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Perspective

The Impact of Corneal Allograft Rejection on The Long-term Outcome of Corneal Transplantation

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FOOTNOTE

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ABSTRACT

Purpose: To examine the influence of corneal allograft rejection on the survival of penetrating corneal transplantation, to review the status of conventional therapies to improve graft survival, and to consider prospects for alternative approaches to reduce the impact of rejection.

Design: Perspective, including prospective, observational cohort study.

Methods: An examination of the literature on human corneal graft rejection and data from the Australian Corneal Graft Registry, reviewed in the context of clinical experience.

Results: Corneal graft outcome is not improving with era. The sequelae of inflammation, whether occurring before corneal transplantation or subsequently, exert a profound influence by predisposing the graft to rejection. Of the developments that have been instrumental in reducing rejection in vascularized organ transplantation, living–related donation is not an option for corneal transplantation. However, HLA matching may be beneficial and requires reassessment. The evidence-base to support the use of systemic immunosuppressive agents in corneal transplantation is thin, and topical glucocorticosteroids remain the drugs of choice to prevent or reverse rejection episodes. Experimental approaches to local allospecific immunosuppression, including the use of antibody-based reagents and gene therapy, are being developed but may be difficult to translate from the laboratory bench to the clinic.

Conclusions: Corneal allograft rejection remains a major cause of graft failure. High-level evidence to vindicate the use of a particular approach or treatment to prevent or treat corneal graft rejection is lacking. In the absence of extensive data from randomized, controlled clinical trials, corneal graft registers and extrapolation from
experimental models provide some clinically useful information.
Corneal transplantation, practiced for a century, falls well short of its full therapeutic potential. The success rate of corneal transplantation is less than is generally appreciated. Allograft rejection is the most common cause of corneal graft. In this centenary year of penetrating corneal transplantation and at a time of unprecedented success in other areas of clinical transplantation, it is timely to consider the current status of the procedure, the way in which outcomes are affected by allograft rejection, and the strategies that can be used to manage rejection. To this end, we have summarized pertinent results from the Australian Corneal Graft Registry, a large repository of corneal transplantation data, reviewed the status of conventional therapies to improve graft survival, and considered the prospects for alternative approaches to reduce the impact of rejection.

The value of transplant registries

Patient registries allow the collection of comprehensive information about transplantation that is difficult to obtain in any other way. Randomized, controlled clinical trials provide the highest level of evidence, but relatively few such trials relating to corneal transplantation have ever been reported. Clinical trials are difficult to perform in the surgical context, and patient registries provide a useful, alternative source of data. They can provide information on the long-term follow-up on large numbers of subjects, enabling robust multivariate analysis to be performed.

The Australian Corneal Graft Registry (ACGR) was established in 1985 to measure the long-term survival of corneal grafts and to identify the factors which influence graft survival. It is administered from the Department of Ophthalmology at the Flinders Medical Centre in Adelaide, Australia. Individual surgeons handled the informed
consent process for each patient according to local legislative requirements, to permit
information to be lodged with the register. The Institutional Ethics Committee of Flinders
University oversees the operations of the register, which are carried out in accordance
with the Declaration of Helsinki. Initially it operated independently. Now it has
collaborative partners through the recently-formed Eye Banks of Australia and New
Zealand Association. The member banks of this organization contribute to the
acquisition of data from surgeons using corneas distributed from the various banks. The
surgeons receive a registration form with each donor cornea and return the form to the
ACGR. They are also sent annual follow-up forms by the ACGR. The process is not
compulsory, but more than 90% of the corneal grafts performed in Australia each year
are registered. Recent analyses included data from 15,000 corneal grafts, some of
which had been followed for almost 20 years. De-identified data, analyses and a
commentary are published every two years in the form of a report, distributed to
contributors and other interested parties around the world.

Registries have limitations – and detractors. The primary data collection is left in
the hands of a large number of contributors – over 500 in the case of the ACGR. In
consequence, there are difficulties in ensuring uniformity of observation. In addition,
there are inevitable losses to follow-up. Registries are not population-based surveys.
Only the data entered can be analyzed, and no epidemiological conclusions can be
drawn. For these reasons, there is division in the evidence-based medicine movement
about the place of registries. The purists, principally those conducting clinical trials,
reject attempts to consider registry data as high-level evidence. However without
registries, there would be little evidence upon which to base clinical transplantation
practice. Registries provide information invaluable to those managing clinical problems.
A more detailed analysis of the benefits and limitations of transplant patient registries has been reviewed elsewhere.\textsuperscript{3}

**MEASURING THE OUTCOMES OF CORNEAL TRANSPLANTATION**

Graft failure is one important measure of the outcome of corneal transplantation. Although not all patients with functioning grafts - or even good levels of post-operative best-corrected visual acuity - are happy with their situation, a non-functioning graft is strongly associated with patient dissatisfaction. Furthermore, graft failure is easily recognized. A corneal graft is either functioning or it is not. Graft failure is thus a crisp end-point for clinical studies. The standard description used in the ACGR is loss of corneal transparency and persistent, increased corneal thickness.

Since the most common reason to undertake corneal transplantation is to improve vision, visual function also needs to be considered as an outcome measure.\textsuperscript{4} However measurements of visual function do not provide clear endpoints. There are a number of reasons for this. Visual acuity measurement is only a surrogate measure for visual disability. Some patients with poor acuity function well and others with excellent acuity function poorly, findings that tend to influence clinical management. Patients who function adequately are not driven to pursue the best possible visual correction, nor to wear it, if inconvenient. Furthermore, the ability of practitioners to provide the best refractive correction is variable.

The outcome of penetrating corneal transplantation is poorer than is generally appreciated. Long-term survival is about 60% at ten years. Survival is similar for both penetrating and lamellar grafts but the outcome for limbal grafts performed for limbal stem cell dysfunction is considerably worse, with a five-year survival of around 35%
CLINICAL MARKERS OF CORNEAL GRAFT OUTCOME

A number of clinical factors are well established to influence the outcome of corneal transplantation. The most obvious of these is the condition that resulted in the need for transplantation in the first place. Patients with keratoconus have favorable outcomes compared with those receiving grafts for acquired disorders such as herpetic eye disease or trauma (Figure 2).

Inflammation is an independent variable associated with corneal graft failure. A corneal graft put into a recipient bed that has never been inflamed has a much greater chance of survival than one placed in a recipient bed that has been inflamed at some stage in the past. Grafts that are put in a recipient bed inflamed at the time of the surgery do worst of all (Figure 3). In the post-operative period, the occurrence of an inflammatory event such as a suture abscess or recurrent herpes simplex virus infection also increases the risk of graft failure.

Neovascularization is an almost invariable consequence of acute or chronic corneal inflammation. The extent of vascularization of the recipient cornea at the time of corneal transplantation correlates strongly with graft survival. The more extensive the vascularization, the greater the risk of graft failure (Figure 4). Similarly, vascularization of the graft in the post-operative period is also associated with an increased risk of graft failure.

Elevated intraocular pressure can also be thought of as a marker of the extent of inflammation. Extensive inflammation can involve the drainage apparatus, reducing the functional reserve capacity of aqueous outflow and resulting in raised intraocular
pressure. A raised intraocular pressure at the time of corneal transplantation is associated with an increased risk of graft failure. So too is a history of raised intraocular pressure at some time in the past, even if it has reduced to normal by the time of surgery (Figure 5). Similarly, an episode of raised intraocular pressure in the post-operative period is associated with an increased risk of graft failure.

Other clinical features associated with an increased risk of graft failure may reflect allosensitization. First grafts fare better than subsequent grafts in the same eye. The more grafts a person has had, the lower the probability of success (Figure 6). Similarly, an episode of rejection in the post-operative period increases the chance of subsequent episodes and ultimate graft failure.

THE MAINTENANCE AND EROSION OF IMMUNE PRIVILEGE

The early success of corneal transplantation was ascribed to the immune privilege of the anterior segment of the eye. The notion is correct – up to a point. Immune privilege is maintained in the normal cornea by a number of mechanisms, but is readily eroded by the sequelae of inflammation.

Accessory cells, including dendritic cells and macrophages, are necessary for an effective immune response to a foreign antigen. The normal human cornea is relatively acellular. However, accessory cells are not completely absent. Langerhans cells are present in the peripheral epithelium, and a few interstitial dendritic cells can be found in the central and peripheral stroma. Major histocompatibility complex (MHC) antigen expression – HLA in man - is limited in the normal cornea. MHC class I determinants are expressed on epithelium, stromal keratocytes and probably on endothelium. Class II determinants are largely restricted to accessory cells. ABO blood-group antigens are
expressed on the epithelium and endothelium. The normal cornea, along with the testis and brain, expresses Fas ligand – a mechanism for inducing apoptosis in lymphocytes finding their way into the cornea. Blood vessels and lymphatics are absent from the normal cornea, which depends upon nutrients from the tear film and aqueous humor. Furthermore the vasculature of the anterior chamber has tight capillaries which sequestrate the aqueous humor from the systemic circulation, thereby forming the blood-aqueous barrier. The aqueous humor also contributes to privilege by way of its immunosuppressive cytokine and peptide profile. It contains relatively high levels of transforming growth factor-β2, calcitonin gene-related peptide, vasoactive intestinal peptide and α-melanocyte-stimulating hormone.

All of these mechanisms contribute to the immune privilege of the anterior chamber and cornea – and all are eroded by inflammation. Inflammation increases the number of accessory cells in the cornea. Even when the clinical evidence of inflammation has disappeared, many years after an inflammatory event, an increased number of accessory cells are present in the cornea. Corneal graft survival in humans correlates with the number of these cells found in the recipient bed at the time of corneal transplantation. Inflammation also increases MHC class I and class II antigen expression. Inflammatory cells produce large amounts of vascular endothelial cell growth factors, resulting in corneal neovascularization and lymphangiogenesis. Inflammation also affects microvascular competence in the anterior chamber, resulting in erosion of the blood-aqueous barrier and exposing the anterior chamber, including the cornea, to systemic influences. The access of pro-inflammatory molecules including cytokines to the aqueous humor contributes to the erosion of privilege.
The influence of inflammation on the cornea, whether before transplantation or subsequently, is thus profound. Inflammatory pathological process decrease the chance of achieving engraftment. In contrast, a normal cornea can be grafted into a normal cornea with little risk of rejection. The closest clinicians come to this is performing corneal grafts on patients with uncomplicated keratoconus. However, once a cornea has been inflamed, it is never the same again.

**THE IMPACT OF ALLOGRAFT REJECTION ON CORNEAL GRAFT SURVIVAL**

Corneal endothelial cell function is plainly critical to corneal graft function – endothelial cell failure results in corneal edema. A number of pathological processes may contribute to graft failure, of which allograft rejection is the most obvious. It is an acute process, manifesting clinically with ocular inflammation, keratic precipitates on the endothelium that are often assembled in a line, and corneal edema.

Rejection is sometimes reversible but often it is not. Even if a rejection episode can be reversed, it carries a poor prognosis for the occurrence of subsequent episodes and ultimate graft failure. Acute corneal allograft rejection is responsible for about one-third of corneal graft failures reported to the ACGR,2 but the true figure may be higher because rejection may go unrecognized. Inflammation around a stitch, infection complicating an epithelial defect, uveitis, or even ocular wall inflammation such as conjunctivitis may precipitate an allograft response. Patients with herpetic infection affecting the corneal graft may be similarly affected. Whatever the cause, the underlying condition may obscure the classical signs of corneal rejection.

The corneal endothelium of a graft may fail for no apparent reason. There can be a gradual, although sometimes fluctuating, development of corneal edema – so-called
late endothelial cell failure - the causative factor of which is uncertain.\textsuperscript{18}

\textbf{CELLULAR AND MOLECULAR MECHANISMS IN CORNEAL ALLOGRAFT REJECTION}

A line of best fit through the accumulated data from many laboratory experiments performed around the world over many years suggests the following sequence of events during corneal allograft rejection (Figure 7).\textsuperscript{19}

\textit{Accumulation of accessory cells}

The tendency to allograft rejection is related to the number of accessory cells in the recipient cornea at the time of corneal transplantation – the higher the count of cells in the recipient button, the greater the chance of subsequent graft failure.\textsuperscript{14} Accessory cells can move into the graft from the surrounding host cornea and are recruited from the circulation into the cornea during inflammation and wound healing. Histocompatibility antigens shed from a transplant may be internalized by host accessory cells and presented to naïve host T cells.\textsuperscript{20} This is referred to as indirect antigen presentation. In an alternative pathway to sensitization, donor accessory cells that have been carried as passenger cells in the donor organ can trigger host T cells directly.\textsuperscript{20} This is referred to as direct antigen presentation. There is evidence that both pathways operate after corneal transplantation.\textsuperscript{21} The therapeutic implication is there is little point in trying to deplete antigen-presenting accessory cells from the graft to increase graft survival, because such interventions will influence the direct pathway only.

\textit{Processing and presentation of donor antigen}

Antigen presentation has been the focus of intense attention from transplant biologists
and much is known of the process at a molecular level. The interaction between the antigen-presenting cell and the host immunocyte (the naïve T cell) occurs within the draining lymph node and is modulated by co-stimulatory interactions between molecules on the surface of the accessory cell and the lymphocyte. The effect of these co-stimulatory interactions may be enhancing or suppressing.

The cornea supposedly lacks formal lymphatic drainage and thus the location at which presentation of cornea-derived alloantigen occurs is open to conjecture. This is of significance for those developing immunosuppressive therapies for corneal transplantation. Antigen presentation is the most vulnerable point of the allograft response, offering the prospect of allospecific suppression, but to which anatomical site should new drugs be targeted? Antigen presentation could occur in the cornea, as the essential elements of the process are there, but mounting experimental evidence suggests that it occurs elsewhere. It might occur in the uvea, the conjunctiva-associated lymphoid tissue, the draining lymph nodes, or beyond. Antigen may leave the eye as soluble antigen or be carried by mobile accessory cells.

*T cell activation, proliferation, and clonal expansion*

Antigen presentation activates naïve T cells, resulting in T cell proliferation and clonal expansion. Clonal expansion is promoted by the influence of interleukin 2 (IL-2). The most potent action of the calcineurin blockers such as cyclosporine and FK 506 is on IL-2-controlled clonal T cell expansion. These two agents have been ineffective in prolonging corneal graft survival when delivered topically, suggesting that clonal expansion does not occur in the cornea.
The effector arm – graft destruction

The efferent arm of the allograft response can destroy all cells of the donor cornea, but it is the corneal endothelium, with its limited replicative capacity, that is the major target of the corneal allograft response. Damage to the graft is primarily cell-mediated; antibody does not seem to play a significant role in corneal graft rejection. The CD4+ T lymphocyte plays a central role in recruiting effector cells into the graft. Damage appears to be wrought by a wide range of cells, including macrophages, polymorphonuclear granulocytes and NK cells, via a range of cytokines including tumor necrosis factor-α and interferon-γ. CD8+ T lymphocytes are also present in rejecting corneal grafts and have the capacity to cause cell damage, but corneal graft rejection still occurs in animals deficient in CD8+ cells.

Current clinical interventions to abrogate corneal allograft rejection

Topical glucocorticosteroids can prevent and reverse corneal allograft rejection. They achieve this through multiple mechanisms, one of the most important of which is probably to inhibit leukocyte migration into the cornea, thereby abrogating the efferent arm of the allograft response. It is unlikely that a more specific intervention would be as effective. There is too much redundancy for interference with any single element of the efferent arm of the response to be useful: block one molecule or cell type and another will take over. Corticosteroids are therefore likely to remain the most effective of the conventional treatments for established corneal graft rejection.

Can interventions used in organ transplantation be applied to the cornea?
The results of transplantation in other areas of medicine have improved remarkably over the last 40 years. Renal transplantation is a valid example: since the procedure was first carried out in the 1960s, there have been steady improvements in outcome. This is in contrast to corneal transplantation, where it has not been possible to demonstrate any improvement in outcome over a similar period. Analysis by era for the last 20 years within the ACGR database shows no tendency for corneal graft survival to increase.

Perhaps this is not surprising, as very little new has been introduced into the clinical practice of corneal transplantation since the adoption of microsurgical techniques and materials decades ago, and the use of topical corticosteroids.

Three major developments have contributed to the 50% improvement in renal graft outcomes in recent years: better histocompatibility matching, improved systemic immunosuppression, and the use of living-related donors. Neither matching nor the use of systemic immunosuppression is widely practiced in corneal transplantation. The use of living-related donors is clearly not justifiable, but both tissue matching and systemic immunosuppression deserve further examination.

Histocompatibility antigen matching in corneal transplantation

Histocompatibility matching for MHC determinants has not found favor amongst clinicians in many parts of the world. This is doubtless in part because the American Collaborative Corneal Transplant Study (CCTS) reported no benefit from HLA class I and class II antigen matching in corneal transplantation, although it did support, surprisingly, a modest benefit from ABO antigen matching. In northern Europe a different attitude prevails. A benefit from MHC matching has been reported from Canada, Holland and Germany, as well as from the United Kingdom. In patients
considered to be of high risk of rejection, the improvement obtained with matching was
of the order of forty percent, compared with those who were less well-matched.\textsuperscript{35}

Why is there such a disparity between the results reported from the USA, Canada
and Europe? Perhaps the results of the CCTS have been too readily accepted. In a
paper published after the clinical results were first reported, a high error rate in the
typing of patients included in the CCTS study was discovered.\textsuperscript{37} This error rate was
about 12\% at the HLA A locus, 20\% at the B locus, and 45\% at the DR locus. Völker-
Dieben and colleagues have since used a mathematical model to demonstrate that an
error rate in DR locus typing of more than 10\% is enough to obscure any chance of
detecting a benefit from matching.\textsuperscript{38}

Perhaps it is time to re-examine the use of HLA matching for corneal
transplantation: the results from Europe cannot be ignored, and molecular techniques
have facilitated ever-more accurate typing. Matching may be the only currently-available
intervention that can improve outcome for high-risk patients without exposing them to
any significant risks. Efforts to find a match may delay surgery, but the possibility of
unacceptably long delays can be assessed prior to surgery by considering the frequency
of a given recipient’s HLA antigens within the population from which donor corneas are
drawn.\textsuperscript{39} How practical it would be to introduce extensive matching programs is another
issue. Histocompatibility matching for corneal transplantation within Europe is performed
in the context of a major international network. Provision of essential infrastructure
funding and large, reasonably homogeneous populations separated by relatively small
distances have contributed to the success of these programs. Whether similar programs
are possible in larger, more fragmented and less densely populated countries – such as
the USA or Australia – is an unresolved issue.
The use of systemic immunosuppression in corneal transplantation

Systemic immunosuppression is not widely applied in corneal transplantation, even for patients at high risk of rejection. There are two reasons for this. The evidence-base for a beneficial effect is limited, and the clinical context of corneal transplantation is different from that of other forms of transplantation in which systemic immunosuppression is readily justified and widely used.

The evidence-base supporting the use of systemic immunosuppression in corneal transplantation is limited, being mostly confined to cyclosporine, and the results are inconsistent. Some investigators have demonstrated a beneficial effect and others have failed to find any improvement in outcome. The efficacy of systemic mycophenolate mofetil in corneal transplantation has yet to be established.

A benefit of systemic immunosuppression in corneal transplantation might well have been expected, given that the corneal allograft reaction is not too dissimilar to organ graft rejection, where the advantages have been obvious. However, the allograft response is more easily suppressed in some tissues than in others. For example, it is more difficult to prolong skin allografts than renal allografts with immunosuppression. It would seem that the cornea is towards the more difficult end of the spectrum with respect to immunosuppression.

There are problems in planning and conducting randomized, controlled clinical trials of systemic immunosuppression for corneal transplantation, because of the serious risks entailed. For the most part, corneal transplantation is carried out on patients who are otherwise well. They may be disabled and frustrated by poor vision, but their general health is not threatened. This contrasts with those requiring a transplant of a solid organ...
such as a kidney, heart, lung, or liver. Such people need an allograft to survive, or in the
case of renal recipients, to escape dialysis. For this group of patients, the risk of a
complication from systemic immunosuppression is the lesser of two evils.

Some patients with poor vision who would benefit from a corneal transplant are
so affected by their predicament, that they are prepared to accept the risks of systemic
immunosuppression. This is a small minority of patients. However, for those who are in
need of immunosuppression and understand the risks involved, there is no evidence
that any one regimen of immunosuppression is any better than any other. In the
absence of data, it is reasonable to extrapolate from protocols used in other organ
transplantation. At present this involves the use of a calcineurin inhibitor such as
cyclosporine or FK506, and an anti-proliferative agent such as azathioprine or
mycophenolate. It is usually preferred that this aspect of the patient’s management be
carried out in collaboration with a physician experienced in immunosuppression for other
clinical indications – such as an internist involved in solid organ transplantation.

Probably as much immunosuppression is needed to prevent corneal graft rejection as is
required to prevent the rejection of any solid organ. Any temptation to use less than this
may not reduce the risk of rejection, but may still expose patients to the side effects of
immunosuppressive agents.

**ALTERNATIVE APPROACHES: THE PURSUIT OF REGIONAL IMMUNOLOGICAL BLOCKADE**

Given that few of the therapeutic interventions that have been applied to other areas of
clinical transplantation are applicable to corneal transplantation, it will be necessary to
develop alternative strategies that are especially suited to the eye. These must take into
account the unique features of the eye, the elements of the corneal allograft response
that are different and can be exploited, and the clinical reality of corneal transplantation.

For example, the eye is readily accessible compared with most other transplantable organs and tissues. It is therefore possible to deliver medication topically in the form of eye drops. In addition, the donor cornea used for transplantation can be manipulated ex vivo over a relatively long period while in storage medium prior to surgery.

New approaches currently being explored in experimental animals are based on developments in molecular medicine – the exploitation of an understanding of the production of proteins by cells and how these proteins function. 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 a gene therapy approach. The anterior segment of the eye is amenable to both approaches.

Topically delivered antibodies to prolong graft survival

Systemic immunosuppressive intervention with polyclonal and monoclonal antibodies directed at key elements of the allograft response is well established in transplantation medicine. Antibodies are currently used to “rescue” vascularized organ grafts undergoing rejection. There are anecdotal reports of this general approach being applied to corneal transplantation, with good results. However, the systemic administration of potent immunosuppressive antibody is not without risk. The use of antibody reagents to generate an allospecific regional blockade would be ideal.

Whole antibody molecules, with a molecular weight of around 150 kDa, cannot pass into the anterior chamber after topical administration to the ocular surface. In order to overcome this problem, we have explored the possibility of using antibody fragments for topical delivery to the eye. Using molecular techniques, antibody fragments
comprising just the variable region domains of the heavy and light chains of an
immunoglobulin molecule can be constructed. Such fragments can be considered as
free antigen-binding domains. For use in experimental systems, we constructed a
murine antibody fragment with a molecular weight of 28kD. The fragment had the same
specificity for antigen as the parental antibody from which it was engineered.

Experiments with corneas mounted in isolated corneal perfusion chambers in vitro
showed that this and similar model antibody fragments could cross the cornea into the
anterior chamber after topical delivery to the ocular surface.\(^{47}\) The fragment was also
shown to be able to penetrate through the corneas of rabbits in vivo. Whether such
antibody constructs will prove to be useful immunosuppressants for corneal
transplantation remains to be determined.

Gene therapy in corneal transplantation

The cornea is well-suited to gene therapy. The tissue is accessible and corneal
endothelial cells are readily transfected. Transfection can be achieved ex vivo, in the
case of a donor cornea, or in vivo by injecting the construct into the anterior chamber.
Either way, high transfection rates can be achieved with appropriate vector systems,
especially with replication-deficient viral vectors. High rates of production of transgenic
protein by corneal endothelium can be achieved and gene expression can be
surprisingly persistent, perhaps because the endothelium (at least in humans) does not
divide. The relative immune privilege of the cornea and anterior chamber may permit the
use of vectors which are otherwise immunogenic.

A number of investigators have reported prolongation of corneal allograft survival
following ex vivo gene transfer to the donor cornea in the mouse, rat, rabbit and sheep.\(^{48}\)
We have found corneal transplantation in the sheep to be a satisfactory model for a number of reasons. The animals are large, robust and outbred – important attributes for preclinical studies on gene therapy. Corneal graft rejection is swift, at around three weeks in untreated animals, and there is no tendency for rejection to be transient or reversible, because the endothelium is non-replicative. In small rodents such as rats and mice, the rejection process may be quite short and healing of the endothelium may occur, so that corneal clarity is restored. A number of ovine-specific reagents are available. When donor ovine corneas were transfected ex vivo with cDNA for ovine interleukin 10 (IL-10) using an adenoviral vector, an impressive and significant prolongation of subsequent corneal allograft survival was observed. IL-10 is an immunomodulatory cytokine with multiple points of action. It affects accessory cell function at an early step in the allograft response. That IL-10 is effective in prolonging graft survival when the endothelium of the grafted cornea is transfected suggests that accessory cells within the cornea and immediate ocular environs are being modulated.

A number of potential problems need to be overcome before gene therapy can be used to prolong human corneal allograft survival. Most investigators in the field have thus far used adenoviral vectors, which may not be optimal. Objections have been raised to these vectors on the basis of potential toxicity, citing the death of a patient in the United States after systemic gene therapy. There are also questions surrounding the period of gene expression which can be expected with adenoviral vectors. Adenoviral vectors remain episomal. Lentiviral and adeno-associated viral vectors integrate into genomic DNA and thus can be expected to provide more prolonged transgene expression.
**Barriers Between the Laboratory and Clinical Practice**

Even if the use of antibody fragments or gene therapy can be shown to be effective in prolonging corneal graft survival in animal models, there remains a long and difficult path to clinical practice. Regulatory requirements are likely to be arduous. High levels of public suspicion concerning genetic manipulation ensure this. Complying with regulatory requirements is likely to be costly and beyond the resources of academic institutions. Commercial partners will be required and they may be difficult to find. The pharmaceutical industry will look at the high levels of investment needed, and expect high returns to cover the investment. The returns from a relatively small clinical domain such as corneal transplantation may not justify such investment. Ophthalmologists and the research programs they support must take the lead, and ensure the developments required for corneal transplantation to fulfill its potential are achieved. Extrapolations from other branches of experimental transplantation are unlikely to be useful: research needs to be carried out specifically in the context of corneal transplantation.

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**References**


**FIGURE LEGENDS**


**FIGURE 5.** The influence of a history of raised intraocular pressure on corneal allograft

**Figure 6.** The influence of repeated keratoplasty in the ipsilateral eye on corneal allograft survival. Kaplan-Meier plots show the survival of penetrating corneal grafts within the Australian Corneal Graft Registry database. Modified with permission from The Australian Corneal Graft Registry Report 2004, eds KA Williams, NB Hornsby, CM Bartlett, HK Holland, A Esterman, DJ Coster. Adelaide: Snap Printing 2004, pp1-192.

**Figure 7.** The mechanisms involved in corneal graft rejection.
Figure 3.

![Graph showing survival probabilities for different conditions](image1)

- Never inflamed
- Inflamed in past, not at graft
- Inflamed at graft, not in past
- Inflamed in past, at graft

Figure 4.

![Graph showing survival probabilities for different vascular status](image2)

- Avascular
- 1 quadrant
- 2 quadrants
- 3 quadrants
- 4 quadrants
Figure 5.

![Graph showing survival probability over years post-graft for different conditions regarding IOP.

Figure 6.

![Graph comparing survival probability over years post-graft for various graft conditions.](image)
Figure 7.

Antigen presenting cell (APC)

Soluble antigen

Antigen processing in rejection-prone corneal allograft

Processed antigen in APC

APC

Lymphocyte

Antigen presentation

IL-1

T cell activation

IL-2

CLONAL EXPANSION

Cyclosporin

FK 506

Effector Cells

Leucocyte-driven corneal allograft destruction

Antigen presentation, T cell activation and clonal expansion can occur in conjunctival associated lymphoid tissue and the draining lymph nodes in the face and neck