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Dermot Pierse Lecture

Mechanisms of Corneal Graft Failure : The Erosion of Corneal Privilege

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Summary

Although corneal transplantation is very successful for keratoconus and some other dystrophies, it is not nearly as successful for acquired corneal opacities. The usual cause of failure is allograft rejection. In high-risk cases a number of strategies are required to decrease the risk of rejection. These include suppression of inflammation, MHC matching, and local and systemic immunosuppression. For the results of corneal transplantation to improve, surgeons must be prepared to choose from a range of approaches, those best suited to the individual patient.

When giving an eponymous lecture, it is customary to begin with a eulogy of the person for whom the lecture is named. This usually involves the lecturer in an exhaustive search for some aspect of the dignitary's life or career not described by previous lecturers who have taken on the same search.

On this occasion, such an approach is both unnecessary and inappropriate. The influence of Dermot Pierse is both contemporary and universal, making it quite unnecessary for us to build up a legend. And it is inappropriate because it is clear to those of us who know him only a little that his humility borders on shyness and that he would not feel comfortable listening to any eulogy which could be honestly and sincerely delivered.

It is appropriate for me to point out, however, that I live and work in a city which is as far from Croydon as any place on the face of the earth. Yet the influence of Dermot Pierse pervades my operating room as if it were his own; his direct influence is there in the instruments, the microscope and the surgical techniques which bear his mark, whether literally or figuratively, and indirectly he has influenced his disciples, who in turn have taught others, myself included.

In choosing to talk about corneal transplantation, I have selected an aspect of ophthalmic surgery which has benefitted enormously from the input of Dermot Pierse and others like him. So impressive has been the improvement in microsurgical techniques that we have reached a point where biological phenomena severely limit the potential of the procedure.

Corneal transplantation has its paradoxes. It is both the most successful and the least successful procedure in clinical transplantation surgery. It is also the most widely practised yet the least understood form of clinical transplantation.

For patients with dystrophic conditions such as keratoconus, the results of corneal transplantation are excellent,[1] at least in

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terms of the graft itself, but for those blinded due to the consequences of keratitis, the outlook is poor.[2] The results are comparable to those for bone marrow transplantation[3] and are considerably worse than those for renal and heart transplantation.[4] The status of the cornea as a privileged site for transplantation is widely acknowledged,[5] but this status is only enjoyed if the ultrastructure of the cornea is undisturbed. Even small changes in the cornea as a result of corneal disease alter this status dramatically. An understanding of what erodes corneal privilege is important if corneal transplantation is to approach its potential as a surgical treatment for patients blind from acquired corneal disease.

A number of steps must be taken to assemble knowledge of the biological phenomena which threaten successful corneal transplantation. These include a search for clinical factors influencing graft survival and the use of animal models and *in vitro* studies.

The clinical factors associated with corneal graft survival can be identified by keeping careful records and applying appropriate actuarial statistics,[6] but more important still is a knowledge of the cellular and molecular mechanisms contributing to graft failure. Knowledge of these factors may point to ways of improving graft survival in high–risk patients.

Current Status of Corneal Transplantation

Corneal transplants are performed for two reasons. First, to improve vision as a means of enhancing a patient's quality of life and, secondly, to preserve the integrity of a globe threatened by corneal disruption. Most corneal grafts are carried out for visual reasons and the results should be assessed in terms of visual outcome. A necessary requirement for good vision is a successful graft, and failure of a graft to function is a prominent cause, although not the only one, of failure to achieve full visual potential. Grafts done for disruptive corneal disease, on the other hand, fall into the high–risk category and success should be measured in terms of preservation of the globe rather than of visual outcome. In such cases, visual outcome may be good but any vision achieved should be considered a bonus.

A Prospective Study of Corneal Transplantation

Useful information about the factors relating to successful transplantation and the patterns of graft failure can be derived from clinical studies. For the results to be meaningful, the relevant data should be collected in a prospective manner, follow–up protocol should be stringent, the end–points crisp, and appropriate statistical analysis should be employed.

A study of 220 grafts in 200 patients operated on at Flinders Medical Centre and followed for 8 years has provided useful information about the outcome of corneal transplantation and the patterns of failure. The grafts were studied for between 6 months and 7 years. Sixty grafts were done for keratoconus; the remainder for acquired corneal disease. The surgery was performed by one surgeon, indications for surgery were consistent, surgical techniques did not vary, and the data were collected on a prospective basis. Follow–up assessments were made every 6 months by the surgeon–in–charge or by the referring ophthalmologist. Graft status was evaluated in terms of best corrected vision, graft clarity and the integrity of the globe. Non–parametric statistical analyses were used with the construction of life tables and survival curves.[6] The data management system was designed specifically for surveillance of the results of corneal transplantation.[7]

The overall survival of grafts in this series was 65 per cent at 5 years. Most graft failures occurred within 3 years but some grafts continue to be lost years after successful grafting (Fig. 1).

The probability of a successful graft depends primarily on the state of the recipient bed. In this respect corneal transplantation differs from other branches of clinical transplantation.

Grafts for keratoconus have the best prognosis with a success rate of 95 per cent, half the patients achieving 6/12 vision with their preferred correction (Fig.2).

The prognosis for patients with acquired disease is much worse. Inflammation of the anterior segment of the eye (Fig. 3) and vascularisation have a profound effect on graft survival (Fig. 4). So, too, does elevated intraocular pressure (I0P) (Fig.5). Elevated I0P, or normal I0P requiring topical medication for maintenance at normal levels, is a contraindication to penetrating keratoplasty. Even if the I0P is normal at the time of surgery but has been noted to be elevated some time in the past, the prognosis for the graft is much worse than if the I0P has never been observed to be raised.

The number of previous grafts is also related to outcome. Initial grafts do much better than subsequent grafts. This effect is most striking (Fig.6).

The pattern of failure is also related to the pre–operative diagnosis. In this series, graft failure in patients grafted for keratoconus was due either to accidental trauma or (in one patient) to self–inflicted injury.

Graft rejection leading to graft failure in patients operated on for keratoconus was not observed in our series but was reported to us in one case followed in a neighbouring country.

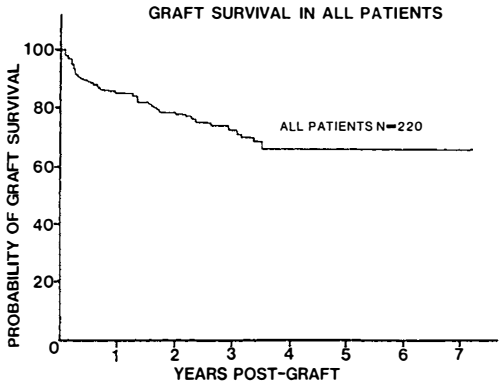

Fig. 1. *The probability of graft survival for a group of 220 patients having penetrating corneal grafts and observed for 6 months to 7 years. For a subgroup of keratoconus patients, the probability of graft survival is increased.*

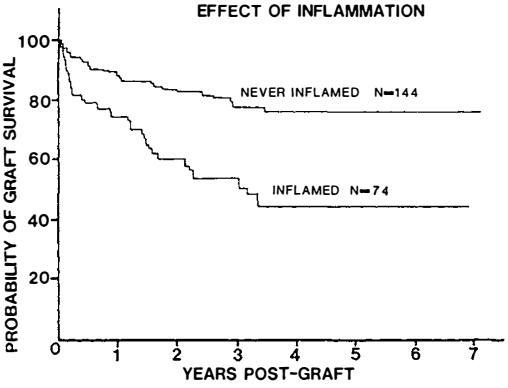

Fig. 3. *Survival curve of penetrating corneal grafts for patients with corneas inflamed or not inflamed at the time of surgery.*

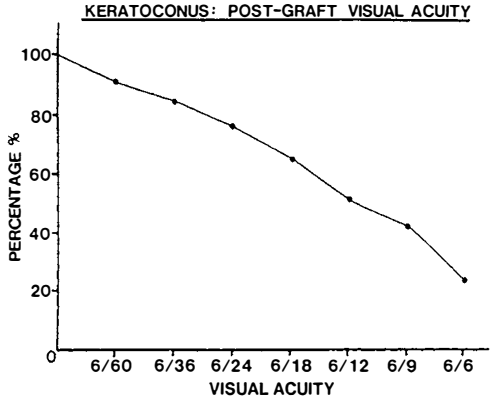

Fig. 2. *Cumulative frequency distribution of visual acuity in patients having corneal grafts for keratoconus and using their preferred correction.*

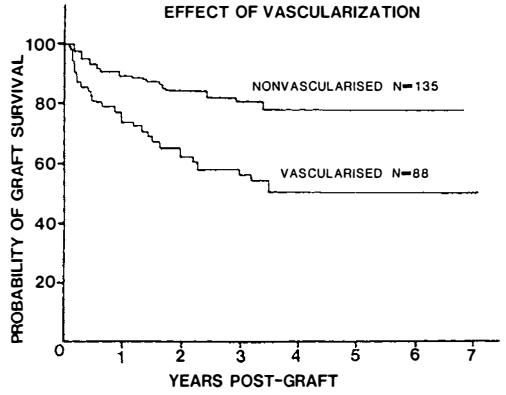

Fig. 4. *Survival curve of penetrating corneal grafts in patients with corneas vascularised or not vascularised at the time of surgery.*

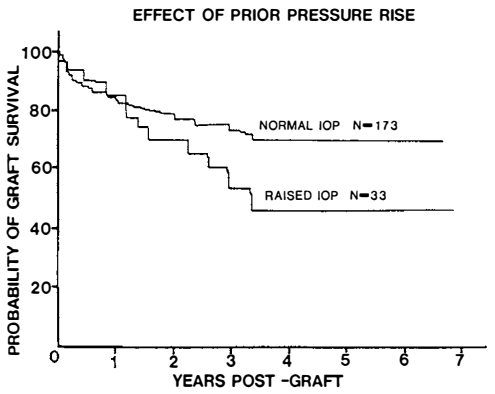

Fig. 5. *Survival curve of penetrating corneal grafts in eyes in which the intraocular pressure had never been observed to be high and in patients where elevations had been observed even though intraocular pressure was normal at the time of surgery.*

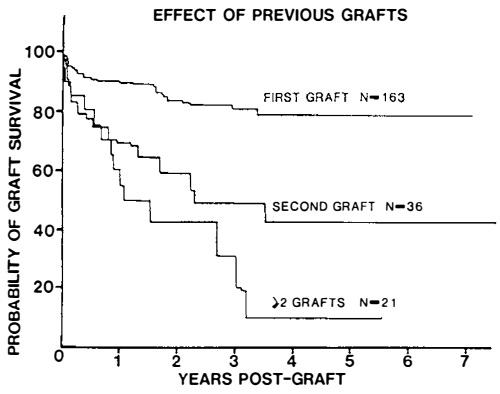

Fig. 6. *The effect of the number of previous corneal grafts on the survival of penetrating corneal grafts.*

Allograft rejection contributed to graft failure in virtually all patients in whom the graft bed was vascularised or inflamed. In patients who had an elevated I0P some time prior to surgery, raised pressure was considered to have contributed to more than half the graft failures although rejection was still the major cause of graft failure.

Allograft rejection is clearly the major obstacle to successful corneal transplantation in patients with acquired disease.

Mechanisms of Allograft Rejection : Structural Considerations

Since the potential of the cornea to reject an allograft is acquired with the occurrence of inflammation, scarring and vascularisation, it is necessary to study the way the normal distribution of antigens and immunoreactive cells is altered with acquired disease.

The normal cornea is a relatively simple structure in the anatomical and immunological sense. This simplicity is a two-edged sword. Observations of immunological events in the cornea are facilitated, but the options for interfering with the immune responses, particularly the allograft response, are limited. Identification of the various donor and recipient entities involved in the allograft reaction is essential. The distribution of major histocompatibility complex (MHC) antigens in the cornea has been extensively studied.[8,9,10,11,12] In some respects there is agreement among investigators but other aspects remain controversial. Corneal epithelium and stromal keratocytes are class I MHC antigen–positive. Langerhans cells, which occur in the central corneal epithelium but are more common peripherally, are class II MHC antigen–positive.[13,14,15] Interstitial dendritic cells, which are found in the central cornea in small numbers, are also more plentiful in the periphery.[12] They too are class II MHC antigen–positive. The status of the corneal endothelium is more controversial. Although a number of groups, including ours, have failed to identify class I or class II antigens on endothelial cells,[8,9,10,12] there are reports of both class I and class II expression on normal cornea and under the influence of inducing agents.[11,16,17]

Inflammation, and the sequelae of inflammation, drastically alter the immunological architecture of the cornea. In corneas which have been scarred and vascularised, there is an increase in the number of bone marrow–derived cells.[12,18] These cells are characterised by the presence of the leucocyte-common antigen and have been termed passenger cells to distinguish them from the normal residential population of the cornea. The majority of these are interstitial dendritic cells. They have a characteristic morphology and express class

II MHC antigens but do not express any T cell or macrophage markers.[19] This population of cells is important in allograft rejection for two reasons. Firstly, class II antigens are potent stimulators of the allograft response. This is clearly important when class II–positive cells are present in the graft because the normal central cornea contains such cells and this is likely to be relevant to sensitisation. Secondly, interstitial dendritic cells can present antigen to T cells, potentiating the initiation of the immune response.[20] This may be important when interstitial cells are present in the graft, or when they are present in the graft bed, as host interstitial dendritic cells can present foreign antigen.

Under some circumstances, the expression of MHC antigens may be altered. With established graft rejection,[21,22] graft versus host disease (23) and other inflammatory conditions,[24] and under the influence of interferons,[25] the expression of MHC antigens is up–regulated. Class I expression on epithelial cells and stromal keratocytes may increase. Endothelium has been reported to express class II MHC antigens under these circumstances.[16,17]

Another consequence of inflammation is vascularisation. Chronic inflammatory disease of the cornea, particularly if associated with oedema, is almost invariably complicated by new vessel development. Once established, vessels probably never regress. A consequence of blood vessel development is facilitated movement of the various elements of the immune response in and out of the cornea.

Mechanisms of Allograft Rejection: Functional Considerations

The corneal allograft reaction comprises a number of distinct processes and any attempt to dissect out the elements of the orchestrated immune response must necessarily be awkward. It is, however, convenient to do so for purposes of description and to identify the various aspects of the reaction which might be amenable to manipulation. At least four major elements of the process can be recognised. They are wound healing and inflammation, sensitisation, clonal expansion of alloreactive immunocytes, and graft destruction.

1. *Wound Healing and Inflammation*

Wound healing is a necessary preliminary to the corneal allograft response and is always associated with inflammation. The more active the process the more likely it is that allograft rejection will follow. In patients with keratoconus, wound healing is slow (wound dehiscence a year after surgery is not rare) but allograft rejection is uncommon. Grafts into vascularised corneas, on the other hand, heal quickly and rejection is common.

Inflammation and wound healing result in accumulation of bone marrow-derived cells in the graft which are important contributors to the genesis of allograft rejection. In addition, vessels grow across the graft-host junction and under some circumstances there is increased expression of MHC antigens in the graft. These events prepare the graft to be rejected.

2. *Sensitisation*

Sensitisation occurs when alloantigen is processed by immunocompetent lymphocytes. This process, which is greatly facilitated by the involvement of accessory cells, results in proliferation of lymphocytes and production of cytokines. The process is summarised in Figure 7. Accessory cells, including interstitial dendritic cells and macrophages, produce interleukin-I in response to exposure to alloantigen. Interleukin-I is necessary for T cell activation, one consequence of which is the production of interleukin-2. Interleukin-2 promotes clonal expansion by a positive feedback mechanism (Figs. 7 and 8). Interstitial dendritic cells in the graft may be of donor or host origin. In other transplantation systems, it has been demonstrated that host dendritic cells can process foreign MHC antigen.[26,27] Corneas vascularised as a result of previous inflammatory disease contain large numbers of instertitial dendritic cells. As wound healing occurs, especially if there is excessive inflammation, the graft is populated with bone marrow–derived cells including interstitial dendritic cells. Since accessory cell

function is important in the initiation of the allograft reaction, disabling these cells, or preventing their accumulation in the graft, may suppress allograft rejection.

3. *Clonal Expansion of Alloreactive Immunocytes*

When alloantigens are presented to an immunocyte, clonal expansion occurs with blastogenesis and release of cytokines. Cytokines have a number of important functions including the encouragement of lymphocyte proliferation, increased MHC antigen expression, and promotion of chemotaxis. These events result in increased foreigness by up-regulation of the MHC antigen expression, increased inflammation with attraction of cells capable of antigen presentation and release of destructive enzymes, and an increase in the number of immunocytes programmed for interaction with the foreign antigen responsible for initiating the process. Interference with this process is the basis of pharmacological immuno–suppressive agents currently employed.

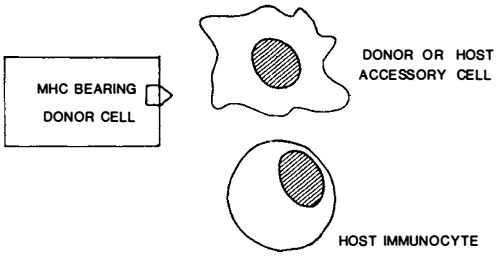

Fig. 7. *Essential requirements for sensitisation: foreign MHC antigen, a host immunocyte, and an accessory cell. The latter may be of graft origin and near the foreign antigen or may be of host origin and present soluble antigen.*

4. *Graft Destruction*

Grafts subjected to an allograft response are destroyed by alloantigen specific and non–specific mechanisms.[28] Cells bearing the foreign antigens to which the immune system is sensitised are subject to assault by a variety of lymphocytes, leukocytes, soluble mediators and perhaps by antibody–related mechanisms.

Both CD4 and CD8 subpopulations of T lymphocytes are involved. Both can be seen in the anterior chamber fluid of eyes during an allograft response. Non–B non–T natural killer cells may also be involved.[29] Evidence of their involvement in rejection in other organ systems is strong but they are seldom seen in the anterior chamber fluid of patients rejecting grafts or in rejecting corneas in animal models.

In addition to lymphoblasts,[30] macrophages[31] and polymorphonuclear granulocytes are also plentiful in and around rejecting grafts. They probably contribute to the process in a non–specific way which is at present unclear. What is clear is that no

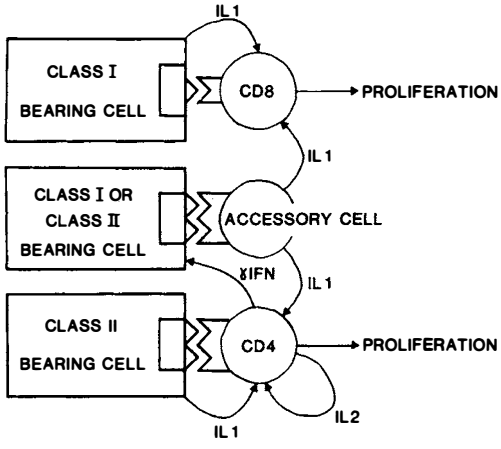

Fig. 8. *A précis of key mechanisms contributing to sensitisation and clonal expansion.*

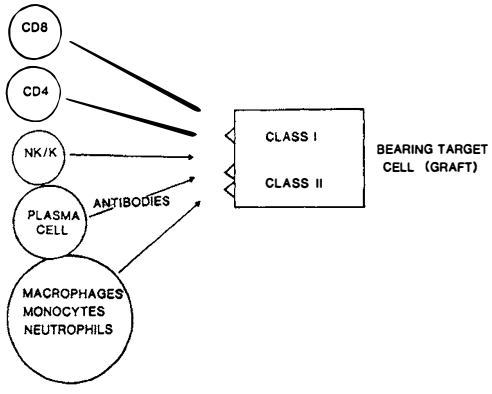

Fig. 9. *A number of factors involving cellular and humoral mechanisms contribute to graft destruction.*

single mechanism of graft destruction can be identified in the cornea or in any other form of allograft rejection (Fig.9).

Options for Suppressing the Corneal Allograft Response

A number of options are available for suppressing the corneal allograft response. Some are more appropriate for clinical practice than are others. The possibilities include reduction of antigenic difference, reduction of antigenic load of donor, decreasing host reactivity, suppression of clonal expansion and interference with allograft destruction.

1. *Reduction of Antigenic Difference*

Reduction of the antigenic difference between donor and host can be achieved by MHC matching. This approach has been well established in some branches of clinical transplantation but remains of unproven value or controversial in others. The value of class I (HLA ABC) matching in high–risk cases is well established for corneal transplantation[32,33,34,35,36] as it is for renal transplantation. The place of class II matching in corneal transplantation is not yet clear.[36,37]

Class I matching for corneal transplantation does present some logistical difficulties. Tissue typing is best done on fresh lymphoid tissue from either spleen or lymph node. These tissues are accessible in the case of renal or multiple organ donors but are seldom available within the usual pool of corneal donors. Typing of post–mortem blood can be done but is not reliable unless the blood is collected very soon after death. This currently causes considerable difficulty because donors are not recognised as such until after death. There is also a problem of cost in some communities but, more importantly, the HLA system is extremely polymorphic and a large pool of donors and recipients is necessary if good matches are to be found.

2. *Reduction of Antigenic Load of Donor*

One way of reducing the antigenic load of a corneal graft is to remove epithelium from the button. This removes a substantial load of class I–bearing epithelial cells and many class II–bearing Langherhan cells from the central cornea. However, there is no good evidence that this prolongs graft survival.[38] Perhaps this is related in some way to the replacement of the donor epithelium with host epithelium which occurs within the first few months after surgery. Quite clearly this manoeuvre does not reduce the load of antigens on the stromal keratocytes or the endothelium, nor does it remove stromal passenger cells.

Another option is to remove passenger cells from the graft. These class II MHC antigen–bearing cells are particularly important when present in a graft. They are potent stimulators of the allograft response since they not only exhibit class II antigens but are capable of producing interleukin–I to potentiate sensitisation.[20]

These cells are selectively removed from tissues maintained in organ culture under oxygen.[39] This is the mechanism by which murine pancreatic islet cell allografts are successfully transplanted in murine models.[40] In this situation, the class II–bearing cells are eliminated from the graft prior to transplantation. Unfortunately this approach has not been successful in prolonging corneal allograft survival in animal models. Perhaps this is due to the paucity of class II–bearing cells in the central cornea used for the graft, but a major problem is the toxic effect of oxygen on the corneal endothelium. The beneficial effect is therefore small and the risk of primary graft failure high.

Ultra–violet light also selectively disarms accessory cells. In experimental animals, UV irradiation of the donor results in a modest prolongation of graft survival.[41,42] However, it is the host cornea which harbours the important cells and to which attention must be directed.

3. *Decreasing Host Reactivity*

In order to decrease the ability of host cells to initiate the allograft response, it is necessary to disable those present in the host bed at the time of surgery and then to prevent late migration of bone marrow–derived cells into the graft as wound healing occurs and during any subsequent episodes of inflammation.

Disarming of accessory cells in the graft bed can be achieved with irradiation of the recipient eye with UVB prior to surgery.[41] Rabbit experiments demonstrate modestly prolonged graft survival after irradiation with a broad band source (313nm, 15J/m^2/sec) for five minutes. The effect was greater than was seen after irradiation of the donor and the effects were cumulative.

Prevention of repopulation of the graft by bone marrow–derived cells of host origin is just as important but more difficult to achieve. Wound healing and inflammation can be suppressed with topical steroids. Administration of topical steroids to animals at risk of graft vascularisation results in an increase in the time taken for vessels to cross the graft margin. This is accompanied by a corresponding increase in graft survival. Similarly, acute intermittent inflammatory episodes can be prevented or treated by appropriate means to lessen the incidence of rejection after episodes of infection of the outer eye or after suture removal.

4. *Suppression of Clonal Expansion*

The essential features of clonal expansion are cell division and production of cytokines. Current pharmacological immunosuppression interferes with both aspects of the response. Three agents are frequently used in this context: corticosteroids, azothioprine and cyclosporin.

Corticosteroids: These inhibit T cell proliferation by preventing accessory cells from releasing interleukin–1 and by blocking interleukin–1–dependent release of interleukin–2 from activated T cells.[43] In addition, corticosteroids act in other ways which are important for suppression of corneal graft rejection. The inhibition of chemotaxis and neovascularisation is important, so too is the stabilisation of neutrophils and other cells capable of inflicting tissue damage in a non–specific way.

Topical corticosteroids have been universally employed in the post–operative management of corneal grafts. Although a number of preparations are available, prednisolone acetate 1% eye drops used four times a day initially, tapering to once daily after 3 months and then continuing for 12 months, is a common dosage schedule. Should rejection occur, the frequency of administration is increased to hourly. Although this approach is widely used it has not been tested against other preparations or dose schedules. There is a remarkable similarity in results between various groups collecting data on a prospective basis, with a high rejection rate in high–risk cases despite the use of topical corticosteroid preparations. In high–risk cases, more effective immunosuppression is required.

Corticosteroids can also be used systemically, either alone or in combination with cyclosporin and/or azathioprine. Multiple drug therapy is more effective and produces fewer side effects than are attributable to the individual agents when used alone in higher doses. It is a reduction in corticosteroids which is especially desirable as long–term complications can be particularly serious.

An effective maintenance dose of corticosteroids is 30 mg/day post-transplant tapering to 20 mg/day within 3 months.

Azathioprine: This is widely used in patients with vital organ allografts. Its place in corneal transplantation is much more limited, but the drug is used when systemic immunosuppression is indicated. Azathioprine impedes cellular proliferation as a result of drug metabolites being incorporated into cellular DNA and altering the synthesis and function of RNA in rapidly dividing cells.

The drug is given continuously at a dose of 2mg/kg/day orally. Doses are monitored by regular surveillance of the blood film. Myelosuppression can occur and cessation of treatment may be necessary.

Cyclosporin: The fungal metobolite cyclosporin interferes with clonal expansion by acting against the helper cell population of T lymphocytes (CD4).[44] The release of interleukin–2 and interferon is inhibited. Suppressor T cells (CD8) are not affected and this may account for the state of specific allogeneic tolerance attributed to the drug.[45]

Although great hopes have been held for the effectiveness of topical preparations of cyclosporin, these have not been realised.

Work in animal models confirms that systemic administration of cyclosporin prolongs graft survival.[46,47,48] Topical preparations prolong graft survival modestly when compared to placebo, but are not nearly as effective in this regard as conventional topical corticosteroids.[49,50,51,52] Furthermore, the effects are not synergistic.[53] Systemic therapy is necessary if the benefits of cyclosporin are to be realised for patients with corneal grafts.

An acceptable dose is 8mg/kg/day in two divided doses at the time of surgery, reducing to 2–4 mg/kg/day within 3 months.

Major side-effects of cyclosporin are nephrotoxicity and hypertension.[54] Blood levels can be determined by high pressure liquid chromatography but levels do not correlate well with nephrotoxicity and the role of blood level assays during chronic maintenance therapy is yet to be defined.

The effectiveness of immunosuppression for clinical transplantation has increased recently with the introduction of cyclosporin and the use of multiple drug therapy. This has resulted in lower doses of the individual drugs accompanied by a reduction in side-effects, particularly the serious side–effects of corticosteroids. Even so, the hazards of systemic immunosuppression preclude this approach in corneal transplantation unless the patient has enough to gain in improved lifestyle from the improved vision to warrant the risks entailed. The risks include potentially fatal complications.

5. *Interference with Allograft Destruction*

Since it is likely that a number of diverse processes bring about graft destruction, no single approach to aborting the process is likely to be effective. Use of intraocular antibodies directed against the relevant cellular elements is under investigation in animal models and in clinical studies.[55] Although this approach shows some promise, major difficulties need to be overcome, including the development of hypersensitivity to the foreign protein. In any event, this approach is more appropriate when attempting to reverse an established rejection episode than in augmenting maintenance immunosuppression.

Current Practice and Anticipated Developments

What is considered optimal practice at present? Careful selection of cases is the key to good results. Corneal transplantation has been practised for many years and the experience gained and the results of clinical studies define quite distinct indications, contraindications, and relative contra-indications.

Corneal transplantation is appropriate when there is grossly irregular astigmatism, as in keratoconus, when the cornea is opaque from oedema, scarring or deposition of macromolecules, or when corneal integrity is threatened or breached by destructive disease.

Raised I0P, mucosal scarring disease, or gross lid abnormalities are so prejudicial to graft survival as to be firm contra–indications to surgery. Of these, only raised I0P is amenable to therapy. It is preferable, however, to have the I0P normal (less than 20 mm Hg) for 6 months prior to transplantation without the need for topical medication. This often means surgical intervention is required for pressure control.

Vascularisation of the recipient cornea, inflammation of the recipient corneal in the past, or a previous corneal graft which has failed, predispose to allograft rejection and graft failure. The risk of failure is high, approximately 50 per cent over 5 years, and the consequences of failure serious in terms of the likelihood of subsequent successful grafts. Reduction of the risk of rejection is achieved by MHC matching and systemic immunosuppression. Since immunosuppression is hazardous, a better option is to avoid grafting at all if possible. Certainly it is better not to graft if vision in the other eye is normal. If one is forced to operate on such cases for visual reasons in order to improve lifestyle, steps are taken to reduce the rate of rejection. These include, at least, matching for class I MHC antigen loci. Immunosuppression requires careful consideration in each individual case. The anticipated benefits of the procedure need to be balanced against the risks of systemic immunosuppression. When this approach can be justified, and this is fortunately uncommon in urban

practice, we prefer maintenance therapy to include azathioprine (2 mg/kg/day) and cyclosporin (2–4 mg/kg/day). Systemic corticosteroids are withheld unless rejection occurs, at which time prednisolone is used at a dose of 0.5 mg/kg/day. These cases are also given topical steroids pre–operatively for 5 days (prednisolone acetate 1% four times/day) and this dosage is maintained for 6 months post–operatively, at which time the situation is reviewed. Should any inflammatory event occur in the first post–operative year, including removal of sutures, the dose of topical steroids is increased temporarily.

The way to improved corneal graft survival is arduous. There will be no quantum leap to universal graft survival. Instead, progress will be made by the summation of lesser gains. The willingness of surgeons to pursue small benefits wherever available will continue to distinguish good transplantation units from average ones.

I wish to acknowledge the contribution of Dr. K.A. Williams, who has been responsible for the corneal transplantation research programme in the Department of Ophthalmology, School of Medicine, Flinders University of South Australia.

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