Corneal Transplantation—Immunologically Privileged Status

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Summary

Despite the relatively high success rate and the word ‘immunologically privileged site’, it has been known that a corneal graft can induce an allograft rejection reaction. This is especially true in the rat where orthotopic penetrating corneal grafts in certain strain combinations are rejected even when transplanted in avascular bed. Reliable microsurgical techniques, together with the availability of inbred or congenic strains and a rapidly developing knowledge of its major histocompatibility complex (MHC) and immune system in general, have made the rat a prime species in which to study the immunological events after corneal grafting.

This review describes recent progress in understanding the immunological mechanisms behind corneal graft rejection. The topics discussed include the rat MHC (RT1) antigens and their distribution in the cornea; different responder status in fully allogeneic strain combinations, including the importance of multiple non-MHC antigens; and the role of antigen-presenting cells (APCs) in corneal graft rejection.

It has been estimated that there are some 28 million blind people in the world and that the majority have lost their sight from corneal opacification.¹ Clinical corneal transplantation was first performed by Kissam in 1838² and has developed with the advent of microsurgery and is now widely accepted as a routine treatment. It has been used to restore the sight of individuals suffering from various conditions whose frequency varies from country to country.

Unlike vascularised organs, donor eyes do not have to be removed from the body immediately after death and may be removed after cessation of heartbeat. An arbitrary time of 8 hours *post mortem* has been suggested as a maximum for enucleating donor eyes because of the viability of corneal endothelium.³ As studies have shown that there is a decreasing endothelial cell count with age,⁴ a

statistical significance of grafts receiving elderly corneas (donors over 75 years) between different age groups is becoming apparent at three to four years after grafting⁵ but there have been many successful grafts from donors aged 80 years or more.⁶ Thus, the age restrictions limiting the donation of organs from grafting do not always apply to the cornea. This relative lack of restriction of donor supply has meant that corneal transplantation has become the most frequently performed transplant operation,⁷ with more than 40,000 cases being reported in the United States and Europe in 1985. Furthermore, the important factors affecting the outcome of corneal transplantation have become apparent; the degree of vascularisation of the recipient's cornea before surgery, the inflammatory or immune status of the recipients at the time of surgery, and possibly the number

of antigenic determinants shared by donor and recipient. In particular, the potential role of donor/recipient histoincompatibility in clinical corneal transplantation has been an area of considerable interest during the past 20 years.⁸ In Flinders University of South Australia, corneal graft survival is approximately 65% at five years and immune reactions are undoubtedly implicated both as the most common post-operative complications and as the major causes of graft failure.⁹ Variation in outcome of corneal grafting will certainly depend upon factors such as the number at risk described above and post-operative management (the centre effect).⁵ Thus when one considers the numbers of patients involved in clinical practice, it is surprising how little experimental research has been devoted to understanding the immune processes involved in corneal grafting compared to the research commitment given to vascularised organ transplants.

This review documents a series of experiments which have been performed on different animals designed to elucidate some of the immunological principles governing the survival or destruction of the allografted cornea.

Classical concept for an 'immunologically privileged' site

In 1948, Medawar considered four possible reasons for the relatively high success of clinical penetrating corneal transplantation:¹⁰

- (1) The dosage of foreign tissue involved in corneal allografting may be ineffectively small. There may indeed (though it has not been demonstrated) be a threshold of dosage below which an allograft cannot immunise its recipient strongly enough to secure its own breakdown.
- (2) Because of the feebleness of immunisation, breakdown (if it occurs) may be delayed in onset and prolonged in execution. Since corneal epithelium is highly mobile, cells of native origin from the periphery of the grafted area may insidiously and imperceptibly replace the foreign epithelium during its protracted breakdown.
- (3) Corneal allografts cannot elicit immunity, even if they could succumb to it.
- (4) Corneal allografts, being unvascularised,

cannot succumb to an immune reaction even if they can initiate one.

In order to investigate these possibilities, Medawar used heterotopic skin allografting as a means for studying the part played by blood and lymph vessels in transplantation immunity in particular regions of the body with major anatomical peculiarities. The region was the anterior chamber of the eye, in which, as *in vitro*, heterotopic skin graft might be kept alive without penetration by blood vessels. The experiment showed that a skin allograft (1 mm × 1 mm) transplanted into the anterior chamber of the eye of a specifically immunised rabbit was destroyed if it was penetrated by blood vessels. Medawar thought the explanation (4) was sufficient for the success of clinical penetrating corneal transplantation and believed this conclusion offered a rational explanation for it.

Medawar's concept of grafting skin to regions of the body with major anatomical peculiarities was developed by Billingham and Boswell.¹¹ They also used a heterotopic skin graft (2 mm × 3 mm) inserted into a small pocket of the avascular cornea of a rabbit which had previously been heavily immunised by its donor's skin. In this experiment, second-set skin grafts were simultaneously transplanted into the avascular intracorneas and to the chest walls of the sensitised rabbits. Although the corneal pocket was not sutured, skin allograft was not exposed to the recipient epithelium, the aqueous humour or cells in the anterior chamber. While the skin grafts to the chest walls showed complete breakdown by the seventh day at the latest,¹² the interlamellar skin grafts into the avascular cornea were still alive up to the 20th day at which time all animals were sacrificed. They found that all the corneal grafts (except those that had either been technically unsuccessful through exposure by perforation of the pocket and those that had become vascularised post-operatively) were still alive when removed from the cornea at up to 20 days. They thought that the interlamellar position of the cornea provided prolongation of corneal graft survival by not allowing the efferent limb of graft rejection to operate efficiently in the specifically immunised rabbits and post-operative vascularisation could break down

this immunologically privileged status of the cornea. They concluded that the absence of recipient vessels in the cornea constituted an absolute barrier to the rejection process. Khodadoust and Silverstein also reported that rabbit lamellar and most of the penetrating corneal allografts obeyed the concept of an 'immunologically privileged site' using non-sensitised and sensitised animals.¹³ They noted that allografts were spared from involvement in the rejection process so long as they remained avascular because of incomplete afferent and efferent limbs for corneal graft rejection. However, it should be emphasised that among these rabbit models there were several fundamental differences; graft tissue, size of the graft, site for grafting, unilateral or bilateral grafting, lamellar and penetrating corneal grafting, method of induction of post-operative vascularisation in the recipient bed, immune status of the recipient. These experiments left unexplained why some orthotopic corneal grafts on a pre-vascularised bed can survive so long, some orthotopic corneal grafts on an avascular bed can be rejected so quickly, a phenomenon often encountered in both clinical and experimental situations.

In clinical penetrating corneal grafts, the time of removal of sutures is dictated by the clinical appearance. In avascular corneas (keratoconus and dystrophies) wound healing is slow and regular, and where nylon sutures have been used at least 12 months may have to elapse to obtain adequate healing between the graft/host junction before they are removed.¹⁴ There are such suture-induced microvascularisation or postoperative chemosis in the recipient avascular bed. Although they may be initial or temporary phenomena, interaction between the donor transplantation antigens and the recipient immune system via blood vessels has occurred at least once in corneal allografts even in the avascular recipient bed.

Factors affecting the outcome of clinical corneal transplantation

It became apparent that while the corneal graft enjoys a certain degree of immunological privilege, it is by no means exempt from the allograft rejection process. This is

because, (a) the barrier of avascularity is not absolute, (b) perfectly avascular beds are rarely available clinically and (c) vascularisation of a hitherto clear graft bed may follow surgery or other insults and set the stage for graft rejection. Allograft rejection is the most common cause of corneal graft failure¹⁴ and there are some factors which are believed to affect the outcome of clinical corneal transplantation:^{7,15,16}

- (1) Degree of pre-vascularisation of the recipient bed.
- (2) Size of donor corneal button.
- (3) Position of the donor corneal button in the recipient bed.
- (4) Preservation methods for the donor corneal button.
- (5) Choice of immunosuppression.
- (6) Immune status of the recipient.
- (7) Histoincompatibilities between donor and recipient (HLA-A, -B, -DR antigens, ABO blood type antigens, Y chromosome antigens).

Factor (1) seems to be consistent with the concept of an 'immunologically privileged site' for transplantation. As far as the concept for clinical corneal transplantation is concerned, if postoperative vascularisation was inevitable even in an avascular bed, all the grafts should have started a rejection reaction when postoperatively vascularised. It should be emphasised that one of the major prognostic clinical criteria for graft success has always been the degree of the pre-vascularisation of the recipient bed at the time of operation, not that of the postoperative vascularisation.^{7,16} In a clinical situation, a linear correlation between corneal graft rejection and the degree of pre-vascularisation was observed in a five-year long-term analysis.⁷ This latent influence of the degree of pre-vascularisation of the recipient bed suggests that the pre-vascularisation may be related to the mechanism of chronic rejection of clinical corneal transplantation. Despite of the concept of an 'immunologically privileged site', the other factors described above indicate the need to consider immunological aspects of corneal graft rejection and to develop various methods to avoid graft rejection. The disparity between the classical concept of an 'immunologically privileged site' for corneal

grafting and the revealing factors which affect the outcome of clinical corneal transplantation clearly indicates that an experimental model which is quite similar to a clinical situation is necessary for a further understanding of clinical corneal transplantation.¹⁷

Recent improvements in microsurgery for orthotopic penetrating corneal transplantation in the inbred rat,¹⁸ in which the rat MHC (RT1) including the associated immune response genes of many strains is well defined in an inbred animal line,¹⁹ have enabled some aspects of orthotopic corneal transplantation to be studied in a more defined manner. In order to take full advantage of the rat model to study the immunological events after orthotopic corneal grafts, the following conditions for experimental corneal transplantation are required.

- (1) A reliable surgical technique for corneal grafting.
- (2) The normal anatomical site for an orthotopic penetrating corneal graft.
- (3) Comparison of the rejection of corneal grafts with that of other tissue or organ grafts.
- (4) The use of inbred or congenic animal lines with a variety of genetically well defined histocompatibilities.

Surgical methods

Full-thickness penetrating corneal grafting in the rat is technically very demanding, but Williams and Coster have described a technique in which interrupted sutures are used to hold the transplanted corneal tissue in place.¹⁸ I have modified their technique by using a continuous interlocking chain suture that ensures minimal suture-induced microvascularisation.^{20,21} In the early stage of the study, most of the causes of technical failure were due to aqueous humour leakage and secondary cataract formation. Improvement in suturing technique is the way to reduce technical failure in rat corneal grafting. Tight attachment between graft and host junctions and a well-balanced, symmetrical tension of the sutures around the perimeter of the graft is the only way to achieve a high success rate in this operation. The use of a continuous chain suture has eliminated most of these problems. This technique has been developed to avoid

aqueous humour leakage and minimises the suture-induced microvascularisation in corneas grafted onto avascular beds. Although the continuous chain sutures were tied off on the intraocular side they were not removed postoperatively. The technique of rat corneal grafting described here has been found to be extremely reliable, with a success rate of 97% in the last 600 grafts performed in this laboratory. Based upon the distance from the graft junction to the limbus, placing a 3 mm diameter button into a rat cornea of 7 mm is comparable to grafting a 7 mm (medium-size) button into a human cornea of 11 mm because of strong convexity of the rat cornea.

The role of transplantation antigens in rat corneal grafting

The rat MHC

The first reproducible evidence for a strong histocompatibility system in the inbred rat came from serological studies.²² The MHC of the laboratory rat, *Rattus norvegicus*, is specifically designated the RT1 system.²³ It is highly polymorphic, and many different haplotypes have been identified among inbred strains. A number of RT1-congenic lines have been developed in different labora-

tories, further contributing to the usefulness of this type of rat in transplantation studies.

The RT1.A, B, C, and D regions have been described as intra-MHC regions and their arrangement of the genes coding for class I and class II antigens is now quite well understood.²³ Single recombination events separated two regions—initially termed A and B—which code for the principal class I and class II antigens respectively.²⁴

All the strong class I alloantigenic activity derives from the RT1.A region and the other weak class I from the RT1.C region²⁵ and possibly from RT1.E region²⁶ or RT1.L and RT1.M.²⁷ The RT1.A antigens are expressed on nearly all body tissues, although there are large quantitative variations. They provoke strong alloimmune responses such as antibody production, graft rejection and cytotoxic T lymphocyte (CTL) generation, but cause only relatively weak graft-versus-host (GVH) reactions and mixed lymphocyte reactions (MLR).^{28,29} In addition, RT1.A antigens serve as restricting elements for CTL responses

against minor transplantation antigens, whereas RT1.C antigens do not.^{30,31} The histocompatibility antigen in RT1.C are of intermediate strength, as shown by skin graft rejection.³² Potent CTL responses can be generated across RT1.C differences when *in vitro* primed lymphocyte populations are employed.^{32, 33} Primary *in vitro* stimulation failed to generate CTL against RT1.C.³³ It is clear that RT1.C is inferior to RT1.A in provoking such responses.

Class II major transplantation antigens are encoded by the RT1.B and D regions,³⁴ which are separable by recombination and are termed B and D.³⁵ It appears that the RT1.B product is the homologue of mouse I-A and RT1.D the homologue of mouse I-E.³⁶ Both code for a and b polypeptide chains of 33kD and 28kD and both carry alloantigenic specificities. It has been proposed that this region be named the RT1.I-region,^{23,37} and the term Ia is often used for rat class II antigens. Like the equivalent class II molecules in the mouse and man, the RT1.B and D region products are the strongest stimulators of MLR, graft versus host (GVH) reaction and acute skin graft rejection.³⁸ Their distribution is more restricted than class I antigens, being found *inter alia* on B cells, some macrophages, dendritic cells, epidermal Langerhans cells, epithelia of thymus, the small intestine, and kidney proximal tubules. They are absent from red cells.^{39,40} Antibodies to RT1.B and D antigens, which may reach a high level after tissue grafting, can be assayed on B lymphocytes by complement-dependent cytotoxic assays or by binding radiomunoassay. The RT1.B and D region genes also function as immune response (Ir) genes. The function of Ir genes which control alloimmune responses to RT1.A antigens has been described and potentially plays an important part in determining graft rejection.^{41,42}

The origins of the various MHC products are defined in the text by designating the subregions by a capital letter in Roman type, followed by the RT1 haplotype source of the MHC by a small letter. For instance, a PVG rat (haplotype c) = RT1^c = RT1.A^bB^cD^cC^c. The congenic rats used in this study (PVG.RT1^a, PVG.R1, PVG.R19 and PVG.R20) are based on PVG strains where intra-MHC structures have been defined.^{24,32}

The blood group antigens in the rat which have been identified so far are RT2, RT3, RT8 and RT9.⁴³⁻⁴⁵ The haplotypes of rat RT4 and RT5 (skin antigens) were previously designated H-4 and H-5.⁴⁶ RT6 (peripheral T cell antigen) codes for T cell-specific differentiation antigens that are expressed on most peripheral T cells, but not on fully differentiated cytotoxic T cells and thymocytes.⁴⁷ RT7 (Pan T cell antigen) is present on all functional T lymphocytes in the rat.⁴⁷

Unless otherwise stated, donors and recipients were always males and weighed between 200–400 g. All rats were obtained from a single supplier, Harlan Olac Ltd (Bicester, Oxon, UK). PVG.R19 and PVG.R20 congenic rats were obtained from the Monoclonal Antibody Centre, AFRC Institute of Animal Physiology and Genetics Research, Babraham, Cambridge, UK.

MHC antigens in rat cornea

Rat cornea contains both class I and class II MHC antigens, as demonstrated by immunohistological staining of corneal sections with general and specific monoclonal antibodies (Mab).^{48,49}

Fresh DA corneas showed that DA class I MHC antigen was distributed extensively in both the central and peripheral corneal areas when the Mab of MN4-91-6 (specific for RT1A^a) was used. Corneal epithelial cells showed strong expression of class I MHC antigen which was most intensively stained in the superficial epithelium. Most corneal endothelial cells also showed positive staining of class I MHC antigen. In the corneal stroma, the keratocyte cells were weakly stained. This distribution pattern of class I MHC expression did not vary significantly from the periphery to the centre in the cornea. DA class I MHC antigen was also distributed extensively in both skin and heart.⁴⁹

Cryostat sections of the normal skin of DA (RT1^a) inbred rats when stained with immunoperoxidase and Mab of F17-23-2 (specific for RT1B^a) and OX-29 (specific for leukocyte-common antigen) showed expression of class II positive dendritic cells, the dendritic cells being found in the dermis and the Langerhans cells in the epidermis with spindly cytoplasmic processes. Cryostat sections of the normal

heart of DA inbred rats showed strong expression of class I antigens in the muscles and vascular endothelium. Serial sections were examined at a 400 fold magnification using a $\times 10$ eye piece and a $\times 40$ objective lens. Central and limbal areas of cornea were assessed separately. An average of nine fields in each of the corneas was counted on each slide using a graticuled eye piece with a square grid, the mean number of dendritic cells counted per field in the centre (0.3 ± 0.5 with F 17-23-2) was less than that in the periphery (3.3 ± 1.4 with F 17-23-2). Cryostat sections of the normal DA corneas showed that the dendritic cells were present in small numbers compared with the DA skins. Cryostat sections of normal AO corneas showed the similar distribution pattern of the class II MHC positive dendritic cells in the cornea with MAbs of OX-3 (specific for RT1B^u) and OX-29 (specific for leukocyte-common antigen). No detectable class II MHC positive cells were found in the corneal epithelium and the corneal endothelium.⁴⁹

During the course of a rejection response, cryostat sections of the rejected DA corneal grafts transplanted on avascular AO recipient beds showed increased expression of the donor class I MHC antigen. The increased expression of the donor class II MHC antigen was also found in the most anterior part of the stroma in the graft. Occasional cells were noted in the graft/host junction and peripheral stroma of the AO recipient bed. Corneal epithelium showed some scattered class II positive cells in the graft. Expression of leukocyte-common antigen in the rejected DA cornea was increased in the donor cornea, the graft/host junction and in the periphery of the AO recipient bed. It was also found that very strong expression of AO (host origin) class II MHC antigens in the DA corneal graft and the graft/host junction and that its relatively increased expression was also observed in the periphery of the AO recipient bed.⁴⁹

An increase in both class I and class II MHC antigens during rejection response has been reported in rat heart.⁵⁰ Also an increase in class II MHC antigens during graft *versus* host reaction has been reported in rat liver.⁵¹ It seems that the increased expression of trans-

plantation antigens during the allograft response is quite a general event including corneal allografts.

Immunogenicity of rat cornea

It was discovered early in this laboratory that, contrary to the concept of immunological privilege, a 3 mm corneal graft placed between genetically differing rat strains might be rejected. That such rejection was under the control of basic transplantation antigens was established as follows. Isografts between DA rats survived indefinitely, fully allogeneic corneal grafts from DA to AO were found to be rejected rapidly in all instances even in an avascular bed (median survival time: 11 days), corneas grafted from parent to F₁ were accepted indefinitely, whereas grafts from F₁ to the parent were rejected (22 days), probably reflecting the reduced antigenicity of the F₁ donors. This is a similar speed to the rejection time seen for other grafts placed in the strain combination of DA to AO. Orthotopic skin grafts were rejected at nine days, heterotopic heart grafts were rejected at six days and orthotopic liver grafts were rejected at 17 days in the same strain combination.^{20,21,52} Histological sections stained by the haematoxylin and eosin method of rejected DA corneas showed a moderate mononuclear cell infiltrate in the stroma, a thin epithelium and often an absence or distortion of the endothelium with marked mononuclear cell infiltrate.^{20,21} Using the rejecter combination, this experiment demonstrates that corneal graft rejection in an avascular bed obeys the basic laws of transplantation, in that grafts between genetically identical animals are accepted, whereas grafts between genetically non-identical animals are rejected with a speed which is dependent on genetic differences between donor and recipient.

Whilst the immunogenicity of transplantation antigens in corneal graft is a necessary requirement for allograft rejection, it seems that the inherited ability of the recipient to respond would also profoundly influence the outcome. This is discussed in the next section using inbred rats.

Genetics of corneal graft rejection in the rat

The fate of rat corneal grafts on an avascular

bed is critically dependent on the particular combination of donor and recipient strains, as summarised in Table I.^{20,21,49} In certain strain combinations, acute rejection occurs (7.5–20 days), in others, rejection is delayed (21–79 days), while in some combinations corneal graft survival is very prolonged (>100 days) or permanent. Thus, a variety of combinations could be found which resulted in corneal graft acceptance. For example, corneal allografts in the combination AUG (RT1^c) to WAG (RT1^u) survived for more than 500 days without any treatment or immunosuppression. Although the cornea behaves as an immunologically favoured tissue, skin, heart or kidney allografts showed acute rejection in the same combination (Table II). In all long-surviving corneal allografts, approximately one third showed a transient rejection and avoided graft rejection. An existence of non-rejector combinations for corneal allografts with suture-induced microvascularisation does not only invalidate the concept of an 'immunologically privileged' site for transplantation as proposed by Medawar¹⁰ and supported by Billingham and Boswell,¹¹ but also casts doubt upon the normal principles of graft rejection genetics as shown by Medawar¹⁰ using skin grafts and the concept of incomplete afferent and efferent limbs for corneal graft rejection as suggested by Khodadoust and Silverstein.¹² The existence of different responder status for the fate of corneal allografts may explain why the outcome of clinical corneal transplantation does not always obey the concept of an 'immunologically privileged site'.

The different responder status described above seems to be compatible with MHC-linked Ir (immune response) genes. Using congenic intra-MHC recombinant strains, MHC-linked Ir genes have been described for immune responses against the class I MHC antigens of PVG.RT1A^a, indicating alloantibody production,⁵³ rejection of skin allografts and generation of cytotoxic T cells.^{41,42} For example, PVG (RT1^c) rats are low responders against PVG.RT1A^a, while PVG.RT1^u and PVG.RT1ⁿ are high responders *in vivo*. The Ir gene alleles were shown by backcross analysis to segregate precisely with RT1 type *in vitro* using heterotopic heart grafts and orthotopic

kidney grafts. On the survival of orthotopic liver grafts in fully allogeneic combinations, PVG (RT1^c) recipients accepted DA (RT1^a) liver grafts indefinitely whereas both AO (RT1^u) and BN (RT1ⁿ) rats rejected the same kind of allografts, usually within 20 days.⁴² According to the responder status restricted by MHC-linked Ir genes, the fate of fully allogeneic liver grafts in rats is restricted to a specific haplotype of donor or recipient and there is an apparent correlation of MHC-linked Ir gene allele with the fate of liver grafts.⁵⁴ The potency of the MHC-linked Ir gene allele can be seen on the survival of orthotopic corneal grafts in some fully allogeneic strain combinations. PVGs accepted 14% of the numbers of DA corneal grafts indefinitely (21 days) whereas both AO (RT1^u) and BN (RT1ⁿ) rats rejected the DA corneal grafts (11 days and 7.5 days respectively), within 18 days.^{20,21} However, unlike liver grafts, the fate of corneal grafts in rats is not always restricted to specific haplotype of either donor or recipient (Table I). There is not always an apparent correlation of MHC-linked Ir gene allele with the fate of corneal grafts in 32 fully allogeneic strain combinations. This suggests that genes in the non-MHC lined backgrounds are important in the fate of corneal allografts.

With a MHC incompatibility (donor/recipient = c/u), corneas grafted from PVG (RT1^c) to AO (RT1^u) were rejected acutely (13 days), corneas grafted from PVG (RT1^c) to WAG (RT1^u) were rejected in delayed fashion (79 days), corneas grafted from PVG (RT1^c) to LOU (RT1^u) showed prolonged survival (>100 days), and corneas grafted from AUG (RT1^c) to WAG (RT1^u) survived indefinitely. This phenomenon was also found in another MHC incompatibility (u/c). Corneas grafted from AO (RT1^u) to PVG (RT1^c) were rejected acutely (11 days), corneas grafted from LOU (RT1^u) to PVG (RT1^c) showed prolonged survival (>100 days), corneas grafted from WAG (RT1^u) to AUG (RT1^c) showed prolonged survival (>100 days), and corneas grafted WAG (RT1^u) to PVG (RT1^c) survived indefinitely, as shown in Table III. Thus, the data showed different characteristic levels of responsiveness to a defined incompatible set of MHC antigens among some fully allogeneic strain combinations.⁴⁹

Table I. Comparison in survival times of orthotopic corneal and liver allografts in the same strain combinations

Rank	Donor strain (RT1)	Recipient strain (RT1)	Survival time of corneal grafts ^a (days)	Survival time of liver grafts ^a (days)	*Ref
1	BN (n)	PVG (c)	7 (×3), 8 (×3)	>100 (×5)	A
2	DA (a)	BN (n)	6, 7 (×4), 8 (×3), 10 (×2)	8, 9, 11, 12 (×2), 13,	A
3	AO (u)	PVG (c)	7 (×2), 10, 12 (×2), 15,	—	—
4	BN (n)	LEW (l)	8, 10 (×3), 13 (×2),	>100 (×4)	A
5	BN (n)	DA (a)	9, 11 (×4), 12,	>100 (×5)	B
6	LEW (l)	BN (n)	6 (×3), 14, 15, 19,	42, 46, 48, 53 (×2),	A
7	BN (n)	AO (u)	9, 10, 11 (×2), 13, 16,	—	—
8	DA (a)	AO (u)	10 (×3), 11 (×7), 14 (×3), 15, 18,	17 (×4)	C
9	PVG (c)	DA (a)	11, 12 (×3), 13 (×3), 14, 15 (×2),	>100 (×9)	B, C
10	LEW (l)	DA (a)	7, 8 (×2), 9, 15, 34,	>100 (×5)	B
11	LEW (l)	AO (u)	10, 11, 12, 14, 16, 20,	—	—
12	PVG (c)	AO (u)	12, 13 (×3), 17 (×2),	—	—
13	AO (u)	DA (a)	11 (×2), 12, 17, 18, 20,	—	—
14	LEW (l)	AUG (c)	11, 12, 14, 17 (×2), 20,	12, 13, 14, 18, 21,	B
15	DA (a)	LEW (l)	10 (×2), 18 (×3), 20,	9, 11 (×2), 12, 13,	A
16	BN (n)	AUG (c)	12, 13 (×3), 19, 25,	47, 49, 50, 53, 63,	A
17	LEW (l)	PVG (c)	9, 10, (×3), 12, 46,	29, 31, 32 (×2), 39,	A
			47, 81, >100,		
18	AUG (c)	DA (a)	14, 15 (×2), 17 (×2), 38,	>100 (×5),	A
19	AUG (c)	LEW (l)	16, 17 (×2), 21, 28,	17 (×2), 18, 19, 20,	A
20	PVG (c)	LEW (l)	15, 19, 20 (×2), 23, 24,	11, 15, 16, 18, 20,	A
21	AO (u)	LEW (l)	14, 16 (×3), 19, 24,	—	—
			26 (×3), 48,		
22	DA (a)	PVG (c)	13 (×2), 15, 16 (×2), 17 (×3), 18 (×2), 19, 20 (×3), 21 (×3), 22, 25, 26 (×3), 27,	>100 (×70),	D
			49, 53, >100 (×4),		
23	PVG (c)	WAG (u)	20, 49, 58, (20), >100 ^b , (58) >100 ^b , >100	—	—
24	AO (u)	BN (n)	16, 20, >100 (×4),	—	—
25	PVG (c)	LOU (u)	50, 50, 53, 55, 84,	—	—
			(58) 100 ^b , >100 (×4),		
26	LOU (u)	PVG (c)	12, 58, (12) >100 ^b , (77) >100 ^b , >100 (×3)	—	—
27	WAG (u)	AUG (c)	14, >(23) 100 ^b , (90) >100 ^b , >100 (×2),	—	—
28	AUG (c)	BN (n)	20, (20), >100 ^b , >100 (×2),	38, 39, 46, 49, 53,	A
29	PVG (c)	BN (n)	69, 70, >100 (×4),	28, 29, 32, 35, 57,	A
30	DA (a)	AUG (c)	64, (18) >100 ^b , (18) >100 ^b , (21) >100 ^b , (27) >100 ^b , (27) >100 ^b ,	10 (×2), 11, 12, 13,	A
			(22) >100 ^b , (22) >100 ^b , (33) >100 ^b , (37) >100 ^b , >100 (×4),	5, 8, 10, 11, 13,	E
31	WAG (u)	PVG (c)	>100 (×6),	16, 18, 70, >100,	
32	AUG (c)	WAG (u)	>100 (×6),	—	—

^aGrafts were examined for a minimum of 100 days post-transplant, and those that had not been rejected in that time were taken as 'accepted'.

^bThese grafts had minor rejections as they showed a transient rejection episode after operation, the end point of which is shown in parentheses, but subsequently they cleared and remained transparent until after the 100 day period of observation. These were also taken as 'accepted'.

*Ref. = References for liver graft data in this table.

A—Zimmermann *et al.* (1983). *Transplant Proc* **25**: 1272.

B—Ulrichs *et al.* (1981) *Eur Surg Res* **13**: 79.

C—Kamada (1982). Ph.D. Thesis University of Cambridge.

D—Kamada and Calne. (1983). *Surgery* **93**: 64.

E—Limmer, Herbertson and Calne. (1980) *Eur Surg Res* **12**: 343.

Table II. *Survival times of WAG corneal, heart and kidney grafts in PVG recipients.*

Tissue/organ type of grafting	Median survival time (days)	Numbers of grafts	Reference
Orthotopic corneal grafts on avascular beds	>100	6	Table 1
Heterotopic auxiliary heart grafts	8	8	A
Orthotopic kidney grafts	9	6	B

Reference in Table II

A. Kamada and Shinomiya (1985). *Immunology* **55**: 85.

B. Kamada (1985). *Transplantation* **39**: 93.

There are two distinct roles proposed for APCs in inducing graft rejection.⁶¹ Direct antigen presentation is provided by APCs of the donor transplanted with the graft and induces acute rejection, whereas indirect presentation is provided by APCs in the recipient which are capable of processing graft antigens and induces chronic rejection. As described in *MHC antigens in rat cornea*, the normal DA corneas showed that the dendritic cells were present in small numbers compared with the DA skins. This indicates the reason why the importance of MHC antigens is reduced on the fate of corneal allografts despite the fully allogeneic situation.

Data show that non-MHC genes on either the donor or the recipient exert more influence on the fate of corneal grafts than do MHC genes. This has an important implication for current clinical practice, as although MHC identity is clearly of importance in graft survival, the tissue typing and matching of donor and recipient MHC may not be sufficient to ensure acceptance. It is necessary to establish the relative importance of the major and minor transplantation antigens in inducing corneal graft rejection. This

is discussed in the next section using congenic rats.

The influence of MHC and non-MHC components on corneal graft rejection

The role of the MHC genes and their products in tissue and organ transplantations have been most extensively studied in mice,^{55,56} rats^{23,25,57} and humans.^{58,59} It was reported that there were strong and weak antigens,⁶⁰ now referred to as major and minor histocompatibility antigens. Whilst MHC-incompatible grafts induced very strong primary alloimmunity, which in most circumstances lead to graft rejection, weak barriers are equated with prolonged allograft survival and chronic rejection.⁶¹ In some experimental and clinical circumstances, it was noted that combinations of minor transplantation antigens could induce rejection similar to that seen with major antigenic incompatibilities.^{62,63}

Recently, the availability of appropriate congenic strains of rats has allowed studies on the influence of the individual immunogenetic components of the MHC (class I and class II antigens) and non-MHC antigens, upon tissue and organ allograft survivals to be investi-

Table III. *Survival times of corneal grafts with a MHC incompatibility*

Incompatibility of the MHC haplotypes	Donor/recipient strain combination	Median (days)	MST $\pm$ SD (days)
c/u	PVG to AO	13	14.2 $\pm$ 2.2
c/u	PVG to WAG	79	71.2 $\pm$ 34.0
c/u	PVG to LOU	>100	79.2 $\pm$ 24.0
c/u	AUG to WAG	>100	>100
u/c	AO to PVG	11	10.5 $\pm$ 3.2
u/c	LOU to PVG	>100	81.4 $\pm$ 13.0
u/c	WAG to AUG	>100	85.7 $\pm$ 35.1
u/c	WAG to PVG	>100	>100

gated. In this section, experiments were performed which were designed to identify which transplantation antigens were important in orthotopic corneal graft rejection. In order to achieve a variety of incompatibilities, the DA to PVG combination was only used here because of the limited availability of congenic strains in the UK. The relative contribution of MHC and non-MHC antigens to orthotopic corneal allografts was investigated using the strains of DA ($A^aB^aD^aC^a$) and PVG ($A^bB^bD^bC^b$), PVG.RT1^a ($A^aB^aD^aC^a$), PVG.R1 ($A^aB^bD^bC^b$), PVG.R19 ($A^aB^aD^bC^b$) and PVG.R20 ($A^bB^bD^bC^a$) rats.³²

By choosing appropriate strain combinations, orthotopic corneal grafts could be performed across the following incompatibilities on avascular beds: full (MHC and non-MHC), MHC [class I (major & medial) and

class II], class I (major), class I (medial), class II, class I (medial) and class II, and non-MHC only. Major and medial class I incompatibilities mean that the RT1.A and RT1.C regions are involved. Medial class I incompatibility alone means that just the RT1.C region is involved. These seven donor/recipient strain combinations which were used in this study to achieve these incompatibilities are shown in Table IV. Between 10 and 29 corneal grafts were performed in each group. In addition, skin and heart grafts were performed across class II incompatibility alone. Ten grafts were used in each experiment.

The data illustrated in Figure 1 show that fully incompatible corneal grafts (DA to PVG; both MHC and non-MHC antigens) were rejected (21 days). In this group, 14% of the grafted corneas became long-term survivors exceeding 100 days post-transplant. Corneal grafts incompatible for MHC alone (PVG.RT1^a to PVG; both class I and class II) were rejected in a median time of 18 days. In this group, 5% of the grafts became long-term survivors. There was a statistical difference between these two groups ($0.01 < p < 0.05$). Corneal grafts incompatible for non-MHC alone (DA to PVG.RT1^a) were slowly rejected (95 days). In this group, 50% of the grafts became long-term survivors. With a class I incompatibility alone, corneal grafts incompatible for both major and medial class I incompatibilities (PVG.RT1^{rl} to PVG) were

rejected (52.5 days). In this group, 36% of the grafted corneas became long-term survivors. In contrast, corneal grafts incompatible for medial class I alone (PVG.R20 to PVG) survived over 100 days. In this group, 90% of the grafts became long-term survivors. The difference in graft survival times between these two class I incompatible groups was significant ($p < 0.01$). Of all the groups, the best survival achieved was surprisingly for those corneas grafted across a pure class II incompatibility (PVG.R19 to PVG.R1). However, when the class II incompatibility was combined with a medial class I incompatibility (PVG.RT1^a to PVG.R1), corneal grafts showed some chronic rejection (25%). Furthermore, when the class II incompatibility was combined with major and medial class I incompatibilities, corneal grafts (PVG.RT1^a

to PVG) showed acute and chronic rejection (95%) with a median survival of 18 days. The difference in corneal graft survival between class I (major and medial) incompatibilities, compared with a class II incompatibility alone, was significant ($p < 0.01$). The seven donor/recipient strain combinations and the survival times of corneas are shown in Table V.

Data illustrated in Figure 1 demonstrate the hierarchy for antigens inducing corneal graft rejection in avascular beds. It would appear that the different survival times were obtained with different class I incompatibility of corneal grafts. Thus, where only a medial class I incompatibility exists, all but one corneal graft survived during the entire observation period (>100 days). Combination of both medial and major class I incompatibilities provides a much more aggressive stimulus to rejection (52.5 days). There are no congenic strains in existence which allow us to investigate a class I major incompatibility in the absence of medial incompatibility. It would also appear that a combination of class II incompatibility with any other incompatibility provides an allogeneic reaction for acute graft rejection in this combination. Unfortunately, the congenic strains which are needed to confirm this hypothesis do not exist, but the comparisons between class II alone (all survivors), class II and medial class I (52 days) and class II and class I (18 days) does support this sugges-

Table IV. Donor/recipient strain combinations used for genetic studies using PVG congenic rats

Donor strain	Recipient strain	Incompatibility of genotype	Area of incompatible components
DA	PVG	A ^b B ^c D ^c C ^c	Full (whole MHC & multiple non MHC)
PVG.RT1 ^a	PVG	A ^b B ^c D ^c C ^c	Whole MHC only (class I and class II)
PVG.R1	PVG	A ^c C ^c	Intra MHC class I (major & medial)
PVG.R20	PVG	C ^c	Intra MHC class I (medial)
PVG.RT1 ^a	PVG.R1	B ^c D ^c C ^c	Intra MHC class I (medial) & class II
PVG.R19	PVG.R1	B ^c D ^c	Intra MHC class II
DA	PVG.RT1 ^a		Multiple non-MHC only

tion. Evidently, both class I and class II MHC incompatibilities in isolation cannot induce the strong alloimmune response that leads to rapid graft rejection. The different survival times obtained with class II incompatibility alone, class II plus medial class I incompatibilities, and class II plus major and medial class I incompatibilities, which means total MHC incompatibility. Unexpectedly, individual class II incompatibility shows the best survival of all and all the corneal grafts survived more than 100 days. This is in contradistinction to the alloaggressive characteristics ascribed to class II MHC products.

Because of these observations, the experi-

ments were extended to include skin and heart grafts performed across a pure class II MHC incompatibility. Surprisingly, the hearts survived indefinitely as did the corneas, whereas the skins rejected acutely (12.5 days; Table VI). However, the survival was not straightforward because all the hearts grafted went through an immunological crisis between days 11 and 15 after transplantation. This took the form of transient rejection: all the hearts stopped once (13 days) and then the heartbeats started again from three to 11 days later, and the grafts survived indefinitely. Transient rejection has been often observed after long-term observation of fully allogeneic

Fig. 1. Hierarchy for antigen-induced corneal graft rejection.

Table V. *The influence of MHC and non-MHC components on corneal graft survival in the combination of DA to PVG (mild responder)*

Area of incompatibility	Corneal graft survival (days)	Median (days)
Full (MHC & non-MHC)	13 (×2), 15, 16 (×2), 17 (×3), 18 (×2), 19, 20 (×3), 21 (×3), 22, 25, 26 (×3), 27, 49, 53, >100 (×4)	21.0
Whole MHC (class I and class II)	6 (×2), 12, 14 (×2), 17 (×3), 18 (×4), 19, 20, 22, 23 (×2), 25, 28, >100	18.0
Intra MHC class I (major & medial)	21, 22, 24, 25, 34, 34, 36, 37, 38 (×2), 39, 66, 67, 88, >100 (×8)	52.5
Intra MHC class I (medial)	32, >100 (×9)	>100
Intra MHC class I (medial) and class II	33, 34, 36, 45, 66, >100 (×15)	>100
Intra MHC class II	>100 (×10)	>100
Multiple non-MHC only	33, 34 (×2), 36 (×2), 38 (×2), 40, 41, 43, 90, >100 (×11)	95.0

corneal grafts²¹ and of liver grafts in the non-rejector combination DA to PVG.⁶⁴ Transient rejection has now been found in heart allografts with a pure class II MHC incompatibility. In any other strain combinations, transient rejection of heart grafts has not been reported.

Using same varieties of strain combinations, hearts grafted across the class I, class II or non-MHC barrier survive indefinitely, whereas skins grafted across the class I, class II or non-MHC barrier are rejected within two weeks.⁶⁵ In the case of cornea, the grafts across the class I barrier are rejected, and the grafts across the class II barrier survive, but the grafts performed across the non-MHC barrier are half way between the two.⁶⁵ This finding on genetic studies between orthotopic corneal grafts and heterotopic heart grafts can be explained by the different quantitative distribution and/or the characteristic difference of the transplantation antigens in each graft and also in each recipient's site for grafting.

The existence of tissue-specific or tissue-restricted histocompatibility antigens may be another explanation.⁶⁶ Strictly defined, tissue-specific antigens are antigens characteristic of one particular tissue or cell.⁶⁶ Some of the histocompatibility antigens, which are other than class II MHC antigens, with limited if not highly specific tissue distribution, are classic tissue-specific antigens, whilst others are alloantigens with limited tissue expression. Much of the evidence that they evoke immune responses that damage or destroy transplanted tissue is incomplete or circumstantial, but some is convincing and includes the immunogenetic characterisation of new antigen systems.⁶⁷⁻⁶⁹ Thus, the role of each component of transplantation antigens is dependent on the situation of a particular type of graft transplanted to a specific site of grafting. This graft/site combination may be critical in the choice of an experimental model for studying transplantation immunology.

Vascular endothelium of the graft can be

Table VI. *The influence of pure class II incompatibility on orthotopic corneal grafts, heterotopic heart grafts and orthotopic skin grafts in the same strain combination of PVG.R19 to PVG.R1*

Type of grafting	Graft survival time (days)	Median (days)
Orthotopic corneal grafts on avascular beds	>100 (×10)	>100
Heterotopic auxiliary heart grafts in the neck	*(11)>100, (12)>100, (12)>100, (12)>100, (13)>100, (13)>100, (14)>100, (14)>100, (14)>100, (15)>100,	>100
Orthotopic skin grafts in the chest wall	11 (×2), 12 (×3), 13 (4×), 14	12.5

* ()>100: These heart grafts had stopped once when they showed a transient rejection episode at the date of () post-operatively, heartbeats started again within a week and the grafts survived over 100 days.

induced to express class II MHC antigens in rodents^{70,71} and humans.⁷² Only avascular donor corneal tissue is suitable in clinical corneal transplantation. Thus, the degree of pre-vascularisation in the recipient's bed is considered as the major factor to be involved in sensitisation via blood vessels as well as presentation of graft antigens to the recipient's immune system. Using monoclonal antibodies, an increased number of dendritic cells was observed in the vascularised or inflamed corneal buttons of the patients.⁷³ This suggests that the presence of large numbers of dendritic cells in the recipient bed may prejudice the outcome of the graft by virtue of an increased indirect antigen presentation caused by the proximity of APCs to foreign antigens in the graft,⁶¹ which would inevitably lead to chronic graft rejection.⁷ This indicates why the degree of prevascularisation is so influential for the outcome of clinical corneal transplantation during long-term observation, because the importance of non-MHC antigens would be revealed by the increased indirect antigen presentation. It was reported in a clinical situation that only in moderate and severely vascularised beds that the beneficial effect of HLA-A and -B matching can be observed in blood group non-O recipients, but this effect does not apply to O recipients at the five year observation period.⁷ However, in non or slightly vascularised beds, HLA-A and -B matching was found to be important irrespective of the ABO compatibility.⁷

Several groups have described the significant effect of HLA-A and -B matching in the particular circumstances defined by high-risk patients with a prior history of graft rejection or significant pre-vascularisation of the recipient bed.^{7,74,75} These studies suggest that good class I MHC (HLA-A, -B) matching is associated with lower corneal allograft rejection. In contrast, 257 patients who had moderate or severely vascularised corneas were matched for HLA-A and -B before transplantation, and the DR matching effect was investigated retrospectively.⁷ They were unable to show any advantage in the combination of HLA-DR matching and HLA-A and -B matching when compared with HLA-A and -B matching alone.

In these experiments, it should be emphasised that corneas grafted across class I or non-MHC incompatibilities do undergo some chronic rejection, unlike the class II incompatibility alone. Thus, these data indicate that even HLA-DR matching alone cannot avoid rejection of corneal grafts. The class I and non-MHC antigens are potential factors which induce chronic rejection in corneal allografts. This chronic rejection may be spontaneously triggered and activated by various conditions. It is suggested that large international data sets with a variety of HLA typing and minor transplantation antigens should be examined to find out whether survival of corneal grafts differs with a single incompatibility at a given locus or with combined incompatibilities at several loci.

The role of antigen-presenting cells in corneal graft rejection

As demonstrated in experiments above, it should be noted that corneal allografts can undergo acute rejection even when transplanted into avascular beds and that antigenic disparity between donor and recipient is an important factor in corneal allograft rejection. Although a small number of class II positive dendritic cells are present in a normal cornea, they may represent the most important stimulus to induce allogeneic reaction.

This section describes experiments designed to investigate the role of APCs present in the cornea on graft survival. Three different factors were studied in relation to the density of APCs;

- (1) size of corneal button,
- (2) position of the corneal button in the recipient bed,
- (3) immunomodulation of APCs by Ultra-violet-B (UV-B) irradiation to donor or recipient corneas and by hypothermic preservation of donor corneas.

Size of corneal button

In 1905, Edward Zirm performed the first penetrating corneal allograft in man and seven months after the operation, he sent to the publisher a case report in which he described that the graft remained clear and transparent.⁷⁶ Using a 5 mm trephine of von Hippel's, he grafted the centre part of the

donor corneal button into the left eye of a 46 years old man in whom both eyes had sustained lime burns which made the recipient beds vascularised. The graft was transplanted on the prevascularised bed with overlaid bridge-sutures. Indeed, despite of the pre-existing vascularisation of the recipient bed following lime burns, acute corneal graft rejection was not observed without any immunosuppression. The experiments described in this section will indicate that acute rejection was probably avoided because of the small (5 mm in diameter) trephine which Zirm used in the initial corneal allograft operation.

The size of the donor corneal button was shown to be of critical importance for the fate of corneal grafts as shown in Figure 2. 4 mm and 3 mm (standard size) diameter corneas grafted in the avascular beds underwent acute rejection (10.5 days and 11 days respectively), whereas 2 mm and 1.5 mm corneas did not undergo acute rejection and survived for over 100 days. Data illustrated in Figure 2 suggested that the smaller the size of the donor cornea, the less was the corneal graft rejection. The 'privilege' in corneal allografting may result not just from the poor degree of vascularity in the recipient bed but also the unique distribution of APCs with this particular tissue itself.

Position of the corneal button in the recipient bed

In this section, full-thickness corneas were grafted orthotopically and unilaterally into the avascular penetrating recipient bed, in order to investigate the contribution to the grafting site of the recipient beds. Using a 2 mm trephine, corneas grafted from the centre of the donor cornea to the centre of the recipient bed survived indefinitely (>100 days). It must be remembered that this is in contrast to the results achieved with 3 mm corneal buttons placed centre to centre which rejected with a median survival of 11 days. The question which these experiments sought to address was why should a relatively small reduction in diameter of the graft causesuch a marked effect in graft survival. Two possibilities were investigated:

- (1) The extra distance needed for blood vessels to 'travel' to reach the graft improved its chances of survival.
- (2) Antigenic stimuli are unevenly distributed throughout the cornea. Thus, a smaller graft from the centre would elicit less of an immune challenge from the recipients.

Therefore, the design of this experiment involved grafting 2 mm corneal buttons from

Fig. 2. Influence of size of the donor corneal button.

Fig. 3. Influence of the site of donor cornea and recipient bed.

periphery to centre and from centre to periphery (Figure 3). 2 mm (non-rejecting size) corneas which were grafted from the centre of the donor cornea to the centre of the recipient bed survived indefinitely (>100 days). Ten corneas grafted from the centre to the periphery showed transient rejection in all instances, and seven corneas were rejected chronically (96 days). In contrast, four of ten corneas grafted from the periphery to the centre showed acute rejection, one cornea was rejected chronically, and others survived indefinitely (99 days).

The results achieved show quite clearly that a corneal button taken from the periphery of the cornea and placed in the centre will elicit acute graft rejection with 40% failing in the first 20 days. Such a result is compatible with a high density of donor antigen-presenting cells being grafted with such a button. In contrast, corneal buttons taken from the centre and placed at the periphery demonstrated no acute rejection, but instead showed chronic rejection (Figure 3). This is consistent with the involvement of recipient antigen-presenting cells, in high concentration at the periphery of the cornea, being involved in inducing this rejection. Such conclusions are entirely

consistent with the clinical study on the involvement of APCs in the rejection of human corneas.⁷⁷

One possible explanation for the different patterns of corneal allografts in these experiments is that the density of dendritic cells is lowest in the central area, but higher in the peripheral area. This unique distribution of dendritic cells in the cornea may also explain why a 2 mm fully allogeneic corneal graft from centre to centre does not elicit sufficient response to induce rejection (>100 days), compared with a 3 mm corneal graft (11 days). The highest distribution in the peripheral area of the recipient bed may explain why a corneal graft from centre to periphery induces chronic rejection. In this situation, the graft can increase the indirect antigen presentation in allograft rejection. In contrast, the highest distribution in the peripheral area of the donor corneal button may explain why a corneal graft from periphery to centre induces acute rejection, because the graft can increase the direct antigen presentation. Thus, a large corneal button carries with it more donor APCs and hence greater risk of acute rejection. A smaller button with fewer APCs has a greater chance of survival. This

means that it is important for successful corneal grafting to use central regions of both donor and recipient corneas in order to minimise its antigenic load. This is a return to the antigen-presenting cell concept, in that 'passenger leukocytes' carried in the donor tissue provide the major immunogenic stimulus for the host.⁷⁸ The immunological significance of these observations have already been demonstrated by Zirm using a small corneal graft (5 mm) in a clinical situation in 1905.⁷⁶ This indicates that it is possible for corneal allografts to alter the antigenicity by treating the tissue to be grafted prior to transplantation.

Immunomodulation of APCs by hypothermic preservation of donor corneas

Donor corneal preservation methods, such as moist chamber, tissue culture medium, organ culture, and cryopreservation, are designed primarily to obtain as many viable cells in the donor cornea as possible. It was reported that no cells bearing HLA-DR antigens were seen in human corneal sections obtained after one week in organ-culture.⁷⁹ The use of the preservation technique to reduce the antigenicity of the donor cornea has been investigated using the moist chamber method.

DA donor eyes were enucleated, placed optic nerve side down on gauze with a little saline. The eyes were kept in a covered glass chamber at 4°C for one week. It is shown in Table VII that fresh (untreated) isografts (DA to DA) survived indefinitely. However, of the eight isografts preserved after the hypothermic preservation, two became opaque and the six other grafts survived indefinitely (>100 days). Fresh (untreated) allografts (DA to AO) were rejected acutely (11 days). However, the survival times of allografts which had undergone the hypothermic preservation (34 days) were prolonged significantly ($p < 0.001$). Histological sections stained by the haematoxylin and eosin method of preserved DA corneas showed a scattered vacuous space in the stroma. Whereas cryostat sections of the preserved DA corneas showed that DA class I MHC antigen was distributed extensively in both central and peripheral corneal area as in the fresh corneas, those of the preserved corneas showed that DA class II positive dendritic

cells were not stained either in the cornea. The removal of the dendritic cells in the preserved cornea was observed histologically.⁴⁹

Immunomodulation of APCs by Ultraviolet-B (UV-B) irradiation

It was reported that rejection of mouse heterotopic corneal grafts was reduced by pretreatment with UV-B irradiation to the epithelial side of the donor eyes.⁸⁰ In this section, another possibility to remove APCs in the cornea using UV-B irradiation prior to transplantation was sought.

Donor irradiation: Enucleated DA donor eyes were divided into two groups. For the epithelial surface irradiation, the whole eye was placed optic nerve side down on moist gauze in a glass chamber. For the endothelial surface irradiation, 3 mm donor corneal buttons were cut out, turned upside down and put back to their original place on the donor corneas. The whole eye was then placed in another glass chamber optic nerve side down. Both chambers (without lids) were exposed to UV-B light sources (6 TL-40W/12 Fluorescent lamps, Philips Light Ltd, Croydon, UK) at a distance of 20 cm from the source for 2.63 minutes (total irradiation: 0.2 J/cm^2). The corneas were grafted into non-irradiated recipient beds of AO rats.

Recipient irradiation: The eyes of the anaesthetised AO recipients to be irradiated were proptosed by traction sutures and the pupils kept open with 1% atropine eye drops. Each animal was carefully put into a box in which a 4–5 mm diameter hole had been made in the upper lid. This hole allowed only one eye to be irradiated (0.2 J/cm^2). DA corneas were grafted into the irradiated recipient beds of AO's.

Corneal buttons irradiated either from the epithelium side or from the endothelium side showed long-term survival after UV-B irradiation treatment (>100 days). In contrast, there was no statistically significant difference between the control group and the group that received UV-B irradiation to the recipient eye. The accepted DA corneas that had been UV-B irradiated prior to grafting showed rare DA class II positive dendritic cells.⁴⁹

It is possible to reduce the severity of allo-

Table VII. *Survival times of orthotopic corneal grafts with/without the hypothermic preservation in the combination of DA to AO*

Donor	Recipient	Preservation (4°C, 1 week)	Corneal graft survival (days)	Median (days)
DA	DA	Fresh	>100 (×10)	>100
DA	DA	Preserved	25, 32, >100 (×6)	>100
DA	AO	Fresh	10 (×3), 11 (×7), 14 (×3), 15, 18	11
DA	AO	Preserved	10, 15, 16, 18 (×2), 32, 40, 57, 61, >100	25

graft rejection by removal of 'APC's' from donor tissue prior to grafting. The APC-depleted graft thus obtains an antigenically privileged status with regard to graft rejection. Data from this study demonstrate clearly the importance of donor APCs in the acute rejection of a corneal graft and suggest that the low density of class II positive dendritic cells observed in the cornea and its uneven distribution are responsible for the unique character of corneal graft survival.

Conclusion

It is clear that clinical corneal transplantation has a long history of success as a surviving allograft. Perhaps because of this it has become one of the most popular transplant operations in clinical practice.⁷ It is, however, one of the most unpopular areas of transplantation immunology today. One of the reasons why it is currently so unpopular with experiments may be that Medawar claimed that the high success of clinical corneal allografts was due to the particularly 'privileged' position of the graft away from the usual cell-mediated immunity associated with the blood and lymphatic vessels.¹⁰ Thus, it was believed by most immunologists that corneal allografts were exempt from the usual histoincompat-

ibility and immunological insults to which other grafts were prone. As a result of this, many ophthalmic surgeons did not consider seriously the problems of corneal graft rejection.

Because interaction between the donor transplantation antigens and the recipient immune system via blood vessels has occurred at least once in corneal allografts even in the avascular recipient bed, corneal allograft rejection represents a major cause of graft failure⁹ and the importance of certain immunological risk factors have become evident in experimental orthotopic corneal grafts in rats.¹⁷

Although the post-operative suture-induced microvascularisation exists in the avascular bed in the rat model, corneal grafts sometimes seem to defy the normal rules of transplantation by survival. The fate of rat orthotopic corneal grafts in 32 fully allogeneic strain combinations is critically dependent on the particular combination of donor and recipient. The existence of different responder status (high, moderate and low) in rats can explain the situations that are faced in clinical corneal transplantation even grafts in an avascular bed. It should be emphasised that, whilst in rejector combinations, corneal grafts

Table VIII. *The influence of Ultraviolet-B (UV-B) irradiation (0.2 J/cm²) to the donor avascular corneal buttons or the recipient avascular corneal beds in orthotopic corneal grafts in the combination of DA to AO*

UV-B irradiation area	Corneal graft survival (days)	Median (days)
Control (no UV-B irradiation)	10 (×3), 11 (×7), 14 (×3), 15, 18	11
Epithelial side of the donor corneas	*(19)>100, (22)>100, >100 (×8)	>100
Endothelial side of the recipient corneas	*(13)>100, (22)>100, >100 (×8)	>100
The anterior segment of the recipient eye	9, 10 (×3), 11 (×5), 14	11

*()>100 = These grafts had minor rejections as they showed a transient rejection episode after operation, the end point of which is shown in parentheses, but subsequently they cleared and remained transparent until after the 100 day period of the experiments.

can undergo acute rejection in avascular beds and obey the basic laws of transplantation, while in some other combinations, corneal grafts can survive indefinitely without any immunosuppressive agents, whilst skin, heart and kidney grafts show acute rejection in the same strain combination. An existence of non-rejector combinations for orthotopic corneal allografts with suture-induced microvascularisation does not only invalidate the concept of an 'immunologically privileged' site for transplantation as proposed by Medawar¹⁰ and supported by Billingham and Boswell,¹¹ but also casts doubt upon the normal principles of graft rejection genetics as proposed by Medawar¹² using rabbit skin grafting and the concept of incomplete afferent and efferent limbs for corneal graft rejection as suggested by Khodadoust and Silverstein¹² using rabbit orthotopic corneal grafting. Furthermore, an analysis of different responder status for corneal grafts does not support the hypothesis that rejection is under the control of MHC-linked Ir genes. This suggests that fully allogeneic corneal grafts do not always obey the role of MHC-linked Ir genes and that multiple non-MHC background genes may play a role in both the survival and the rejection of orthotopic corneal grafts. This shift of immunological importance might be related to the functional imperfection of antigen presentation in corneal allografts.

In order to investigate the importance of histoincompatibility in corneal graft rejection, it was possible to undertake experiments based on a mild rejector combination (DA to PVG) because of the availability of congenic rats. It was demonstrated that corneal grafts with the class II incompatibility alone enjoyed prolonged survival beyond 100 days. In contrast, class I or non-MHC incompatible corneal grafts caused chronic rejection. When class I and class II components were combined, there was a significant allogeneic relation leading to corneal graft rejection. It seems evident that the ability of antigens to induce corneal graft rejection is determined largely by class I MHC products in conjunction with class II MHC products.

In a rejector (high responder) combination DA to AO, experiments were undertaken to show how to avoid acute corneal graft rejection.

As demonstrated by experiments of size of corneal button and the site for corneal grafting, both the relative lack of MHC class II positive dendritic cells and their uneven distribution in the cornea can explain why corneal graft survival is multifactorial. Thus, the corneal graft has the character of a 'naturally' APC-reduced graft. This allows corneal grafts to avoid the worst effects of the allograft reaction with approximately one third of corneal grafts surviving indefinitely in the fully allogeneic strain combinations and possibly avoid the dominant role of MHC linked Ir-genes. Even though there is a relative lack of class II MHC positive dendritic cells in the cornea, the eccentric corneal graft experiment showed that a critical factor influencing corneal graft rejection is the presence and the high density of such dendritic cells in the graft (donor APCs). Thus, removal or inactivation of donor APCs could be effectively used to prevent corneal graft rejection by interrupting the immunological induction of the allograft rejection reaction.

Use of a normal size trephine (3 mm) and pretreatment with hypothermic preservation of UV-B irradiation resulted in the reduction of allograft rejection, because fewer APCs or inactivated APCs in the donor corneal button reduced its antigen-presenting capacity.

Although the importance of APCs seems to be different in either donor or recipient corneas in inducing corneal graft rejection, a relationship between donor's and recipient's APCs is of particular interest because of the possible induction of donor-specific immunosuppression (DSI).⁸¹ However, when the APCs in both donor and recipient corneas are involved in graft rejection, the immune reaction would be very violent.

Finally, the work on corneal transplantation in the rat can be used to improve corneal transplantation in man. As APCs have been shown to play such an important role in the allograft situation, every effort should be made to reduce the numbers of APCs in the donor tissue or to place the graft in such a position as to reduce this effect. This can be achieved by:

- (1) Using as small a corneal disc as is compatible with a good visual result (i.e. a clear central corneal disc without undue astigmatism).

- (2) Trying to prevent the positioning of an eccentric graft and avoiding the limbus in the graft. That is, the donor corneal button from a small eye ball (e.g. newborn or child) should not be used. Also, the recipient bed of a small eye ball should, if possible, be avoided for corneal grafting, because the corneal limbus (where there is a relatively high distribution of APCs) is involved in both instances.
- (3) Trying to reduce corneal pre-vascularisation as far as possible by intensive pretreatment of the patient and possibly by surgical occlusion of large preexisting corneal vessels.
- (4) Using suture techniques which do not induce postoperative vascularisation and above all, by avoiding prominent knots or irregular corneal suturing which will induce a neovascular reaction.
- (5) Using techniques to reduce the APCs of the donor such as organ culture of donor tissue, hypothermic preservation, or possible pretreatment of the graft with UV-B irradiation. However, the donor corneal button should not be over-irradiated because of the possibility of damage to each of the corneal layers. The recipient eye should not be irradiated, because this can induce an inflammatory reaction in the recipient bed.

Using these techniques, it should be unnecessary to use systemic immunosuppression in these patients. Pretreatment of the donor tissue should be considered in all primary corneal allografts. However, once second-set rejection occurred in high-risk situations (for example, corneal re-grafting and a subsequent graft in the contralateral eye), site-specific immunosuppressive regimes become essential.⁸² New approaches aimed at suppressing the rejection such as using administration of anti-T cell MAb into the anterior chamber in addition to the local administration of immunosuppressive agents should be considered.⁸³

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