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Coster, D.J. & Williams, K.A., 2005. The impact of corneal allograft rejection on the long-term outcome of corneal transplantation. American Journal of Ophthalmology, 140(6), 1112-1122.

which has been published in final form at

DOI:

http://dx.doi.org/10.1016/j.ajo.2005.07.024

1 **Perspective**

2 **The Impact of Corneal Allograft Rejection on The Long-term**

3 **Outcome of Corneal Transplantation**

4

5 **DOUGLAS J COSTER, FRACO, AND KERYN A WILLIAMS, PHD**

6 Department of Ophthalmology, Flinders University, Adelaide, Australia

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8 Key words: corneal transplantation, allograft rejection, outcomes

9 Word count for text: 5255 excluding Abstract and References

10 Figures and tables: 7 figures

11 Address for correspondence: Professor D. J. Coster, Department of Ophthalmology,

12 Flinders Medical Centre, Bedford Park, SA 5042, Australia; fax: 618 8277 0899; email:

13 doug.coster@flinders.edu.au

14

1 **ABSTRACT**

2 **Purpose:** To examine the influence of corneal allograft rejection on the survival of

3 penetrating corneal transplantation, to review the status of conventional therapies to

4 improve graft survival, and to consider prospects for alternative approaches to reduce

5 the impact of rejection.

6 Design: Perspective, including prospective, observational cohort study.

7 **Methods:** An examination of the literature on human corneal graft rejection and data

8 from the Australian Corneal Graft Registry, reviewed in the context of clinical

9 experience.

10 **Results:** Corneal graft outcome is not improving with era. The sequelae of inflammation,

11 whether occurring before corneal transplantation or subsequently, exert a profound

12 influence by predisposing the graft to rejection. Of the developments that have been

13 instrumental in reducing rejection in vascularized organ transplantation, living–related

14 donation is not an option for corneal transplantation. However, HLA matching may be

15 beneficial and requires reassessment. The evidence-base to support the use of

16 systemic immunosuppressive agents in corneal transplantation is thin, and topical

17 glucocorticosteroids remain the drugs of choice to prevent or reverse rejection episodes.

18 Experimental approaches to local allospecific immunosuppression, including the use of

19 antibody-based reagents and gene therapy, are being developed but may be difficult to

20 translate from the laboratory bench to the clinic.

21 **Conclusions**: Corneal allograft rejection remains a major cause of graft failure. High-

22 level evidence to vindicate the use of a particular approach or treatment to prevent or

23 treat corneal graft rejection is lacking. In the absence of extensive data from

24 randomized, controlled clinical trials, corneal graft registers and extrapolation from

1   experimental models provide some clinically useful information.

2

1   Corneal transplantation, practiced for a century, falls well short of its full therapeutic

2   potential. The success rate of corneal transplantation is less than is generally

3   appreciated. Allograft rejection is the most common cause of corneal graft. In this

4   centenary year of penetrating corneal transplantation[1] and at a time of unprecedented

5   success in other areas of clinical transplantation, it is timely to consider the current

6   status of the procedure, the way in which outcomes are affected by allograft rejection,

7   and the strategies that can be used to manage rejection. To this end, we have

8   summarized pertinent results from the Australian Corneal Graft Registry, a large

9   repository of corneal transplantation data, reviewed the status of conventional therapies

10   to improve graft survival, and considered the prospects for alternative approaches to

11   reduce the impact of rejection.

12

13   **THE VALUE OF TRANSPLANT REGISTRIES**

14   Patient registries allow the collection of comprehensive information about transplantation

15   that is difficult to obtain in any other way. Randomized, controlled clinical trials provide

16   the highest level of evidence, but relatively few such trials relating to corneal

17   transplantation have ever been reported. Clinical trials are difficult to perform in the

18   surgical context, and patient registries provide a useful, alternative source of data. They

19   can provide information on the long-term follow-up on large numbers of subjects,

20   enabling robust multivariate analysis to be performed.

21   The Australian Corneal Graft Registry (ACGR) was established in 1985 to

22   measure the long-term survival of corneal grafts and to identify the factors which

23   influence graft survival.[2] It is administered from the Department of Ophthalmology at the

24   Flinders Medical Centre in Adelaide, Australia. Individual surgeons handled the informed

1 consent process for each patient according to local legislative requirements, to permit

2 information to be lodged with the register. The Institutional Ethics Committee of Flinders

3 University oversees the operations of the register, which are carried out in accordance

4 with the Declaration of Helsinki. Initially it operated independently. Now it has

5 collaborative partners through the recently-formed Eye Banks of Australia and New

6 Zealand Association. The member banks of this organization contribute to the

7 acquisition of data from surgeons using corneas distributed from the various banks. The

8 surgeons receive a registration form with each donor cornea and return the form to the

9 ACGR. They are also sent annual follow-up forms by the ACGR. The process is not

10 compulsory, but more than 90% of the corneal grafts performed in Australia each year

11 are registered. Recent analyses included data from 15,000 corneal grafts, some of

12 which had been followed for almost 20 years. De-identified data, analyses and a

13 commentary are published every two years in the form of a report, distributed to

14 contributors and other interested parties around the world.

15 Registries have limitations – and detractors. The primary data collection is left in

16 the hands of a large number of contributors – over 500 in the case of the ACGR. In

17 consequence, there are difficulties in ensuring uniformity of observation. In addition,

18 there are inevitable losses to follow-up. Registries are not population-based surveys.

19 Only the data entered can be analyzed, and no epidemiological conclusions can be

20 drawn. For these reasons, there is division in the evidence-based medicine movement

21 about the place of registries. The purists, principally those conducting clinical trials,

22 reject attempts to consider registry data as high-level evidence. However without

23 registries, there would be little evidence upon which to base clinical transplantation

24 practice. Registries provide information invaluable to those managing clinical problems.

1   A more detailed analysis of the benefits and limitations of transplant patient registries

2   has been reviewed elsewhere.[3]

3

4   **MEASURING THE OUTCOMES OF CORNEAL TRANSPLANTATION**

5   Graft failure is one important measure of the outcome of corneal transplantation.

6   Although not all patients with functioning grafts - or even good levels of post-operative

7   best-corrected visual acuity - are happy with their situation, a non-functioning graft is

8   strongly associated with patient dissatisfaction. Furthermore, graft failure is easily

9   recognized. A corneal graft is either functioning or it is not. Graft failure is thus a crisp

10   end-point for clinical studies. The standard description used in the ACGR is loss of

11   corneal transparency and persistent, increased corneal thickness.

12   Since the most common reason to undertake corneal transplantation is to

13   improve vision, visual function also needs to be considered as an outcome measure.[4]

14   However measurements of visual function do not provide clear endpoints. There are a

15   number of reasons for this. Visual acuity measurement is only a surrogate measure for

16   visual disability. Some patients with poor acuity function well and others with excellent

17   acuity function poorly, findings that tend to influence clinical management. Patients who

18   function adequately are not driven to pursue the best possible visual correction, nor to

19   wear it, if inconvenient. Furthermore, the ability of practitioners to provide the best

20   refractive correction is variable.

21   The outcome of penetrating corneal transplantation is poorer than is generally

22   appreciated. Long-term survival is about 60% at ten years. Survival is similar for both

23   penetrating and lamellar grafts but the outcome for limbal grafts performed for limbal

24   stem cell dysfunction is considerably worse, with a five-year survival of around 35%

1   (Figure 1).

2

3   **CLINICAL MARKERS OF CORNEAL GRAFT OUTCOME**

4   A number of clinical factors are well established to influence the outcome of corneal

5   transplantation. The most obvious of these is the condition that resulted in the need for

6   transplantation in the first place. Patients with keratoconus have favorable outcomes

7   compared with those receiving grafts for acquired disorders such as herpetic eye

8   disease or trauma (Figure 2).

9   Inflammation is an independent variable associated with corneal graft failure. A

10   corneal graft put into a recipient bed that has never been inflamed has a much greater

11   chance of survival than one placed in a recipient bed that has been inflamed at some

12   stage in the past. Grafts that are put in a recipient bed inflamed at the time of the

13   surgery do worst of all (Figure 3). In the post-operative period, the occurrence of an

14   inflammatory event such as a suture abscess or recurrent herpes simplex virus infection

15   also increases the risk of graft failure.

16   Neovascularization is an almost invariable consequence of acute or chronic

17   corneal inflammation. The extent of vascularization of the recipient cornea at the time of

18   corneal transplantation correlates strongly with graft survival. The more extensive the

19   vascularization, the greater the risk of graft failure (Figure 4). Similarly, vascularization of

20   the graft in the post-operative period is also associated with an increased risk of graft

21   failure.

22   Elevated intraocular pressure can also be thought of as a marker of the extent of

23   inflammation. Extensive inflammation can involve the drainage apparatus, reducing the

24   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.[5] The notion is correct – up to a point. Immune privilege is maintained in the normal cornea by a number of mechanisms,[6] 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.[7,8] 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.[9] Class II determinants are largely restricted to accessory cells.[7-10] ABO blood-group antigens are

expressed on the epithelium and endothelium.[11] 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.[12] 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.[13] The aqueous humor also contributes to privilege by

way of its immunosuppressive cytokine and peptide profile.[6] 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.[10] 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.[14] Inflammation also increases MHC class I and class II antigen

expression.[15] Inflammatory cells produce large amounts of vascular endothelial cell

growth factors, resulting in corneal neovascularization and lymphangiogenesis.[16]

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.[17,18] The access of pro-inflammatory molecules including

cytokines to the aqueous humor contributes to the erosion of privilege.

1 The influence of inflammation on the cornea, whether before transplantation or

2 subsequently, is thus profound. Inflammatory pathological process decrease the chance

3 of achieving engraftment. In contrast, a normal cornea can be grafted into a normal

4 cornea with little risk of rejection. The closest clinicians come to this is performing

5 corneal grafts on patients with uncomplicated keratoconus. However, once a cornea has

6 been inflamed, it is never the same again.

7

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

9 Corneal endothelial cell function is plainly critical to corneal graft function – endothelial

10 cell failure results in corneal edema. A number of pathological processes may contribute

11 to graft failure, of which allograft rejection is the most obvious. It is an acute process,

12 manifesting clinically with ocular inflammation, keratic precipitates on the endothelium

13 that are often assembled in a line, and corneal edema.

14 Rejection is sometimes reversible but often it is not. Even if a rejection episode

15 can be reversed, it carries a poor prognosis for the occurrence of subsequent episodes

16 and ultimate graft failure. Acute corneal allograft rejection is responsible for about one-

17 third of corneal graft failures reported to the ACGR,[2] but the true figure may be higher

18 because rejection may go unrecognized. Inflammation around a stitch, infection

19 complicating an epithelial defect, uveitis, or even ocular wall inflammation such as

20 conjunctivitis may precipitate an allograft response. Patients with herpetic infection

21 affecting the corneal graft may be similarly affected. Whatever the cause, the underlying

22 condition may obscure the classical signs of corneal rejection.

23 The corneal endothelium of a graft may fail for no apparent reason. There can be

24 a gradual, although sometimes fluctuating, development of corneal edema – so-called

1   late endothelial cell failure - the causative factor of which is uncertain.[18]

2

3   **CELLULAR AND MOLECULAR MECHANISMS IN CORNEAL ALLOGRAFT REJECTION**

4   A line of best fit through the accumulated data from many laboratory experiments

5   performed around the world over many years suggests the following sequence of events

6   during corneal allograft rejection (Figure 7).[19]

7

8   *Accumulation of accessory cells*

9   The tendency to allograft rejection is related to the number of accessory cells in the

10   recipient cornea at the time of corneal transplantation – the higher the count of cells in

11   the recipient button, the greater the chance of subsequent graft failure.[14] Accessory cells

12   can move into the graft from the surrounding host cornea and are recruited from the

13   circulation into the cornea during inflammation and wound healing. Histocompatibility

14   antigens shed from a transplant may be internalized by host accessory cells and

15   presented to naïve host T cells.[20] This is referred to as indirect antigen presentation. In

16   an alternative pathway to sensitization, donor accessory cells that have been carried as

17   passenger cells in the donor organ can trigger host T cells directly.[20] This is referred to

18   as direct antigen presentation. There is evidence that both pathways operate after

19   corneal transplantation.[21] The therapeutic implication is there is little point in trying to

20   deplete antigen-presenting accessory cells from the graft to increase graft survival,

21   because such interventions will influence the direct pathway only.

22

23   *Processing and presentation of donor antigen*

24   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.[22] 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,[23] the conjunctiva-

associated lymphoid tissue,[24] the draining lymph nodes,[25] or beyond. Antigen may leave

the eye as soluble antigen[26] or be carried by mobile accessory cells.[27]

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.[28] 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.[29]

CURRENT CLINICAL INTERVENTIONS TO ABROGATE CORNEAL ALLOGRAFT REJECTION

Topical glucocorticosteroids can prevent and reverse corneal allograft rejection. They achieve this through multiple mechanisms,[28] 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.[30] 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.[31] 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.[32] In northern Europe a different attitude prevails. A benefit from MHC matching has been reported from Canada,[33] Holland[34] and Germany,[35] as well as from the United Kingdom.[36] 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.[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.[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.[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.[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.

1

2 *The use of systemic immunosuppression in corneal transplantation*

3 Systemic immunosuppression is not widely applied in corneal transplantation, even for

4 patients at high risk of rejection. There are two reasons for this. The evidence-base for a

5 beneficial effect is limited, and the clinical context of corneal transplantation is different

6 from that of other forms of transplantation in which systemic immunosuppression is

7 readily justified and widely used.

8 The evidence-base supporting the use of systemic immunosuppression in corneal

9 transplantation is limited, being mostly confined to cyclosporine, and the results are

10 inconsistent.[40-44] Some investigators have demonstrated a beneficial effect[40,41] and

11 others[42-44] have failed to find any improvement in outcome. The efficacy of systemic

12 mycophenolate mofetil in corneal transplantation has yet to be established.

13 A benefit of systemic immunosuppression in corneal transplantation might well

14 have been expected, given that the corneal allograft reaction is not too dissimilar to

15 organ graft rejection, where the advantages have been obvious. However, the allograft

16 response is more easily suppressed in some tissues than in others. For example, it is

17 more difficult to prolong skin allografts than renal allografts with immunosuppression. It

18 would seem that the cornea is towards the more difficult end of the spectrum with

19 respect to immunosuppression.

20 There are problems in planning and conducting randomized, controlled clinical

21 trials of systemic immunosuppression for corneal transplantation, because of the serious

22 risks entailed.[45] For the most part, corneal transplantation is carried out on patients who

23 are otherwise well. They may be disabled and frustrated by poor vision, but their general

24 health is not threatened. This contrasts with those requiring a transplant of a solid organ

1 such as a kidney, heart, lung, or liver. Such people need an allograft to survive, or in the

2 case of renal recipients, to escape dialysis. For this group of patients, the risk of a

3 complication from systemic immunosuppression is the lesser of two evils.

4 Some patients with poor vision who would benefit from a corneal transplant are

5 so affected by their predicament, that they are prepared to accept the risks of systemic

6 immunosuppression. This is a small minority of patients. However, for those who are in

7 need of immunosuppression and understand the risks involved, there is no evidence

8 that any one regimen of immunosuppression is any better than any other. In the

9 absence of data, it is reasonable to extrapolate from protocols used in other organ

10 transplantation. At present this involves the use of a calcineurin inhibitor such as

11 cyclosporine or FK506, and an anti-proliferative agent such as azathioprine or

12 mycophenolate. It is usually preferred that this aspect of the patient's management be

13 carried out in collaboration with a physician experienced in immunosuppression for other

14 clinical indications – such as an internist involved in solid organ transplantation.

15 Probably as much immunosuppression is needed to prevent corneal graft rejection as is

16 required to prevent the rejection of any solid organ. Any temptation to use less than this

17 may not reduce the risk of rejection, but may still expose patients to the side effects of

18 immunosuppressive agents.

19

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

21 Given that few of the therapeutic interventions that have been applied to other areas of

22 clinical transplantation are applicable to corneal transplantation, it will be necessary to

23 develop alternative strategies that are especially suited to the eye. These must take into

24 account the unique features of the eye, the elements of the corneal allograft response

1   that are different and can be exploited, and the clinical reality of corneal transplantation.

2   For example, the eye is readily accessible compared with most other transplantable

3   organs and tissues. It is therefore possible to deliver medication topically in the form of

4   eye drops. In addition, the donor cornea used for transplantation can be manipulated *ex*

5   *vivo* over a relatively long period while in storage medium prior to surgery.

6   New approaches currently being explored in experimental animals are based on

7   developments in molecular medicine – the exploitation of an understanding of the

8   production of proteins by cells and how these proteins function. Two broad approaches

9   are applicable: first, the production of peptides or small proteins that can be delivered to

10 the eye topically and second, the *in vivo* production of proteins by a gene therapy

11 approach. The anterior segment of the eye is amenable to both approaches.

12

13 *Topically delivered antibodies to prolong graft survival*

14 Systemic immunosuppressive intervention with polyclonal and monoclonal antibodies

15 directed at key elements of the allograft response is well established in transplantation

16 medicine. Antibodies are currently used to "rescue" vascularized organ grafts

17 undergoing rejection. There are anecdotal reports of this general approach being

18 applied to corneal transplantation, with good results.[46] However, the systemic

19 administration of potent immunosuppressive antibody is not without risk. The use of

20 antibody reagents to generate an allospecific regional blockade would be ideal.

21 Whole antibody molecules, with a molecular weight of around 150 kDa, cannot

22 pass into the anterior chamber after topical administration to the ocular surface. In order

23 to overcome this problem, we have explored the possibility of using antibody fragments

24 for topical delivery to the eye.[47] 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.[49] 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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FIGURE LEGENDS

FIGURE 1. Long term corneal allograft survival. Kaplan-Meier plots show the survival of penetrating, lamellar and limbal 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 2. The influence of indication for transplantation 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 3. The influence of ipsilateral ocular inflammation prior to, or at the time of corneal transplantation, 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.

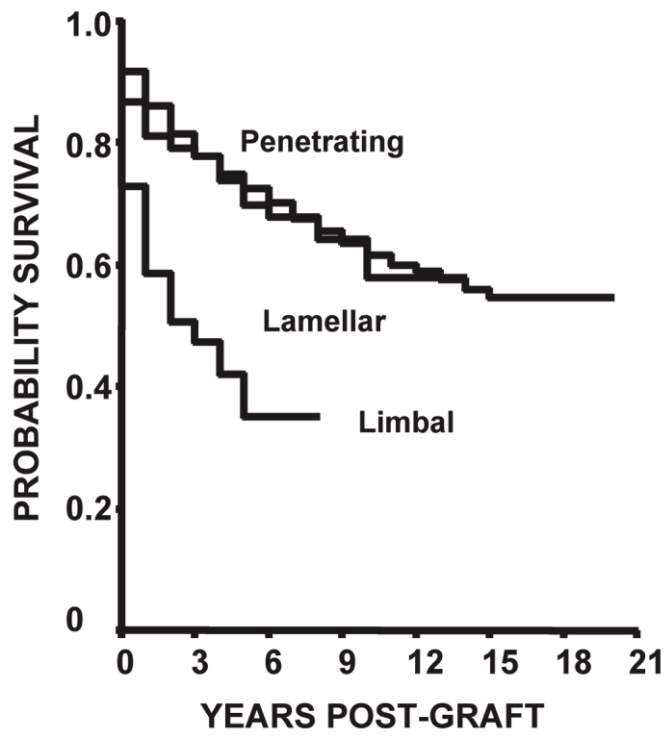**FIGURE 4**. The influence of corneal neovascularization at the time of transplantation on corneal allograft survival. Kaplan-Meier plots show the survival of penetrating corneal grafts within the Australian Corneal Graft Registry database. Modified with permission from The Australian Corneal Graft Registry Report 2004, eds KA Williams, NB Hornsby, CM Bartlett, HK Holland, A Esterman, DJ Coster. Adelaide: Snap Printing 2004, pp1-192.

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

1   survival. Kaplan-Meier plots show the survival of penetrating corneal grafts within the

2   Australian Corneal Graft Registry database. Modified with permission from The

3   Australian Corneal Graft Registry Report 2004, eds KA Williams, NB Hornsby, CM

4   Bartlett, HK Holland, A Esterman, DJ Coster. Adelaide: Snap Printing 2004, pp1-192.

5   **FIGURE 6**. The influence of repeated keratoplasty in the ipsilateral eye on corneal

6   allograft survival. Kaplan-Meier plots show the survival of penetrating corneal grafts

7   within the Australian Corneal Graft Registry database. Modified with permission from

8   The Australian Corneal Graft Registry Report 2004, eds KA Williams, NB Hornsby, CM

9   Bartlett, HK Holland, A Esterman, DJ Coster. Adelaide: Snap Printing 2004, pp1-192.

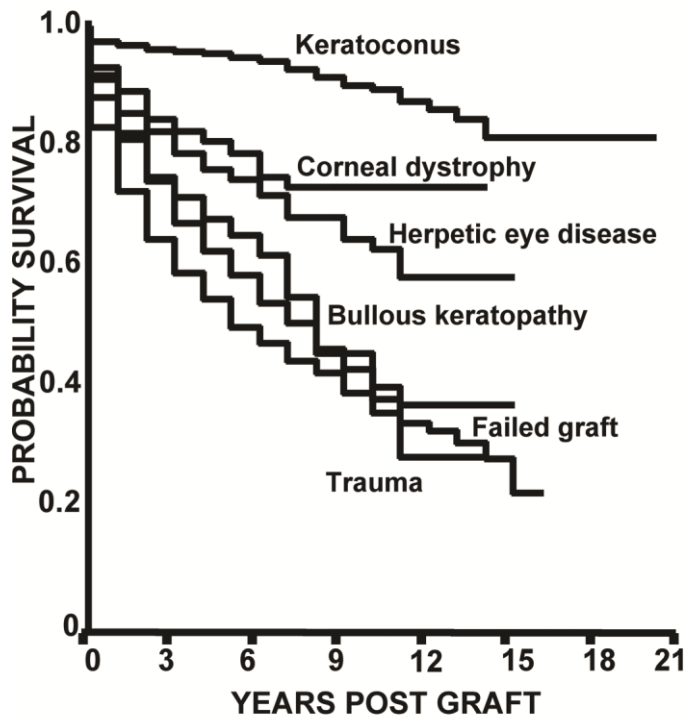10   **FIGURE 7**. The mechanisms involved in corneal graft rejection.
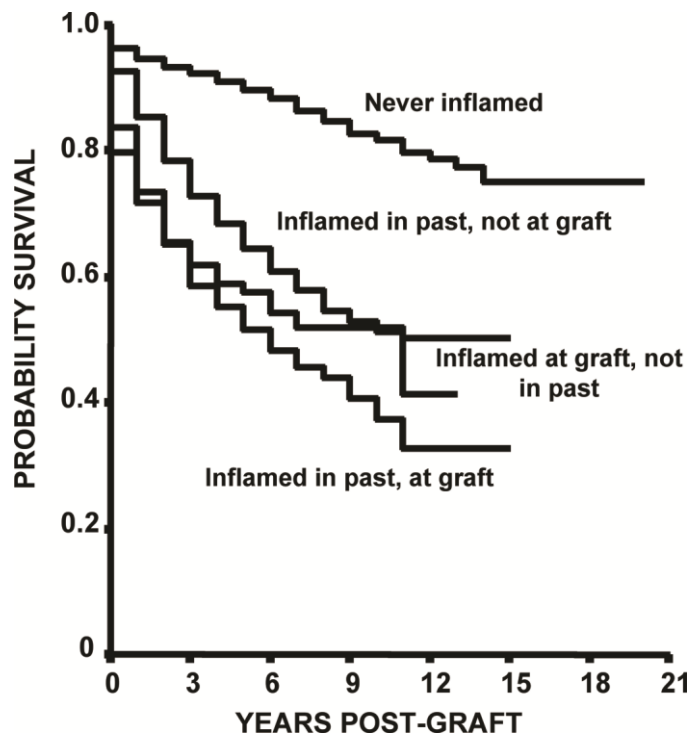
11

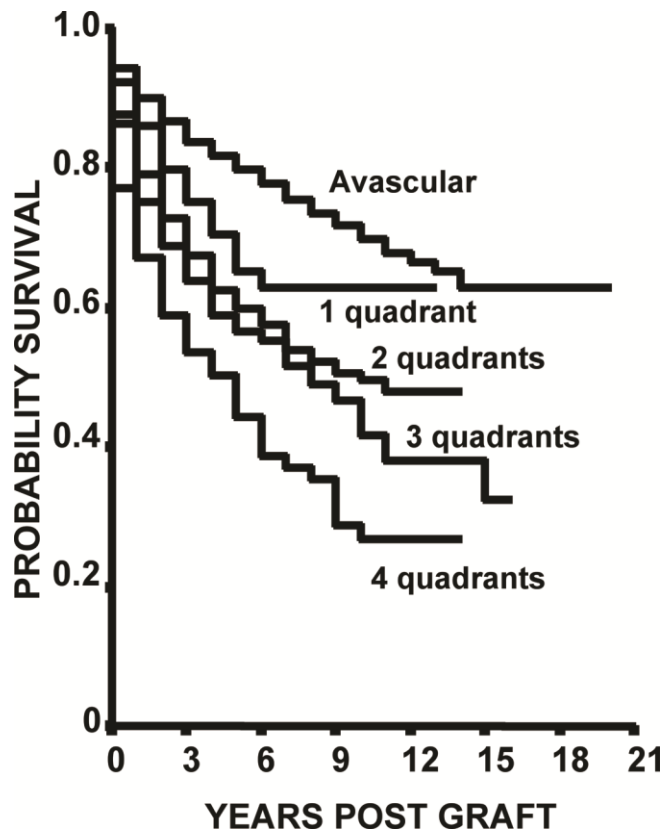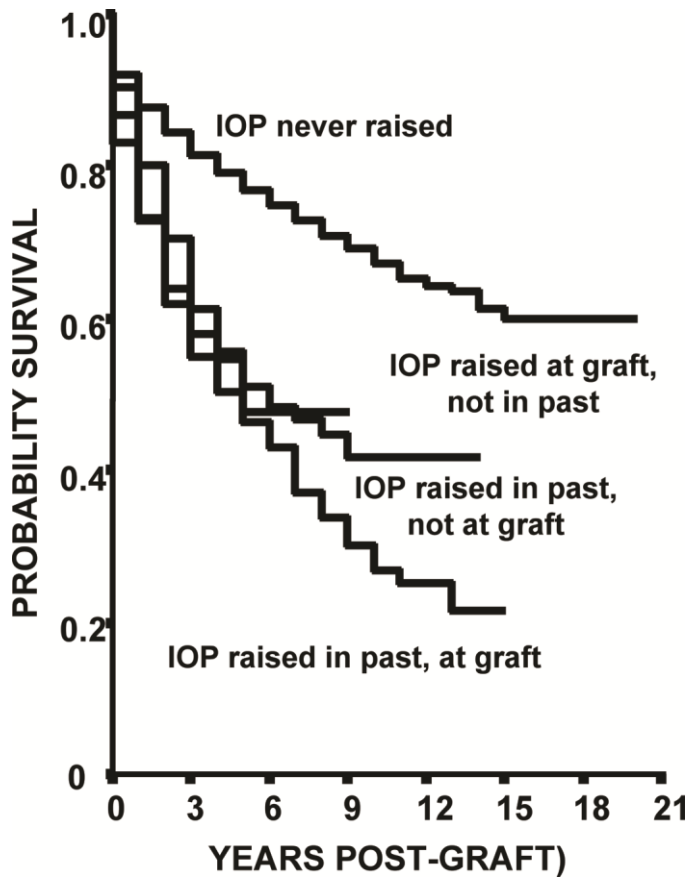1   Figure 1.

2

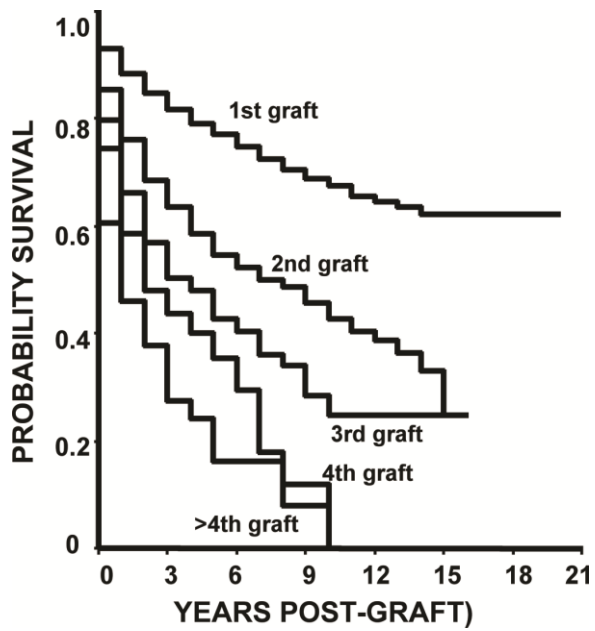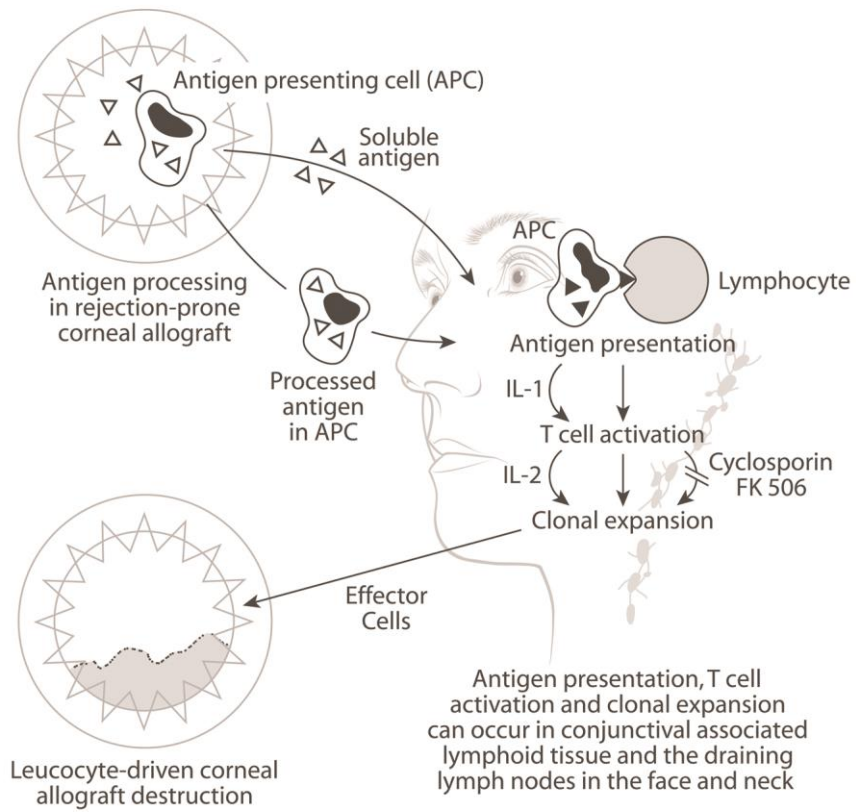3   Figure 2.

4

1   Figure 3.

2

3   Figure 4.

4

1   Figure 5.

2

3   Figure 6.

4

1   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

Cyclosporin FK 506

Clonal expansion

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

2