PERSPECTIVE

Corneal allograft rejection

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The cornea is avascular, enjoys relative immune privilege, and immunosuppressive treatment can be directly applied: yet data from all available reports indicate that allogeneic rejection is the commonest cause of corneal graft failure. In Australia, 5 year actuarial corneal graft survival is 72%: irreversible rejection accounts for at least 33% of all graft failures.¹ Graft survival is much shorter in certain high risk groups, with rejection being the primary cause of failure in a higher proportion.²⁻⁴ Moreover, graft survival figures give no indication of the number of patients denied the opportunity of a graft in the first place because of perceived high risk of rejection, and in corneal surgery at the present time the main strategy to reduce the impact of graft rejection is to avoid grafts in high risk cases.

The fact that rejection can be diagnosed, and its clinical course studied, by direct observation of corneal grafts obviates the need for histopathological study of biopsy specimens of graft. Biopsy of rejecting cornea could not be justified ethically. This constraint does not apply to human solid organ transplants: investigation of immunohistochemical, ultrastructural, and molecular aspects of rejection of kidney and other organs is well established and can guide clinical management. The virtual inaccessibility of rejecting human cornea from the biopsy standpoint, and the fact that grafts are seldom removed at the time of rejection, has two consequences for the investigation of mechanisms of graft rejection. Firstly, the corneal grafts on which pathological studies have been reported represent late or burnt out rejection. Secondly, most information on the sequence of events in rejecting corneas has been obtained from experimental animal models. Thus, while corneal graft rejection presents clinical appearances familiar to ophthalmologists, our knowledge of pathology and mechanisms is comparatively fragmentary.

Incidence, clinical, and pathological features of corneal graft rejection

Corneal graft rejection usually arises as an apparently isolated event, not associated with any other clinical abnormality. It is frequently the case, however, that rejection follows intercurrent inflammation such as that induced by suture loosening, suture track infection, or recurrent herpetic infection. Reported incidence of rejection in any series will depend on the indications for graft and other factors, but in the largest reported cohort of graft recipients, 18% have undergone at least one rejection episode.¹ Graft rejection of all types has been shown to have higher incidence in patients younger than 50 years than in those over 50.⁵ Endothelial rejection was shown, in the same series of graft recipients, to occur at an average interval of 8 months after graft, with a range of 2 weeks to 29 months. While rejection is unusual later than this time, unequivocal endothelial graft rejection has been observed by the author to occur as late as 9 years after graft. It is a manifestation of the relative immune privilege of the cornea that, in contrast, most solid organ transplants that

Figure 1 Endothelial rejection. A horizontal endothelial rejection line, scattered keratic precipitates and folds in Descemet's membrane are shown.

succumb to rejection are lost within the first 3 months after graft.

The signs of corneal graft rejection most often seen in clinical practice indicate endothelial rejection. Earliest signs are limbal injection, aqueous cells, and small graft keratic precipitates. An endothelial rejection line is seen in longer established rejection, advancing across the graft and associated with corneal oedema (Fig 1). Epithelial rejection lines and the nummular subepithelial infiltrates indicative of stromal graft rejection (Fig 2) are seen less frequently. These may be associated with only minor symptoms and are not usually accompanied by aqueous inflammation, unless followed after a short interval by endothelial rejection. In some graft recipients, the rejection of individual cell layers of the cornea thus described may overlap.³ In irreversible rejection the entire graft may become oedematous and opaque. On rare

Figure 2 Stromal rejection. The scattered anterior stromal infiltrates shown are restricted to donor cornea. Visible aqueous inflammation is minimal or absent in such cases.

Figure 3A Surviving rat corneal allograft. A clear Wistar-Furth strain donor cornea photographed 52 days after graft in a Fischer 344 strain recipient. Some recipient corneal vascularisation to the graft interface is seen.

occasions, the rejection may be so potent that sloughing of the corneal stroma may occur.

Graft oedema indicates endothelial pump dysfunction, with an increase in thickness during a rejection episode occurring consequent to immunologically induced endothelial cell injury. Graft pachymetry is helpful in following the response to therapy of rejection, once the graft begins to deturgesce after the initial acute decompensation. Measurement is particularly helpful from about 4 days to 4 weeks after initiation of treatment. One study of graft pachymetry on 234 patients with rejection found 77% to have increased thickness and 47% to have a thickness increase of more than 10%. The increase in thickness detected at the time of graft rejection was found to be greater in those grafts that went on to fail, compared with those in whom rejection was reversed.⁶

Studies of rejected human corneal grafts have found thinned or denuded endothelium, a retrocorneal inflammatory membrane in some cases, vascularisation, superficial stromal scarring, loss of Bowman's zone, and a cellular infiltrate of mixed composition.^{7,8} The predominant cells are macrophages and T lymphocytes, the majority of which carry helper T cell surface determinants.⁸ HLA class I and II antigens are expressed on corneal endothelial cells, stromal keratocytes, and basal epithelial cells in rejected grafts.^{9,10} Intercellular adhesion molecule 1 (ICAM-1) is expressed on epithelial cells, keratocytes, and endothelial cells, particularly at foci of mononuclear cell aggregation.^{10,11} Vascular cell adhesion molecule 1 (VCAM-1) and the neutrophil ligand E-selectin have been found in some vascularised rejected grafts to be expressed on vascular endothelial cells.^{10,11} Only corneal graft specimens obtained some months after rejection have been reported and pathological studies must be interpreted with this in mind. Furthermore, the extent to which some findings reported represent alloantigen recognition rather than rejection per se is uncertain because of the small numbers reported.

Experimental corneal allograft rejection

Review of animal corneal transplantation will here be

Figure 3B Rat corneal allograft rejection. Graft oedema and vascularisation shown 47 days after graft, 3 days after onset of rejection.

restricted to experimental models of orthotopic full thickness allografts (homologous to clinical grafts) on which studies of rejection have been reported. The rabbit is the most widely used animal model because of the similar corneal size and appearance of graft rejection to that of humans, and the ease of post-graft examination with a slit-lamp microscope. In rabbits, unlike other animals, it is however necessary to overcome the corneal immune privilege of the normal eye in order to induce rejection. This is effected by donor strain skin grafting following corneal graft, transplantation into pre-vascularised cornea, or into an eccentric bed near the limbus. Rabbits were used in the early studies of rejection and Maumenee's report in 1951 presented the first evidence that late failure of corneal grafts could be caused by sensitisation of the host to donor antigens.¹² Several descriptions of rabbit corneal graft rejection have subsequently been reported.¹³⁻¹⁵ Other animal models which have been described are the cat,¹⁶ inbred rat,¹⁷⁻¹⁹ inbred mouse²⁰⁻²² and sheep (Williams KA *et al*, submitted for publication).

Corneal graft rejection in animals resembles to varying degrees the appearance observed in humans. In rabbit and sheep eyes, aqueous inflammation usually indicates incipient graft rejection, which causes spreading stromal oedema and opacification. Epithelial and endothelial rejection lines are observed in a proportion of rejecting rabbit^{14,23,24} and sheep grafts. Endothelial rejection lines have been shown by Khodadoust and Silverstein to represent a line of lymphocytes destroying endothelial cells.¹⁴ Graft rejection in rats is characterised by graft oedema and vascularisation (Figs 3A and 3B)¹⁷: rejection lines are rarely, if ever, visible.

In untreated rejecting rabbit allografts, the cellular infiltrate within the graft is heterogeneous, containing macrophages, lymphocytes, plasma cells, and neutrophils.^{14,23} Immunohistochemical study by Williams and colleagues has shown that half of the leucocytes are T lymphocytes, two thirds bore MHC class II markers, and one fifth carried myeloid cell markers.²⁴ In the same experiment, cells migrating in the aqueous were similar to those found in the graft itself, with a trend to increasing proportions of T and myeloid cells as rejection progressed.

The availability of inbred rat strains, and the wider

available range of monoclonal antibodies to rat cell surface antigens and cytokines has allowed more detailed analysis of corneal graft rejection in this animal. Moreover, the rat cornea closely resembles the human cornea in terms of major histocompatibility complex (MHC) expression.²⁵ Tissue sections show increased expression of MHC class I antigen and donor and recipient MHC class II antigen in the graft.^{26,27} Neutrophils, macrophages, CD4+, and smaller numbers of CD8+ cells infiltrate the graft,²⁸ most lymphocytes expressing the interleukin 2 (IL-2) receptor.²⁷ Leucocytes in the graft stroma express tumour necrosis factor, interferon gamma, IL-1 and IL-2, but not IL-4; de novo expression occurs of the adhesion molecules P-selectin on limbal vessels and ICAM-1 on the corneal endothelium; later in rejection there is an intense anterior chamber reaction with attachment to endothelium of T lymphocytes, monocytes, and granulocytes (Larkin *et al*, submitted for publication). Corneal graft rejection in the rat can be summarised as showing characteristics of a classic delayed type hypersensitivity response, accompanied by local production of proinflammatory cytokines.

As in the case of rat, the mouse has particular advantages over larger animals because of a large amount of information on its immune system, the wide range of immunological reagents for investigative use, and the availability of inbred strains. The small size of the mouse eye presents a formidable challenge in corneal surgical technique, with a reported failure rate of 30–40% due to surgical complications. Murine grafts undergoing rejection showed stromal infiltration with mononuclear cells and granulocytes, vascularisation and disruption of the stromal architecture, and loss of the endothelial layer.^{21,22}

Treatment of corneal allograft rejection

Corneal graft clarity depends primarily on survival and function of the non-replicative endothelial monolayer. If rejection begins, it must be reversed before irreparable damage is done to the endothelium. Topical corticosteroids remain the mainstay of immunosuppression for prevention of, and treatment of, established rejection, but this therapy fails to reverse all rejection episodes. Boisjoly and colleagues have reported that 18 grafts in a series of 28 with rejection failed following rejection therapy.²⁹ There is little information on the optimum steroid dosage and route of administration. At Moorfields Eye Hospital, patients with graft rejection are usually treated with hourly topical dexamethasone 0.1%, with, in some cases, subconjunctival betamethasone 4 mg daily until the episode has been reversed. On an empirical basis, some treat the more severe cases of endothelial rejection with systemic, in addition to local, steroid. Use of the following have been reported: oral prednisolone 80 mg daily for 5 to 7 days, then tapering doses,⁵ pulse intravenous methylprednisolone 125 mg with oral prednisolone 1 mg/kg,³⁰ intravenous methylprednisolone 250 mg³¹ and 500 mg.³² However a recent survey of the graft rejection treatment preferences of 137 members of the Castroviejo Society showed a remarkable variation.³³

One study on corneal graft rejection, by Hill and colleagues, compared (a) a single intravenous dose of methylprednisolone 500 mg with (b) 60–80 mg oral prednisolone daily, both given in addition to hourly topical prednisolone acetate 1%. It was found that those graft recipients treated with intravenous steroid had superior graft survival and less chance of a further rejection, when compared with those treated with oral steroid, only if they presented within 8 days of onset. No difference in outcome was found in those who presented later than day 8, or in the overall study population.³⁴ While intravenous methylprednisolone is the standard first line treatment of solid organ graft rejection, it is difficult

to justify the use of potentially toxic systemic steroids in corneal rejection when a high proportion can be successfully treated with topical preparations.

There is an evident need for safe alternative immunosuppressive agents to treat graft recipients with steroid resistant rejection. Many of the published research studies on cyclosporin have investigated prevention rather than treatment of rejection, and investigated corneal penetration with different vehicles for topical administration in experimental models. Chen and colleagues examined rabbit corneal allograft survival following treatment with cyclosporin begun at the earliest sign of rejection and continued for 60 days: grafts treated with cyclosporin in collagen shields survived longer than those treated with drops in an olive oil vehicle.³⁵ Topical cyclosporin A 0.025% in an alpha cyclodextrin vehicle has been reported to suppress rabbit allograft rejection if commenced early in the episode.³⁶ Topical FK506 administered in the same vehicle has also been shown to reverse established rejection in the rabbit model (Mills RA, personal communication).

Antibody therapy, which is frequently used in the treatment of solid organ graft rejection, has not yet been adopted in clinical corneal transplantation. This is despite the appealing prospect of local administration. Direct application of antibody to the eye might be effective in reversing rejection if expansion and maturation of alloantigen specific T lymphocytes occurs *in situ* rather than in the central lymphatic system (lymph nodes, blood, spleen); it also might diminish the problem of host response to systemically administered non-human proteins. Earliest reports described attempts to prevent, rather than treat, rejection episodes. Rabbit corneal allograft survival was prolonged by systemically administered heterologous antilymphocyte serum,^{37,38} but not by an anti-rabbit pan-T cell monoclonal antibody F(ab')₂ fragment ricin A chain conjugate.³⁹ No effect on survival was observed with subconjunctival⁴⁰ or topical⁴¹ antilymphocyte serum or subconjunctival anti-T cell monoclonal antibody.³⁹

There have been interesting reports of local administration of monoclonal antibody to reverse corneal graft rejection. Ippoliti and Fronterre described a favourable effect of intracameral injection of mouse monoclonal antibodies to human T cell surface antigens as an adjunct to oral steroid in patients with graft rejection. The injections caused no adverse effects.^{42,43} Williams and colleagues examined the effect on rabbit corneal allograft rejection of intracameral injection of mouse monoclonal antibodies against rabbit T cell, myeloid, or MHC class II antigens.²⁴ Reversal of rejection, in the absence of any other immunosuppression, occurred in the majority of recipient animals which received the T cell and myeloid antibodies, but the anti-class II antibody had no effect.

It is unclear whether the mechanisms of action of locally administered antibody are as simple as might be supposed, and these need to be thoroughly unravelled in order to direct the path of future clinical studies. We need a detailed understanding of the mechanisms underlying graft tolerance in experimental models of corneal allotransplantation. In general terms, it will be difficult to apply the information on immunosuppression obtained from animal models into clinical management. This is because corticosteroids, the principal immunosuppressive agents, are effective in reversal of a high proportion of corneal allograft rejection episodes and cannot be excluded from a treatment protocol without compelling reasons.

There is wide agreement that corneal allograft rejection is T cell mediated, but information on the precise mechanisms responsible for initiation of rejection and graft destruction is still fragmentary. Published studies support the concept that in circumstances causing graft inflammation, such as suture loosening, production by activated T cells of lymphokines

such as interferon gamma may result in local induction of MHC class I and II antigens on graft endothelium, keratocytes, and basal epithelium. Consequently, donor cells become targets for immune destruction by lymphocytes and other less alloantigen specific inflammatory cells in the clinical syndrome recognised as graft rejection.

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