-type cells predominantly produce interleukin-2 (IL-2) and interferon-γ (IFN-γ), which mediate cellular immunity involving cytotoxic T lymphocyte (CTL) and delayed type hypersensitivity (DTH). Th2-type cells produce IL-4, IL-6, IL-10, and IL-13, which may mediate graft tolerance and humoral immunity. The specific corneal allograft immune response is mediated by CD4+ T cells, either through the Th1 or Th2 pathway. In contrast to solid organ transplantation, a CTL immune response is not necessary for corneal allograft rejection.

**Clinical presentation of graft rejection**

As a consequence of HLA antigen distribution in all cellular layers, corneal allograft rejection can occur in each of the three main layers independently or simultaneously. Corneal graft rejection most commonly occurs during the first year after surgery. Fortunately, most episodes of graft rejection do not cause irreversible graft failure if recognised early and treated adequately. Graft rejection is usually associated with blurring of vision, discomfort and redness of eye, but in mild cases of graft rejection patients may be asymptomatic.

**Epithelial rejection** is considered relatively unimportant, as the donor epithelium can be replaced by recipient epithelium derived from the limbus without further consequences. It occurs within 1 to 13 months postoperatively, at a rate of 1–10%, as after this time the donor epithelium is replaced by host epithelium. Clinical manifestation of epithelial rejection includes the appearance of an elevated epithelial rejection line, which consists of damaged donor epithelial cells. It usually starts from the periphery, stains with fluorescein and migrates towards the centre of the graft like the Khodadoust line.

**Subepithelial rejection** presents as subepithelial infiltrates of 0.2–0.5 mm in diameter located in the anterior stroma (Fig. 3). They occur within 2–24 months after the centre of the graft like the Khodadoust line. Of great importance for the differential diagnosis with epithelial HSV keratitis is the fact that reinnervation of the cornea cannot be expected between 3–6 months after keratoplasty.
after keratoplasty and resemble infiltrates similar to those in adenoviral keratitis. Photophobia can be the only symptom of subepithelial rejection. This is the second most common form of corneal graft rejection with a reported incidence of 2–15%[6]. Subepithelial graft rejection leaves no sequelae if treated, but it may precede the more severe endothelial rejection[16].

**Stromal rejection** is relatively rare (1–2%)[6]. It is characterised by peripheral stromal infiltrates and haze in a previously clear graft. Stromal rejection usually occurs simultaneously with endothelial rejection.

**Endothelial rejection** is the most common form of graft rejection with reported rates of 8–37%[6]. In terms of corneal function, this type of rejection is of the greatest interest. The endothelial monolayer functions to pump water out of the stroma of the cornea. In the absence of this pump the cornea swells, the collagen fibres are disoriented and the cornea loses transparency. Human endothelial cells are non replicative, and thus, donor cell loss is irreversible. During endothelial rejection it is possible to visualise linear or multifocal deposits of leucocytes adhering to the endothelium associated with segmental corneal edema. An endothelial rejection line (i.e., Khodadoust line) starts in the periphery of the graft, usually in the vicinity of stromal vessels, and moves towards the centre of the graft (Fig.4). Differential diagnosis includes HSV/HZV endothelitis, especially in cases of diffuse endothelial rejection.

Hence, although intensive treatment with topical steroids reverses the acute inflammation in most patients, the goal is to reverse the rejection episode as early as possible to minimise endothelial cell loss.

**Prevention of graft rejection**

Two major strategies are used to improve corneal graft survival:

* decrease of the recipient’s sensibility by reduction of antigen difference between donor and recipient (HLA matching);
* reduction of the recipient’s afferent or efferent immune response by pharmacological modulation.

**HLA matching**

In clinical practise, HLA matching between donor and recipient is not routinely performed, even when growing evidence shows a benefit on graft outcome (Table 2). Recently, several studies support a significant benefit for HLA–A, –B, and –DR matching both in low and high risk patients[8,52]. However, results of the two

<table>
<thead>
<tr>
<th>Authors</th>
<th>Number of patients</th>
<th>Study design</th>
<th>Follow-up (yr)</th>
<th>Prognosis</th>
<th>Assayed antigens / matching priorities</th>
<th>other</th>
<th>Criteria</th>
<th>HLA-typing</th>
<th>Results / comments</th>
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</thead>
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<tr>
<td>Böringer et al. /8/, 2006</td>
<td>229</td>
<td>retrospective</td>
<td>2</td>
<td>Normal–high–risk</td>
<td>HLA class I and II</td>
<td>A1, H-Y, IR</td>
<td>serological</td>
<td>HA-3, HA-3: PCR</td>
<td>H-Y matching is important to graft outcome in human PKP, while HA-3 did not show statistical significance.</td>
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<td>Beekhuis et al. /5/, 2003</td>
<td>303</td>
<td>retrospective</td>
<td>4</td>
<td>high–risk</td>
<td>A, B</td>
<td>irrev. IR</td>
<td>serological</td>
<td></td>
<td>significant beneficial effects of HLA–A and B matching on graft survival; superior effect of HLA split matching at 3–12 yr follow-up. HLA–AB and DR matching significantly lowers significantly rate of immune reaction for normal– and high–risk patients; no significant benefit of HLA split matching.</td>
</tr>
<tr>
<td>Khaireddin et al. /19/, 2003</td>
<td>459</td>
<td>retrospective</td>
<td>3</td>
<td>Normal–high–risk separate</td>
<td>A, B, DR</td>
<td>IR</td>
<td>serological</td>
<td></td>
<td>significant beneficial effects of HLA–A, B and DR matching on graft survival for both groups.</td>
</tr>
</tbody>
</table>

**Figure 4.** Endothelial graft rejection

**Table 2.** Monocentric clinical trials: HLA Matching

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**References:**

1. Böringer et al., /8/, 2006
2. Beekhuis et al., /5/, 2003
3. Khaireddin et al., /19/, 2003
4. Völker–Dieben et al., /52/, 2000
5. Roy et al., /45/, 1997

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<th>Follow-up (yr)</th>
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large multicentre matching trials in patients at high risk of rejection have revealed no clear justification for at least MHC antigen matching. The prospective randomised “CCTS study” has shown no benefit of HLA class I and class II–DR matching on graft survival, while a second study has shown a small, but significant, benefit of class I matching and increased risk of rejection with class II matching. The validity of the “CCTS study” has been discussed because of high error rates in the matching procedures and concomitant high doses of topical corticosteroids. The “CCTS study” demonstrated that aggressive local immunosuppressive therapy reduces the incidence of graft rejections after transplantation and thus masks the beneficial HLA matching effect. However, it may increase the risk of steroid related complications. It has been known for sometime that certain HLA mismatches differ in immunogenicity and may have influence on graft survival in kidney transplantation and in keratoplasty, but this fact just recently got adequate attention. Moreover, studies using rodent models of keratoplasty have shown that, in some strain combinations, allografts carrying minor histocompatibility (H) alloantigens are more likely to be rejected than those bearing MHC alloantigens. Immunogenicity of minor H antigens arises as a result of their presentation on the plasma membrane in the context of HLA I or II, where they are recognized by alloimmune T cells. To date, relatively small number of human minor H loci have been described. Recently, a clinical study designed to assess any benefit of matching the broadly expressed gender (H–Y) and HA–3 antigens in HLA1 donor positive human keratoplasty showed that HLA–A1/H–Y matching may further improve prognosis. Generally, male grafts can be subjected to alloimmune reactivity in female recipients, since H–Y minor H antigen derives from a Y–chromosome encoded gene.

HLA matching cannot replace immunosuppressive therapy, since higher matches are difficult to achieve, especially in heterogeneous populations, and other factors, such as minor transplantation antigens, are also important. Nevertheless, it can definitely reduce the dose and consequently the negative side effects of immunosuppressive therapy. Therefore, HLA matching should be considered routinely in keratoplasty.

Immunomodulatory agents in keratoplasty

No generally accepted “guidelines” on the prevention and treatment of corneal allograft rejection are available. Several surveys of corneal transplant societies indicate that a broad pattern of clinical practises exist. It can be agreed, however, that a certain subpopulation of patients can be classified as “high risk” individuals that should be treated differently.

Well known risk factors for corneal graft rejection include:
- more than 2/4 vascularization of the recipient’s cornea
- preoperative inflammation of the anterior segment
- keratoplasty à chaud
- anterior synechiae
- large graft (>7.7 mm) diameter
- grafts near the limbus
- multiple graft

Corticosteroids

Corticosteroids are the mainstay of prevention and treatment corneal graft rejection since the introduction of these agents in 1950s. Unfortunately, the optimal dose of topical steroids for patients having corneal graft has not been agreed upon and there is considerable variation in the way clinicians use these drugs. In patients without risk factors for transplant rejection, topical postoperative treatment with corticosteroids is considered as sufficient. Main advantages include immediate and potent anti-inflammatory effects, favourable pharmacological properties and low price. Steroids rapidly permeate the cellular membrane and act intracellularly through glucocorticoid–responsive elements. Biological effects include down regulation of interleukin (IL)–1, IL–3, IL–6 and IL–8 which are secreted predominantly by antigen–presenting cells. In addition, reduction of IL–1 and IL–6 leads to inhibition of IL–2 secretion and subsequent T cell inhibition. Furthermore, the broad spectrum of antiphlogistic properties is important for the postoperative use of corticosteroids in corneal grafts. They reduce the permeability of the vascular endothelium (blood–aqueous barrier, blood–retina barrier) and inhibit migration of monocytes. Experimental data demonstrate a dose–dependent inhibitory effect on cytokine production. Already very low doses (10–100 nM) inhibit resting cells, whereas far higher doses are necessary to block also activated cells.

Prednisolone acetate is the drug of choice for most clinical scenarios, but the use of lotepredal has increased, especially for low risk grafts and phakic patients. It is important to emphasize that the biological effect of local steroid therapy is mainly anti-inflammatory. Although steroids are commonly applied locally for postoperative treatment after keratoplasty, there are different opinions about dose and duration of treatment. There are no controlled clinical studies from which a “gold standard” could be derived. In “normal risk” patients topical application of 1% prednisolone acetate q.i.d. has been suggested for the first 2–4 weeks, reduced to 1 drop/day after 3 months and then continued up to the 12th postoperative month. For “high risk” patients recommendations vary for prednisolone–acetate...
drops from b.i.d. to up to 24 times per day. Even when topical corticosteroids are used close to maximal tolerated dose, they are only partially effective. The rejection rate for high risk patients remains unacceptably high despite high doses of topical corticosteroids.

Systemic treatment with corticosteroids has not only antiphlogistic but also immuno-suppressive effects. Suggestions for intensity and duration of the treatment vary as much as in the case of local treatment. For the perioperative period some authors suggest 100–250 mg/day prednisolone intravenously. Prolonged monotherapy with systemic corticosteroids should be avoided because of the high potential of side effects. In a randomised prospective study there was no additional benefit for normal risk patients that received of systemic fluocortolone for a 3 week period plus topical prednisolone acetate when compared to sole local treatment.

Well-known undesired side effects of local corticosteroids are altered wound healing, increased risk for infection, reactivation of herpes virus keratitis, secondary glaucoma and cataract formation. Cataract formation has been observed already after a cumulative dose of 740 drops of 0.1% dexamethasone. Systemic steroid therapy provokes all the side effects of local therapy and may additionally lead to severe metabolic alterations. The ophthalmologist must be aware of both ocular and systemic toxicity in patients who are receiving systemic steroids. Systemic complications may include peptic ulceration, osteoporosis, diabetes mellitus, hypertension, compression fracture, aseptic necrosis of the femoral head, muscle and skin atrophy, mental changes, and growth retardation in children.

**Management of corneal graft rejection**

Despite prophylactic postoperative medical management, corneal graft rejection still occurs. Thus, besides preventive measures, early recognition and effective treatment of graft rejection remain critical for the success of corneal transplantation. The treatment of corneal graft rejection varies according to the clinical presentation. **Epithelial and stromal rejection** is usually treated on an outpatient basis with frequent topical steroids, such as prednisolone acetate 1% every 2 hours. However, in patients with an **endothelial rejection**, the strategy should be more aggressive. A recent study, designed to show the practice patterns for corneal graft rejection, demonstrated that up to 42% participants preferred to admit these patients into a hospital and treat them with topical prednisolone 1% every hour. In addition, systemic steroids were administered and preferred over subconjunctival injections. The value of systemic steroids remains unclear, because the results of different studies are contradictory.

**Calcineurin inhibitors (Cyclosporine A, Tacrolimus)**

Inhibition of calcineurin in the intracellular signalling pathway interferes with DNA–transcription and thus leads to a decreased function of immune cells. Calcineurin inhibitors are potent immunosuppressive drugs acting predominantly through inhibition of T cell activation. While FK506 is 50–100 times more potent than cyclosporin A (CsA), it still shares its toxicity and many of its side effects.

**Cyclosporine A**

Graft prognosis has improved considerably since the introduction of systemic immunosuppresion with CsA in the postoperative treatment of high risk keratoplasty. CsA acts mainly by inhibiting cytokine synthesis, especially of IL–2 but also IL–4 and tumor necrosis factor. Subsequently, it causes suppression of T cells activation and proliferation that is reversible when the treatment is stopped. This drug acts dose–dependently on the afferent and efferent arc of the immune response. The effective concentration (MHC50) of CsA is 10–20 ng/ml for IL–2 production and 20–50ng/ml for the proliferation of cytotoxic lymphocytes.

A number of experimental and clinical studies dealt with topically applied CsA, but pharmacokinetic properties of CsA as a lipophilic compound limit the benefit of topical therapy. Due to its lipophilic characteristic and poor water solubility, the intra–ocular concentrations of topical formulations vary over a wide range, but concentrations in aqueous humor remain very low. Improved solvent preparations like cyclodextrin, azone, collagen lenses and liposomes led to higher intraocular concentrations. Subsequent experimental studies using these preparations demonstrated a significant therapeutic effect. Another strategy to enhance the bioavailability of the lipophilic CsA is the synthesis of an inactive hydrophilic chemically modified molecule, which can be converted, within the tissue, into the active form after enzymatic transformation. Bourges et al. demonstrated that topically instilled CsA hydrosoluble prodrug was as effective as intramuscular injection for the prevention of corneal graft rejection in rats.
The results of a prospective clinical study by Price et al.39 suggest that topical CsA 0.05% is not as effective as the use of prednisolone acetate 1% for prevention of graft rejection episodes in low risk transplants. If cyclosporine 0.05% was not effective for preventing graft rejection in low risk patients, it would certainly not be expected to be effective in more demanding high risk patients. Perry et al.30 suggested that topical CsA (0.5%) application may substitute local steroids in secondary glaucoma responders, but at the same time an increased rejection rate in CsA-treated patients occurred.

Systemic application of CsA was more favourable. Several uncontrolled clinical studies showed a positive effect on the graft survival in high-risk cases13. There are a number of controlled and uncontrolled clinical studies that support posttransplant use of CsA. A prospective study showed that combined CsA treatment with topical dexamethasone was superior to topical corticosteroid treatment alone. However, different opinions exist on the duration of CsA application. Though a positive effect of a short-term therapy (3 months) was reported in one study26, there are later results showing a best efficacy with 1 year of CsA treatment. The inefficacy of a short-term (3 months) CsA treatment was confirmed by others. In addition, some studies could not confirm a benefit of this approach at all38.

The usual dosage of CsA is between 3 and 5mg/kg per day. However, severe side effects occur in up to 40% patients. The most common complications include nephrotoxicity and hepatotoxicity, alteration in glucose metabolism, hypertension, gingival hyperplasia, and hypertrichiasis. Because of the potentially serious complications, patients must be monitored closely. Regular blood monitoring, including complete blood count, renal and liver function test, as well as blood pressure control should be performed monthly (Table 3). To minimize side effects, CsA must be administered according to blood trough levels, which should not exceed 150ng/ml.

Taken together, CsA is still the most frequently used immunomodulatory agent for the treatment of high-risk keratoplasty patients38.

**Tacrolimus (FK506)**

The mechanism of action of tacrolimus is similar to CsA, but inhibition of lymphocyte proliferation is 10–100-fold stronger compared to CsA. Like CsA, FK506 is a lipophilic compound with similar limitation following local application, but intraocular drug levels are higher than with CsA34. A prospective pilot study demonstrated that topical FK506 was at least as effective as topical steroids in preventing graft rejection in low risk patients. However, local discomfort limited further use43. The most common listed side effects include superficial punctuate keratitis, injection of the conjunctiva, burning, slowly healing erosion, and slight superficial opacification. In another noncomparative case series tacrolimus was effective in the prevention of rejection in patients with high-risk corneal and limbal grafts49.

According to a recently published study18, systemic tacrolimus was relatively safe and effective in prolonging graft survival in high risk keratoplasty patients. The main side effect noted with tacrolimus was hypertension. However, considerably side effects such as raised serum creatinine, pancreatitis, and lymphopenia were also noted in the same study18. A high rate of undesired side effects and narrow therapeutic index have limited the clinical use of FK506 in other fields of

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**Table 3. Immunomodulatory agents in keratoplasty**

<table>
<thead>
<tr>
<th>Effect</th>
<th>Initial dosage</th>
<th>Maximum dosage</th>
<th>Initiation of effects</th>
<th>Common side effects</th>
<th>Control parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-cell inhibitor</td>
<td>3–5 mg/kg/d</td>
<td>10 mg/kg/d</td>
<td>2–6 weeks</td>
<td>renal, arterial, tremor, hirsutism, gingival hyperplasia</td>
<td>creatinine level monthly (potentially C2 level) RR–control white blood count</td>
</tr>
<tr>
<td>T-cell inhibitor</td>
<td>0.15–0.3 mg/kg/d</td>
<td>0.30 mg/kg/d</td>
<td>2–8 weeks</td>
<td>bone marrow depression, haematuria, infection</td>
<td>white blood count 1–4 weekly urine analysis</td>
</tr>
<tr>
<td>Purine–synthesis</td>
<td>2x500 mg/d</td>
<td>2x1,5 g/d</td>
<td>2–12 weeks</td>
<td>diarrhoea, gastro–intestinal function, infection, neutropenia</td>
<td>white blood count liver enzymes</td>
</tr>
<tr>
<td>T-cell inhibitor</td>
<td>2x1000mg/d</td>
<td>2x1000mg/d</td>
<td>2–8 weeks</td>
<td>thrombocitopenia, anemia, rise in LDH, Chl, diarrhea, infection</td>
<td>Complete blood count HDL, LDL, Chl 1–4 weekly urine analysis</td>
</tr>
</tbody>
</table>
transplantation medicine. Predominantly side effects are nephrotoxicity and neurotoxicity (Table 3).

**Mycophenolate Mofetil (MMF)**

MMF had been introduced initially for psoriasis some 30 years ago. Mycophenolate mofetil interferes with purine synthesis. After oral intake, MMF is transformed into mycophenolic acid (MPA), the biologically active compound acting as a potent inhibitor of T and B lymphocyte proliferation. In 2001 it was demonstrated in a small randomised study that MMF was equally effective as CsA in high-risk keratoplasty patients. In a subsequent retrospective study, Birnbaum et al. showed an even stronger effect of MMF compared with CsA in preventing immune reaction after high risk keratoplasty, despite a shorter MMF administration comparing with CsA. In regard to drug safety, the MMF treated patients presented fewer side effects. A recent survey of German transplant centers revealed a good acceptance of this agent in high risk recipients. Typical side effects are gastrointestinal symptoms, tachycardia, arthralgia, severe systemic infections, and hepatotoxicity. Interestingly, MMF has also antiviral activity that may support corneal graft survival in patients with HSV–associated keratitis. Blood monitoring is largely unnecessary, the drug is administered at a fixed dose of 2x1g per day (table 3).

**Rapamycin**

Rapamycin (sirolimus, Rapamune) is a bacterial macrolide isolated from *Streptomyces hygroscopicus*, which has antifungal and immunosuppressive properties. The mechanism of action is not calcineurin–inhibiting, and is therefore not expected to be nephrotoxic. Rapamycin decreases IL–2–mediated activation of T-lymphocytes, and also inhibits growth factor–induced proliferation of fibroblasts, endothelial cells and smooth muscle cells. Subsequent prospective pilot study demonstrated that rapamycin and MMF, used monotherapeutically following high–risk keratoplasty, were comparably effective regarding immune reaction–free graft survival. However, a broad spectrum of side effects from rapamycin was observed as well. The most frequent side effects in this study were hypercholesterinemia, furunculosis, exanthema, hypertriglyceridemia, elevation in LDH, and gastrointestinal disturbances (Table 3).

**Future aspects: immunological and gene therapeutic approaches**

New approaches currently being explored in experimental animals are based on developments in molecular medicine. Two broad approaches are applicable: first, the production of peptides or small proteins that can be delivered to the eye topically, and second, the in vivo production of proteins by gene therapy. The pathophysiologic basis of modern immune pharmacology is focused on T cell modulation and the interaction with antigen–presenting cells.

**Monoclonal antibodies**

Antibody–based therapy has been established in most branches of clinical transplantation, but it has not found a place in corneal transplantation to date. Because of their high specificity and selective biological effects, monoclonal antibodies (mab) hold great promise as pharmacological agents. Treatment of allograft rejection was one of the first major fields for polyclonal and monoclonal antibodies. Understanding the immunobiology of transplantation led to the application of antibodies targeting structures including the T cell receptor, CD3 molecule, IL–2 receptor and CD4 molecule of T helper cells. Other strategies focus on costimulatory molecules, cell adhesion molecules (LFA–1, ICAM–1) or proinflammatory cytokines (TNF). In spite of a multitude of experimental studies in corneal transplantation, there are only a few clinical studies.

Ippoliti and Fronterre injected anti–CD3 and anti–CD6 mab into the anterior chamber of patients with acute corneal graft rejection refractive to steroid treatment and were able to reverse rejection. In another study, a pan–T cell antibody (CAMPATH–1H) which is well known as a strong T cell depleting agent, was successfully applied (systemically) in patients with graft rejection. The only report on preventive application of monoclonal antibodies was targeting activated T cells by using basilicimab, an anti–CD25 mab. Perioperative application of systemically administered mab in combination with systemic CsA was well tolerated and led to a rejection free long term survival in five high risk patients. Recently, anti TNF therapy (infliximab) demonstrated a favourable clinical course in patients with rheumatic corneal ulceration. Problems that limit the broader use of monoclonal antibodies include “first–dose anaphylactic reactions”, production of antibodies against the therapeutic antibody by the recipient and high costs.

**Gene therapy**

Current immunosuppressive treatment protocols to prevent graft rejection have to be administered for a prolonged or even lifetime period and may lead to severe side effects. Therefore, less toxic alternative approaches are needed for corneal transplantation. Another future option to prolong corneal graft survival is to modify the recipient’s immune response by gene transfer. The cornea is a suitable candidate for a gene–based immunomodulatory approach for several reasons. These include the relatively simple histological structure of the cornea, its accessibility for examination and manipulation, its ability to be maintained in ex vivo culture for several weeks, and the relative immune privilege of the anterior chamber.

A variety of delivery vehicles such as adenovirus, retrovirus and liposomes have successfully developed for gene transfer into ocular tissues. Adenovirus (Ad) medi-
adequate gene transfer is an excellent strategy for gene therapy because of its high transduction efficiency. It is of particular interest that Ad specifically target the endothelium of the cornea whereas epithelium and stroma cells remain unaffected.

T-cell activation requires additional costimulation between molecules expressed on the T cell and APC surface. Binding of CD28 on the T cell surface to ligands CD80 and 86 on the APC surface is an essential signal for the activation of T cells. A strategy to prolong corneal graft survival by inhibiting costimulatory signals through CD28 has been examined in rat experimental keratoplasty using AdCTLA4-Ig gene therapy. The experiments demonstrated that local ex vivo gene transfer only moderately delayed allograft rejection whereas systemic CTLA4Ig administration significantly prolonged allograft survival. Several studies targeting different structures such as immunomodulatory cytokines (vIL-10, IL-12-p40) in experimental corneal grafting were promising and able to prolong transplant survival, whereas others were not successful.

Recently, Gong et al. reported for the first time the potential of local nerve growth factor (NGF) gene therapy to prolong corneal graft survival in an experimental rat transplantation model. Moreover, immunomodulatory therapy further improves graft survival and demonstrates that both anti-inflammatory and cytoprotective mechanisms are involved in the prevention of corneal allograft rejection.

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Corneal graft rejection


