Gene Therapy Approaches to Prevent Corneal Graft Rejection: Where Do We Stand?

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Key Words
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Abstract
Cornea transplantation (penetrating keratoplasty) is the most frequently performed transplant procedure in humans. Despite advances in microsurgery and immunosuppressive treatment protocols, a significant number of corneal grafts still undergo immune-mediated allograft rejection. Topical treatment with corticosteroids is currently the gold standard and while this treatment is effective in many corneal transplant patients, it is much less effective in 'high-risk' patients with previous episodes of neovascularisation or graft rejection. Therefore, alternative approaches such as genetic modification of donor corneas are needed to prevent corneal transplant rejection. Cornea transplantation holds the unique advantage in that gene therapy can be used to modify allografts ex vivo prior to transplantation. Many preclinical studies using local (and systemic) gene transfer have been performed to date and many different gene transfer vehicles (gene therapy vectors) and therapeutic strategies (immunomodulatory or graft-protective) have been investigated to prevent corneal allograft rejection. The most recent gene therapy applications to prevent corneal allograft rejection will be reviewed in this article. Moreover, it will be discussed why the development of clinical trials for the genetic modification of corneal grafts prior to transplantation is lagging behind of those for the treatment of inherited retinal diseases.

Introduction

Cornea transplantation (penetrating keratoplasty) is the most often performed transplant procedure in humans. Failure of corneal transplants is often not considered significant in the absence of risk factors. This can usually be explained by the immune privilege of the avascular cornea, which is grafted to an immune-privileged site, the anterior chamber of the eye. However, this immune privilege is not robust as it can be overcome and graft rejection is not uncommon. Reports on the incidence of graft rejection after penetrating keratoplasty vary between 5 and 40% [1]. With regard to long-term observations, the 5-year prognosis for penetrating keratoplasty is even inferior to solid organ transplantation and estimated to be approximately 50% [2]. Topical treatment with corticosteroids is currently the gold standard in corneal transplantation in patients [3]. While this treatment is effective in many patients, it is much less effective in 'high-risk' patients with previous episodes of
neovascularisation or graft rejection [4]. Systemic immunosuppression has reduced the rejection rate of corneal allografts; however, prolonged use of these agents can produce significant side effects that may limit their use in a non-life-threatening indication. Therefore, alternative approaches are needed to prevent corneal transplant rejection such as genetic modification of donor corneas.

**Genetic Modification of Corneal Allografts prior to Transplantation**

Cornea transplantation is unique in that gene therapy can be used to modify allografts ex vivo prior to transplantation. In contrast to other tissues that are routinely transplanted, the cornea can be kept in culture for up to 4 weeks without significant loss of function which allows sufficient time for ex vivo manipulation. However, it has also been reported that endothelial cell loss during storage is mediated by apoptosis which may contribute to early graft loss after transplantation [5, 6]. Local expression of therapeutic genes may be sufficient to achieve a therapeutic effect thus making systemic (genetic) intervention either unnecessary or required only at reduced doses thereby avoiding undesired side effects. In this article, the most recent gene therapy applications to prevent immune-mediated corneal allograft rejection will be briefly summarised (table 1). Genetic modification of corneal tissues with the view of improving endothelial viability during storage has been published recently [7, 8]. However, gene therapy for endothelial protection or for treatment of corneal diseases has been reviewed recently and will not be discussed in this review [9, 10].

The majority of preclinical gene-therapeutic studies to improve organ graft survival including corneal grafts utilise recombinant viral vectors derived from adenoviruses (Ad), lentiviruses (LV) or adeno-associated viruses (AAV) as gene transfer vehicles [11–13]. Non-viral gene transfer would avoid many issues concerning unwanted immune responses against the viral vector and/or the therapeutic gene and also with regard to general safety aspects such as reversion to potentially pathogenic wild-type virus. The efficiency of gene transfer into corneal cells, however, is reportedly low [13]. In contrast, viral vectors are generally characterized by their high transduction efficiency of different cell types and tissues, although transduction efficiency may vary, depending on the cell type. Ad vectors predominantly transduce the corneal endothelium, whereas the epithelium and stroma are refractive to Ad-mediated gene transfer [14–17]. Due to the genetic DNA backbone of the Ad vector used in many studies, Ad-transduced cells will still express adenoviral proteins. These antigens can be presented via major histocompatibility complex molecules on endothelial cells or keratocytes and stimulate

<table>
<thead>
<tr>
<th>Vector</th>
<th>Treatment</th>
<th>Therapeutic gene</th>
<th>Promoter</th>
<th>Function/effect</th>
<th>Animal model</th>
<th>Results</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus</td>
<td>Ex vivo and systemic gene transfer</td>
<td>ICOSIg fusion gene</td>
<td>CMV</td>
<td>Inhibition of costimulation by blocking the interaction between ICOS and ICOS-L</td>
<td>Rat</td>
<td>No prolongation of graft survival</td>
<td>[31]</td>
</tr>
<tr>
<td>Lentivirus</td>
<td>Ex vivo gene transfer</td>
<td>PD-L1</td>
<td>Ubiquitin</td>
<td>Modulates graft infiltrating cells expressing PD-1</td>
<td>Rat</td>
<td>Significantly prolongs allograft survival</td>
<td>[40]</td>
</tr>
<tr>
<td>Lentivirus</td>
<td>Ex vivo gene transfer</td>
<td>Ovine IL-10</td>
<td>SV40</td>
<td>Inhibits Th1 immune response, down-regulates APC activity</td>
<td>Sheep</td>
<td>Moderate prolongation of graft survival</td>
<td>[45]</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>Ex vivo and systemic gene transfer</td>
<td>vIL-10</td>
<td>CMV</td>
<td>Inhibits Th1 immune response, down-regulates APC activity</td>
<td>Rat</td>
<td>Only systemic expression leads to prolongation of graft survival</td>
<td>[44]</td>
</tr>
<tr>
<td>Plasmid/</td>
<td>Ex vivo gene transfer</td>
<td>vIL-10</td>
<td>CMV</td>
<td>Inhibits Th1 immune response, down-regulates APC activity</td>
<td>Rat</td>
<td>Only marginal prolongation of graft survival</td>
<td>[44]</td>
</tr>
<tr>
<td>liposomes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenovirus</td>
<td>Ex vivo gene transfer and systemic expression</td>
<td>IL-12p40</td>
<td>CMV</td>
<td>Modulates IL-12 function by its natural inhibitor IL-12p40</td>
<td>Rat</td>
<td>No prolongation of graft survival</td>
<td>[47]</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>Ex vivo gene transfer and systemic expression</td>
<td>NGF</td>
<td>CMV</td>
<td>Modulates immune response, plays role in corneal wound healing</td>
<td>Rat</td>
<td>Only ex vivo gene transfer prolongs graft survival</td>
<td>[52]</td>
</tr>
</tbody>
</table>

IL-12p40 = Interleukin-12 p40 subunit; Th1 = T helper 1; Th2 = T helper 2; IL-10 = interleukin-10; vIL-10 = Epstein Barr Virus derived IL-10; APC = antigen-presenting cell; ICOSIg = inducible costimulator-immunoglobulin; ICOS-L = inducible costimulator-ligand.
T-cell-mediated immunity. This may result in short-term, transient expression of the therapeutic gene which might be sufficient or even desired in certain circumstances when long-term expression of the therapeutic gene might not be beneficial. Unlike Ad, AAV and LV vectors are generally considered as gene therapy vehicles leading to long-term gene expression in target cells due to their low immunogenic profile and their integration into the cellular genome [18]. Although integration does not seem to lead to pathological consequences when using AAV vectors, cellular integration of lentiviral vectors in chromosomal areas harbouring tumour genes has led to insertional mutagenesis and tumour formation [19]. LV vectors used for gene therapy in cornea transplantation are either derived from primate LV (HIV or SIV) or from non-primate LV (equine infectious anaemia virus) by removing most of the viral regulatory elements and have been shown to efficiently transduce corneal endothelium and keratocytes (signal 1) and costimulatory molecules present on antigen-presenting cells which can either transmit positive or negative signals into T cells (signal 2) [28]. Interfering with the CD28-B7.1(CD80)/B7.2(CD86) pathway has been shown to be important in inhibiting T cell activation and preventing graft rejection [11]. Modulation of other costimulatory pathways has also been studied for their potential to prevent corneal allograft rejection with various degrees of success. ICOS (Inducible Costimulator) is an inducible costimulatory receptor expressed by activated T cells and memory T cells [29]. Blocking the interaction of ICOS with its receptor has been shown to be an effective strategy to prevent autoimmune disease [30] or allogeneic graft rejection; however, the timing of treatment seems to be critical. We have analysed the effect of Ad-mediated overexpression of soluble ICOSIg (AdICOSIg) and neither local nor systemic administration of AdICOSIg prolonged corneal graft survival [31]. This may indicate that the inhibition of primary immune responses is more important in order to prolong corneal graft survival than inhibiting memory cells by ICOSIg.

Recently the PD-1/PD-L1 pathway has been shown to play a role in the negative regulation of immune responses (fig. 1) [32, 33]. Programmed cell death (PD) ligand 1 (PD-L1) and programmed cell death ligand 2 (PD-L2) are known ligands for PD-1. PD-L1 has been detected on lymphoid cells including monocytes, antigen-presenting cells and B cells, as well as in non-lymphoid tissues such as the heart, lung, placenta, kidney, liver and cornea [34–37]. It has been documented that blockage of either PD-1 or PD-L1 using antibodies leads to accelerated graft rejection in mouse corneal allograft models [38]. Furthermore, systemic application of a soluble PD-L1-Ig fusion protein was able to prolong allogeneic graft survival in a mouse corneal transplant model [39] highlighting the importance of this pathway. We showed that LV-mediated overexpression of PD-L1 in cultured corneas significantly prevents corneal graft rejection by modulating graft-infiltrating cells [40]. This striking result was associated with a reduction of natural killer T cells and cytotoxic CD8+ T cell infiltration, and was also accompanied by at-

**Gene-Therapeutic Applications to Prevent Corneal Allograft Rejection**

Several different experimental approaches have been investigated in recent years to promote corneal allograft survival which include ex vivo viral and non-viral gene transfer in cultured corneas, instromstral vector injection, systemic gene transfer pre- and post-corneal transplant and tolerogenic approaches. Similar gene therapeutic strategies are currently being investigated in the related field of solid organ transplantation. While some of these strategies such as systemic gene transfer have been proven successful in preclinical models but are unlikely to be transferred into a clinical scenario in non-life-threatening disease (side effects of systemic application, difficulty to control), we will be focussing in this article mainly on reviewing ex vivo gene transfer approaches as a safer method for using viral vectors.

**Costimulatory Blockade and Inhibitory Receptor Expression**

The immune system and in particular allo-activated T cells play a central role during corneal allograft rejection. Many studies indicate that rejection of corneal allografts is mediated predominantly by CD4+ T lymphocytes [26, 27]. In order to become fully activated, T cells need to engage via the T cell receptor with major histocompatibility complex class I or II molecules (signal 1) and costimulatory molecules present on antigen-presenting cells which can either transmit positive or negative signals into T cells (signal 2) [28]. Interfering with the CD28-B7.1(CD80)/B7.2(CD86) pathway has been shown to be important in inhibiting T cell activation and preventing graft rejection [11]. Modulation of other costimulatory pathways has also been studied for their potential to prevent corneal allograft rejection with various degrees of success. ICOS (Inducible Costimulator) is an inducible costimulatory receptor expressed by activated T cells and memory T cells [29]. Blocking the interaction of ICOS with its receptor has been shown to be an effective strategy to prevent autoimmune disease [30] or allogeneic graft rejection; however, the timing of treatment seems to be critical. We have analysed the effect of Ad-mediated overexpression of soluble ICOSIg (AdICOSIg) and neither local nor systemic administration of AdICOSIg prolonged corneal graft survival [31]. This may indicate that the inhibition of primary immune responses is more important in order to prolong corneal graft survival than inhibiting memory cells by ICOSIg.

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tenuation of pro-inflammatory cytokine expression [40]. Interestingly, the neovascularisation of transplanted corneas seemed to be unaffected by this therapy.

**Overexpression of Immunomodulatory Cytokines and Growth Factors**

Interleukin (IL)-10, and its Epstein-Barr virus-encoded IL-10 homologue viral IL-10 (vIL-10), is probably the most studied cytokine due to its potent immunomodulatory properties such as diminishing the antigen-presenting capacity of monocytes and inhibition of monokine production such as IL-1, IL-6, IL-8, and TNF-α [41, 42]. It is well documented that gene transfer of IL-10 leads to prolonged graft survival in different transplant models including the cornea [14, 43]. It has been shown that Ad-mediated IL-10 gene transfer in cultured corneas leads to significant prolongation of graft survival in an outbred sheep corneal transplant model [14]. Subsequent studies investigated both non-viral (liposomes) and viral (Ad) genetic modification of cultured corneas in a high-responder major histocompatibility complex class I/II mismatched rat transplant model. Although neither strategy led to prolongation of corneal graft survival, systemic administration of AdvIL-10 in transplant recipients significantly prolonged allograft survival [44]. Recently, Parker et al. [45] investigated LV-mediated gene transfer of ovine IL-10 in cultured corneas in the sheep transplant model. LVovineIL-10 gene transfer was less efficient in prolonging corneal graft survival compared to Ad-mediated gene transfer. LVovineIL-10 gene transfer seems to be less efficient in producing IL-10 compared to AdvIL-10 although a different promoter was used in this study (SV40 vs. CMV in the previous study), which makes the results difficult to compare. Overall, IL-10 gene transfer seems to be beneficial but it will need further research to fully evaluate the potential of IL-10 gene therapy in cornea transplantation. Besides IL-10 gene transfer, inhibition of the pro-inflammatory cytokine IL-12 by overexpression of the IL-12p40 inhibitory subunit has also been investigated in different animal models of cornea transplantation and also showed varying results [46, 47].

A number of neurotropic growth factors and their associated receptors have been found in the anterior segment of the eye. Among these nerve growth factor (NGF), neurotrophin-3 and -4 and their receptors are present in the cornea [48]. It has been reported that NGF is able to modulate an immune response from a predominantly Th1 type to a Th2 type [49, 50]. Moreover, it has been described recently that NGF is present in the anterior segment of the eye and plays an important role in tissue main-
Gene Therapy Approaches to Prevent Corneal Graft Rejection

Conclusions and Future Outlook

Although multiple successful gene-therapeutic strategies to prevent corneal allograft rejection have been investigated, to the best of our knowledge no clinical trials have emerged from these studies. This is somewhat surprising because ex vivo gene transfer in cultured corneas allows local tissue manipulation in a culture dish followed by removal of excessive gene transfer vehicles before transplantation thereby reducing viral exposure of the graft recipient. Moreover, the risk of insertional mutagenesis as it happened when integrating gene therapy vectors used to modify hematopoietic stem cells might be lower if differentiated cells were the target cells [53]. This may further support corneal gene therapy using LV vectors as corneal buttons may contain few stem cells only. In addition, rapid development of improved vectors with reduced potential for induction of chromosomal aberrations should help to move corneal gene therapy towards clinical trials [18]. A key problem in further developing gene therapy in organ transplantation seems to be the multitude of suitable genes which could be successfully applied to prolong graft survival. This is in contrast to the recent successful gene therapy clinical trials for retinal diseases in which single gene defects are targeted [54]. Currently it is not clear which therapeutic gene should best be used for gene therapy in cornea transplantation. Gene transfer of secreted molecules such as immunomodulatory cytokines seems to be able to produce good results; however, it is not clear how these secreted molecules might affect neighbouring cells in the eye after transplantation. In this context, expression of membrane-bound therapeutic molecules such as PD-L1 might have an advantage as these will only interact with infiltrating immune cells but not with other cell types thereby reducing the risk of unwanted or unexpected side effects. As it emerges from currently available data, a gene-therapeutic strategy focusing on local (corneal) manipulation combining immunomodulatory/anti-inflammatory with anti-apoptotic gene transfer to prevent endothelial cell loss could be the most promising gene therapy approach for a clinical trial to prevent corneal allograft rejection in the near future.

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