Corneal Graft Failure
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Introduction to the problems of corneal graft failure

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The clinical importance of corneal grafting lies in the fact that successful transplantation can restore excellent sight to persons suffering from corneal blindness.

The potential scope of corneal grafting is indicated by World Health Organization returns which show that corneal disease throughout the world accounts for more blindness than any other form of eye disease. But this potential cannot at present be realized because the vast bulk of corneal blindness results from trachoma and other external eye disease in people living in areas that lack even the most elementary ophthalmic care and in which it is not possible at present to provide adequate after-care to ensure a reasonable prognosis for clarity of the graft.

There are no accurate figures published on the number of corneal grafts being performed but it is estimated that 2000 are done in the United States and 500 in Britain annually, with an estimate of 1–2 per 100 000 population each year in certain areas.

However, even in the most favoured medical environments it is failure of the graft to maintain clarity, rather than operative difficulty, which limits the applicability of grafting. This limitation is well exemplified by the poor outlook for continuing clarity of grafts placed in grossly damaged and vascularized corneas after severe chemical injury.

Thus we face enormously important challenges to identify and obviate the causes of corneal graft failure, in order to extend the scope of grafting and, it is hoped, to simplify the postoperative regimes to make grafting rewardingly available in underdeveloped areas where the need is greatest.

In the past, the absence of adequate physiological understanding of the corneal functions allowed surgeons to engage in corneal grafting without due appreciation of the most important fact that full-thickness corneal trans-
plantation is organ transplantation, not the insertion of a rather inert piece of transparent tissue. Personal participation in physiological experiments on animal corneas has, above all else, taught surgeons that when transplanting cornea we are transplanting a highly dynamic organ, and that the endothelial pump is exquisitely delicate and liable to damage during storage and transplantation.

It has become so clear that corneal grafts can fail because of poor quality of donor material, or damage to the endothelium during transportation and during transplantation, or because of other errors of surgical technique, that many surgeons have doubted the occurrence of corneal allograft reactions in clinical practice. But surgeons who may doubt the existence and the importance of immunological reactions in jeopardizing graft clarity, especially in cases with a poor prognosis, have only to consider the difference in outlook for a total corneal replacement using autogenous cornea and using homoplastic material. Very large autotransplants generally remain clear whereas very large homotransplants (allografts) generally opacify.

But it is not only surgeons who have overlooked important factors leading to corneal graft failure. It is probably true to say that most immunologists have expected corneal allograft reactions, if they occur, to lead to an all-or-nothing rejection such as occurs with skin grafts. Certainly many immunologists have had the erroneous idea that the cornea is so privileged that allograft reactions do not occur in it and so have thought that corneal transplantation is an immunologically uninteresting business depending only on a complicated exercise of surgical minutiae.

The fact is that the progressive refinements of surgical technique with the introduction of surgical microscopes, continuous monofilament nylon suture and obsessional care of the endothelium during transplantation by maintaining a cushion of air behind it during suturing, have all contributed to improving the prognosis and enabling us to graft less favourable cases, with less and less immunological privilege. So today, although there are still many other problems to be rewardingly tackled, we do see allograft reactions as the ultimate limitation to corneal transplantation.

It is therefore the purpose of this symposium to bring together immunologists, biologists and clinicians to converge on the problems and privileges of corneal transplantation in the light of detailed clinical and physiological knowledge of the cornea and the broader understanding of the immunological and therapeutic aspects of transplantation in general.

It is hoped that this will lead to greater facility in recognizing allograft reactions, to their avoidance and to better treatment when they occur. The group of workers from Baltimore have so beautifully defined what these
INTRODUCTION AND OBJECTIVES

reactions look like in the experimental animal. But their certain recognition is often not so easy in clinical practice because they frequently supervene as a complication of a variety of defects of the graft resulting from poor quality of or damage to donor endothelium, a wide variety of surgical deviations and host disease, or release from immunosuppression, all combining to erode a situation of marginal immunological privilege.

It is thus of great importance that workers of different backgrounds, coming together to consider corneal graft failure, have an appreciation of the complexity of the clinical situation in which there is commonly a concurrence of several factors each of which has contributed to failure of the graft. It is for this reason that good evidence for the existence of one pathway to graft failure in no way detracts from the likelihood of other pathways being relevant, or of the possibility of synergism of multiple pathways leading to failure.

In clinical practice it seems likely that, when grafting into a nearly normal cornea, one can get away with a few defects in the total technique such as the use of moderately sick and mismatched tissue together with a moderately rough surgical procedure, and still have a clear graft most of the time; whereas, if the recipient cornea is more severely diseased, it may demand the most meticulous operative technique as well as a degree of matching of tissue in perfect functional condition and also a more profound umbrella of immunosuppression in order to give a worthwhile prognosis for clarity.

There are also other forms of interdependence in the problems before us. If it emerges that tissue matching significantly improves the prognosis, the general application of this will depend on the availability of a nearly perfect method for the non-damaging short-term storage of donor corneas. Otherwise we shall be at risk, rather expensively, of substituting one pathway to graft failure for another, with little overall improvement. Similarly, if more profound immunosuppression is required for the more severely diseased recipient corneas, we shall have to be assured that the drugs and regimes used do not materially worsen the prognosis for the host disease. This is a particular problem with herpes simplex keratitis in that recrudescence and extension of viral replication may be favoured by the procedures that lessen host response to foreign tissue.

These interdependencies should make for a stimulating and productive symposium for they mean that we each need to pay particular attention to the other man's hobby horse, for that may well play an important part in determining the role of our own, and together they may determine the outcome of our graft.
Clinical patterns of corneal graft failure

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Abstract  Corneal transplants fail or become opaque for several different reasons. The first is the immediate opacification of the graft, occurring from the first day after corneal transplantation to the second or third week. In technically successful transplants this is usually due to faulty endothelium on the donor button. Other obvious early failures are due to technical difficulties in the operative procedure, including apposition of the donor material, flat anterior chambers and trauma of the lens. Infections may result from infected donor material or stitch abscesses during the immediate postoperative period. An interesting phenomenon needing further investigation is the higher percentage of immediate corneal oedema that occurs in technically successful corneal grafts in aphakic eyes.

The incidence of corneal opacification as a result of an immune response varies considerably, depending on the condition of the recipient cornea. Such an immune response may occur as early as 2–3 weeks after transplantation or as late as 15 years after operation. In penetrating corneal grafts the reaction begins with an accumulation of lymphocytic cells on the posterior surface of the corneal button and increases to a circumcorneal injection and a positive aqueous ray. Destruction of the endothelial cells is shown by diffuse stromal corneal oedema. The recognition of this reaction is important clinically for if detected in its earliest phases the destruction of endothelial cells can frequently be suppressed with steroids. Stromal and epithelial tissues may also be injured by the immune response but they are not as critical to the clarity of the graft as are the endothelial cells. Opacification of the transplant from the immune response occurs more frequently when the recipient cornea is vascularized than when it is avascular. A late manifestation of this type of response is the development of a fibroblastic membrane on the posterior surface of the corneal button. This has been referred to as a stromal fibroblastic downgrowth or ingrowth but clinical and experimental evidence suggests that this fibroblastic proliferation is merely a late manifestation of the immune response.

Clinical and experimental evidence suggests that the endothelial cells of the donor button survive for as long as the graft remains clear. There is suggestive clinical evidence that the endothelium on the donor button does not survive as long in eyes that have been grafted for corneal oedema as does the transplanted endothelium in eyes operated upon for keratoconus or corneal dystrophies.
One of the several methods that may be used to classify corneal graft failure or opacification is to divide the cases into groups according to the time that opacification begins. Obviously there will be an overlap into various periods, but roughly they can be arranged into early failures from the first postoperative day to the third week, failures occurring in an intermediate period of three weeks to two years, and late failure from three to fifteen years after grafting.

**EARLY FAILURE**

*Defective endothelium*

The most common cause of early graft failure in penetrating corneal transplants is defective endothelium on the donor material. This may result from a disease of the donor endothelium, trauma at the time of surgery, or autolysis. Some surgeons think that prolonged debilitation of the patient before death contributes to a rapid deterioration of the endothelial cells. Endothelial dystrophies are more common in elderly persons than in younger, and I have the definite clinical impression that donor corneal material obtained from patients 15-50 years of age is more favourable than that obtained from patients of 60 years and older. It is for these reasons that I prefer to use donor corneal material no longer than 24 hours after the death of the patient, and preferably from an individual 15-50 years of age.

The endothelium, as has been shown by Polack (1972a), is extremely sensitive to even minute trauma. Thus, removal of the cornea from the donor eye by means of scissors damages the peripheral endothelium of the donor button. Barrie Jones has shown that the endothelium can be damaged merely by moving it over the surface of the donor iris while cutting the button, or over the recipient iris while suturing the graft in place (Jones & Rice 1969). Certainly allowing the endothelium to dry, or irrigating it with normal saline, or touching it on the drapes will markedly reduce the chances of obtaining a clear corneal graft.

It has been my impression that the endothelium does not fare as well when grafted into an aphakic eye as into an eye with the lens in place. On several occasions I have used a pair of corneal buttons from a single donor, transplanting one to an aphakic eye and the other to an eye with the lens intact, and have obtained a clear graft on the first day in the phakic patient and noted definite stromal oedema in the donor button in the aphakic eye. This is not due to vitreous touch to the donor, for vitrectomies were done in these patients. It is
possible that the replacement of 1 or 2 ml of removed vitreous by balanced salt solution may be sufficient to produce this endothelial damage.

The clinical manifestations of this type of corneal graft failure are quite characteristic. On the first postoperative day there is a moderate striate keratopathy in the donor button with a 'mackerel clouding' opacification of the posterior stroma. On slit-lamp examination the anterior stroma and epithelium appear normal. Over the ensuing weeks those patients with milder endothelial damage may recover and obtain a clear corneal graft of normal thickness. Frequently, however, the stromal oedema progresses and by the end of a week to two weeks, epithelial bedewing may be noted.

In two patients, who developed early corneal opacification, I have removed the donor button within a week after the initial corneal transplant, and on histological examination there has been total loss of endothelium in one case, and apparent damage to the endothelium in the second. In one other patient with this type of corneal opacification, when the button remained for a period of a year before regrafting, the inner surface of the donor cornea was covered by a multilayered fibrous membrane.

Kaufman et al. (1965) have attempted to improve the selection of donor material by examining the endothelium of one of a pair of donor eyes before using the fellow eye for transplantation.

An observation on a patient with an unusual sequence of events suggests that the endothelium of the donor button is much more sensitive at the time of transplantation than it is several months after grafting. The case illustrating this point was a 19-year-old white male who had a corneal transplant because of advanced keratoconus. The donor button was taken from a 50-year-old patient who had died approximately 24 hours previously. During the first few postoperative days there was a very mild striate keratopathy of the donor button, but this cleared completely over a period of three to four weeks, and by four weeks after operation the patient's visual acuity could be corrected to 20/30. His corneal graft was entirely normal thickness. There was no evidence of stromal or epithelial oedema. Two and a half months after transplantation the patient was struck in the eye by a plastic object. All of the 10-0 monofilament interrupted nylon sutures that held the graft in place in the superior part of the wound were broken and when the patient was seen in the accident room six hours after the injury his graft was hinged by the remaining sutures at the six o'clock position. The main portion of the donor button was protruding between the upper and lower lids. Since no fresh corneal material was immediately available, the graft was resutured. During the first postoperative week the transplant appeared about 2 1/2 times normal thickness and was almost completely opaque. However, during the following month, the corneal oedema
disappeared and the patient’s vision returned to 20/50. Similar trauma to the endothelium of the donor material at the time of corneal grafting would have certainly led to a permanent opacification of the transplant.

Early endothelial failure has been discussed in some detail because in non-vascularized corneas, particularly in aphakic eyes, this is one of the most common causes of graft opacification.

Defective wound closure

In the past, defective wound closure was a major problem in corneal grafting and frequently resulted in high astigmatism. In other instances, the iris either prolapsed or became adherent to the posterior surface of the cornea. This brought blood vessels into the area of the graft, increasing the chance of an allograft reaction. It also produced peripheral angle closure from iris adhesions and increased the incidence of postoperative glaucoma. This complication is now an extreme rarity owing to the more accurate cutting of the corneal buttons with disposable trephines, exact tailoring of the wound under the operating microscope, and the use of 10-0 monofilament suture material for exact apposition of the wound edges. The improved surgical technique makes flat anterior chambers a rarity and wound dehiscence an extremely rare event. The suture material produces so little reaction that corneal wound healing occurs very slowly and the sutures must be left in place for at least six months to avoid disruption of the wound when they are removed. If either a running suture is pulled to a proper tightness or each knot of an interrupted suture is buried in the corneal stroma, the epithelium will cover the surface of the sutures so that it does not stain with fluorescein in the postoperative period. Because of this the patients are entirely comfortable after the first three to four postoperative days.

Infection

Although the corneal buttons are seldom sterile at the time of transplantation, panophthalmitis is an extremely rare complication. If the fine monofilament suture material either is not properly buried or for some reason erodes through the corneal epithelium, it is a potential source of a stitch abscess.

Cystoid maculopathy

If opacification of the corneal graft is considered as the only cause of graft failure, cystoid maculopathy should not enter this discussion. However, from
the patient's point of view, if he has a clear graft and cannot see, he considers the operative procedure a failure. Cystoid maculopathy is a definite clinical entity (Maumenee & Emery 1972; Gass 1970). It occurs most frequently after cataract extraction but is also a definite complication of corneal grafting, particularly in aphakic eyes. The finding of cystoid changes on slit-lamp examination of the macular area and the typical fluorescein leakage pattern is diagnostic for this condition. The exact pathogenesis of the macular change is not known. Fortunately, in many patients this lesion heals spontaneously, but in other instances the loss of central vision may be permanent.

**Glaucoma**

As already mentioned, flat anterior chambers and secondary glaucoma were not infrequent causes of graft failure before the use of modern surgical techniques. Such complications are extremely rare today. On the other hand, because of our success rate in operating on patients with oedematous corneas, particularly in those patients where corneal oedema has occurred secondarily to cataract extraction, glaucoma is a definite complication which must be dealt with. Most frequently the elevated intraocular pressure is present before the time of corneal grafting. In some instances, however, Kaufman feels that elevated intraocular pressure immediately after corneal transplantation is the result of the operative procedure (Kaufman et al. 1970). Irrespective of its cause, elevated intraocular pressure causes further embarrassment to the donor endothelium and may produce early opacification of the transplant unless the intraocular pressure is reduced.

**Cataract**

Patients with endothelial dystrophy and corneal oedema who require a corneal transplant frequently have some degree of lenticular opacity before the time of grafting. The mechanical trauma to the eye which occurs during the grafting procedure frequently causes the lenticular opacity to progress rather rapidly after transplantation. Because of this, some authors have advocated the use of a combined cataract extraction and corneal transplant at the same procedure. I have obtained better results by performing a corneal transplant and waiting an interval of a year before removing the cataract (Stark & Maumenee 1973).
INTERMEDIATE FAILURE

Allograft reaction

The primary cause of opacification of a clear corneal graft three weeks to five years after operation is an allograft reaction. This response may occur as early as six to eight days after the operation, but such cases are frequently patients who have had previous corneal grafts where the recipient cornea is heavily vascularized and opaque. These early graft failures are usually not recorded as definite allograft responses because it is difficult to be certain that the clouding is not due to faulty donor material or operative trauma. If the graft remains clear for three weeks after operation, however, it is unlikely that surgical trauma or the condition of the donor material will be the cause of failure.

At the opposite end of the time interval, I have seen two allograft reactions after ten years and know of another that occurred 15 years after operation. Since the endothelial cells may survive for the life of the patient, and since these cells do not take on the antigenic nature of the host, the allergic response may occur at even longer periods after transplantation.

Many excellent studies of the allograft reaction have been made in experimental animals by Polack (1972b) and by Khodadoust & Silverstein (1969a). These authors have carefully recorded the clinical and histopathological course of events which occur in the opacification of a graft. Khodadoust & Silverstein's experiments have been particularly helpful for they have shown that the corneal epithelium, stroma and endothelium may be rejected separately or in concert. Careful study of clinical material has shown that the responses in man may be quite similar to those found in experimental animals.

Corneal vascularization is extremely important in the pathogenesis of the allograft reaction. Grafts placed in avascular corneas seldom show this response unless the recipient cornea becomes vascularized during the postoperative period. On occasion, however, a typical reaction may occur in a completely unvascularized recipient cornea. On the other hand, in those patients with dense stromal vascularization, particularly after chemical burns, it is almost impossible to avoid an immune response.

The endothelium in a penetrating corneal transplant is by far the most important part of the donor material. A typical allograft reaction begins with a slight circumcorneal injection, a mild aqueous flare containing cells, and a few dust-like deposits or irregularities on the endothelial surface. These endothelial changes may be located near a tuft of vessels that have grown into the recipient cornea. If the response is not halted by steroid therapy within a
few days, or a shorter period of time, a rejection line will develop on the endothelial surface. This has been shown by Khodadoust & Silverstein (1969a) in experimental animals to represent a line of lymphocytes which are destroying endothelial cells. As the line advances from the edge of the donor tissue the stroma overlying the area of destroyed endothelium becomes oedematous. Blood vessels may then grow into the donor cornea. The entire graft may become oedematous and opaque. On rare occasions the response may be so violent that sloughing of the corneal stroma may occur. Eventually the inner surface of the donor material will become covered with a retrocorneal fibrous membrane.

In other instances, the response may be more violent in onset and the entire donor endothelium will develop a roughened appearance. A typical rejection line may never be observed in these cases. In some eyes with an even milder reaction this may be the course of events.

It is interesting to study carefully eyes in which an allograft reaction occurs where the recipient cornea is relatively clear. It is noted in these cases that the endothelial changes and deposits occur only in the donor cornea. I have previously reported an example of this localized response in a man who had had a 7 mm penetrating graft for a corneal opacity following an infection with herpes simplex (Maumenee 1962). The transplant was successful and was completely clear. The visual acuity was corrected to 20/20 postoperatively. However, there was a second Descemet's membrane behind the transplant, which in effect formed two anterior chambers, one behind the graft and the other behind the membrane and the rest of the cornea. Over a period of approximately nine months, blood vessels which had been present at the margin of the transplant grew onto the surface of this membrane. The patient then developed an allograft reaction; keratic deposits were present on the posterior surface of the graft, and the aqueous humour in the pocket between the membrane and the graft showed a positive ray with cells. No keratic deposits could be seen on the posterior surface of the recipient cornea and the aqueous humour in the anterior chamber appeared clear. The next month the inflammatory reaction subsided, the graft became clear, and the visual acuity returned to 20/20. The pocket behind the transplant collapsed. The patient has now been followed for approximately 15 years and there have been no recurrences of the clouding of the graft.

Some observers have suggested that the retrocorneal membranes found histologically on corneal grafts that had failed many months previously were due to an overgrowth of stromal keratocytes from the wound edges (Rycroft 1963). I have been of the opinion, however, that this fibrous membrane was merely a manifestation of the allograft reaction (Maumenee 1965). Recently
we have been able to produce such membranes in animal eyes with intact Descemet's membranes by repeatedly freezing the central portion of the cornea (Michels et al. 1972). There is good circumstantial evidence that such membranes arose from a metaplasia of the endothelial cells. The possibility that inflammatory monocytes may have contributed to the membrane could not be ruled out. Other tangential information which supports the concept that stromal fibroblasts do not produce the retrocorneal membrane are: the membrane is almost always confined to the donor button; it does not grow out of trephine holes made for the control of glaucoma; it did not develop from wounds that were markedly misaligned when overlying sutures were used; and, finally, it is unreasonable to expect stromal fibroblasts suddenly to cover the posterior surface of the donor button when they have been dormant for as long as three or four years.

Steroid therapy is frequently quite effective in reversing endothelial damage if it is used early in the course of the allograft reaction. However, if a large area of endothelium has been destroyed the graft may remain oedematous even though the active process has been halted. Certainly steroids are of no value once a retrocorneal membrane has developed.

**Stroma**

An inflammatory response may occur as a result of an immune reaction to the stromal tissue (Maumenee 1962). Khodadoust has shown that this reaction may appear as a greyish line of inflammatory cell infiltrate in the mid-stromal tissue (Khodadoust & Silverstein 1969a). This has a very similar appearance to the Wesseley reaction in corneal tissue (Germuth et al. 1962). Destruction of the stromal cells, however, may not produce a permanent opacification of the graft, for it has been shown that large areas of corneal stromal cells may be destroyed and the cornea return to its normal transparency (Maumenee & Kornblueth 1949).

**Epithelial rejection**

Epithelial rejection may cause only a mild irritation of the eye with a slight opacification of the anterior surface of the graft. Epithelial defects of the cornea are known to regenerate quite rapidly. Thus, the destroyed epithelium may be replaced from the recipient without causing appreciable damage to the donor button. Khodadoust & Silverstein (1969b) were the first to call attention
to this aspect of the corneal allograft response. In some instances, however, epithelial rejection appears to be much more important, for in these cases the recipient epithelium fails to cover the graft, or if the epithelium regenerates, it does not adhere firmly to the donor cornea and thus becomes repeatedly denuded. In rejections of this type, soft corneal contact lenses are of palliative value.

The rejection phenomenon in each of the layers has been described separately but they may occur in unison. The multiple layer response is probably more frequent than the single layer response, particularly in the end stages of the rejection phenomenon.

Lamellar corneal grafts

The allograft reaction has been thought not to occur in lamellar corneal transplants (Kornblueth & Nelken 1958). However, I have noted that a moderate percentage of lamellar corneal grafts become cloudy because of lipid deposits at the donor-recipient interface and in the donor tissue (Maumenee 1962). There is no experimental evidence in animals to confirm the assumption that this is a result of an allograft response, nor has it been possible to prevent the development of these lipid deposits with steroid therapy. However, they do not occur in auto-lamellar grafts or in avascular corneas. Since Khodadoust & Silverstein (1969a) confirmed the observation that stromal tissue may respond to an allograft reaction, it does seem possible that the lipid deposits may be a manifestation of chronic irritation of the blood vessels in the cornea by the immune response.

Recently it has been observed that the epithelium becomes persistently denuded over lamellar corneal grafts. Again, this is related to vascularization of the recipient cornea and is probably a rejection phenomenon.

Other causes for intermediate failure

Other causes of failure of the graft during the intermediate period are relatively rare. These failures occur from traumatic dehiscence of the wound and vitreous touch to the endothelium in penetrating grafts in aphakic eyes. Infection may also occur in the area of the sutures, if one of the sutures becomes exposed or if the patient develops a severe conjunctivitis. Fortunately this complication is extremely rare, but it has assumed more significance with the use of the monofilament fine sutures which are being left in the cornea for periods of six months or longer.
Opacification of a transplant after many years of a successful graft may occur as a result of malfunction of the donor endothelium, or from an invasion of the donor cornea by disease of the host. Penetrating keratoplasties for keratoconus have been known to remain clear for 15–25 years. On the other hand, Stocker reports that the donor endothelium grafted into eyes with endothelial dystrophy survives for only 10–15 years (Stocker & Irish 1969). I have observed several patients, operated upon because of oedematous corneas, whose grafts were completely clear for periods of four to five years, after which the transplants became totally oedematous. These eyes did not show the inflammatory response that occurs in patients with the allograft rejection, but there was a gradual deterioration similar to that seen in Fuchs' endothelial dystrophy. I have had one patient who maintained a crystal-clear graft for almost 25 years, and whose endothelium gradually failed. In the latter case, as in several other transplants that became oedematous at an earlier period, histological examination of the corneal button at the time of regrafting revealed a defective endothelium.

The most frequent condition in which the disease of the recipient appears to invade the donor cornea is stromal herpetic keratitis. This breakdown of the donor tissue usually occurs within the first year or two years after transplantation. It begins in the region adjacent to the residual recipient stromal disease and gradually progresses to an ulceration of the donor cornea. The reason for the involvement of the donor cornea in this process is not clearly understood at present.

I have not seen clear-cut examples of major corneal dystrophies, such as the macular, granular or lattice dystrophies, involve the donor corneal material.

In summary, corneal grafts may fail from a great variety of causes. In spite of an appreciable overlap, it has been suggested that the causes of failure may be classified according to the time at which the graft becomes opaque. The most common cause of failure in the early period, from the time of grafting to three weeks, is faulty donor endothelium. The most common cause of failure in the intermediate period, three weeks to five years, is the allograft reaction. In the late stage, endothelial deterioration accounts for the majority of the opaque grafts.

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Discussion

Billingham: When changes are observed that you suspect to be of immunological aetiology, is there any evidence that mononuclear cells gather in the aqueous humour?

Maumenee: We have no definite clinical evidence of this. We see an aqueous flare, with particulate matter in the aqueous which we strongly suspect to consist of inflammatory cells.

Billingham: In seeking to explain the origin of the secondary retrocorneal
membrane one should not neglect the work of M. Allgöwer (see Russell & Billingham 1962) and others which suggests that a significant contribution to the fibroblast population in a healing wound is made by cells of haematological origin—probably monocytes which undergo a transformation. Consistent with this premise is the finding that leucocytes from peripheral blood are capable of producing connective tissue networks when cultured in vitro for a few weeks (see Allgöwer & Hullinger 1960). The formation of a retrocorneal membrane might be attributable to the activity of cells of vascular origin.

Maumenee: I cannot rule this possibility out. One piece of evidence that these cells may be of endothelial origin is that we see typical retrocorneal membranes in other conditions, such as vitreous adhesion at the back of the cornea. Secondly, if the cornea is frozen and thawed and examined in flat preparation, as has been done by Dr Ali Khodadoust and also Dr J. A. Capella (1972), the endothelial cells which migrate to close the defect look like fibroblasts. Corneal endothelial cells are in fact mesothelial cells, not true endothelial cells; so it is reasonable that they could undergo a certain degree of metaplasia.

Polack: Why then should inflammation be necessary for the formation of the retrocorneal membrane?

Maumenee: Inflammation is not essential. Repeated freezing of the cornea is also enough to produce the retrocorneal membrane (Michels et al. 1972).

Brent: Dr Maumenee, in patients suspected of having rejected corneal allografts, has the immunological status towards donor antigens been investigated? Now that one can freeze lymphoid tissues from the donor this might be a useful tool in studying the reactivity of the recipient's blood leucocytes to donor antigens. One might also look for the appearance in the recipients of allo-antibodies to HL-A antigens of the donor.

Maumenee: Dr Walter Stark of the Wilmer Institute (now at the National Institutes of Health) is typing patients before corneal grafting and studying their sera to see whether they show such antibody responses.

Brent: In those cases in which presumed graft rejection is reversed by steroids, for how long does the treatment have to be continued in order to ensure the final success of the graft? Is a relatively short course of treatment sufficient or does steroid therapy have to be maintained more or less for the duration?

Maumenee: Treatment depends on the stage at which the rejection response is seen and to some extent on the time that has elapsed since transplantation. A reaction occurring several years after grafting is much less severe than one occurring in a heavily vascularized cornea two or three weeks after grafting. If we observe a slight or medium reaction within a few days of the onset of an aqueous flare and give intensive topical steroids (1% dexamethasone five or six
times a day, together with 50 or 60 mg prednisone daily) the reaction is controlled within three or four days, depending on the extent of corneal oedema. It takes that length of time for the endothelium to repair. I usually keep patients on this therapy for two to three weeks and then taper it off. After this they may never, as far as I know, have another immune response. Other patients have had up to four or five reactions, or cloudings of the graft, which respond each time to treatment with steroids if the endothelium has not been too much destroyed.

It appears that as long as the eye is not inflamed the allograft reaction does not develop. Some patients have had a reaction after an attack of influenza or conjunctivitis or after a foreign body entering the cornea. An inflammatory reaction in the eye can therefore trigger, or at least precipitate, this type of rejection.

Brent: How do you explain the fact that relatively short courses of locally administered steroids have this dramatic effect, when presumably the reaction is a consequence of immunological events that have occurred elsewhere? The locally applied steroids may subdue the lymphocytes that happen to have made their way into the cornea, but you would surely expect the reaction to recur unless you disconnect the cornea, at the same time, from the whole immune system?

Maumenee: One patient had an endothelial failure 25 years after corneal grafting. I did a second graft and he recovered normal visual acuity for two or three months, after which a typical immune response occurred that was, for various reasons, not treated immediately. When I saw him the corneal blood vessels were markedly increased. The patient was put on intensive steroid therapy; the vessels practically disappeared from the cornea, which cleared. This is true of the corneal vascularization: the vessels can dilate or constrict. This patient has since had one more attack and again after steroid therapy his corneal graft is clear. Thus, a graft which has caused a response, and reacted to it, may again become ‘isolated’ from the host. The cornea is a partially privileged site and a partially privileged tissue; it can initiate and respond to immunological reactions but in such a weak way that unless an inflammatory response is present to release the antigen, the cornea stays relatively isolated. Dr Khodadoust has investigated this. When silk suture material was left in the cornea at the edge of the graft longer than usual, to cause irritation, the percentage of allograft responses was increased in proportion to the length of time the suture material remained (Khodadoust & Silverstein 1969a, b).

Brent: This question of the privileged nature of the site is perhaps a semantic problem. One has always regarded the anterior surface of the eye as a privileged site because, in its normal state, it is avascular; vascularization of the cornea is,
then, a secondary feature which runs counter to this particular definition. A site that supports 90% of corneal allografts, as appears to be the case with avascular corneas, could reasonably be regarded as privileged.

Silverstein: It is not a semantic question: the cornea is a privileged site. Your point about disconnecting the cornea from the body is probably correct, because the privilege is both afferent and efferent. If you 'disconnect' in even the sensitized host (which steroids do eminently well in the cornea) you can obviate much of the problem.

Maumenee: Vascularization is not essential for the allograft reaction to occur, however.

Fine: You said that the allograft reaction occurs in proportion to the degree of vascularization, but is there good evidence for this? Do you not see transplants into corneas with one vessel that have very severe reactions and others into tissue with many vessels that do perfectly well? Experimentally you have shown that only one small blood vessel coming into the line of union can start an allograft reaction.

Maumenee: I have not made a statistical analysis of cases, but this is my clinical impression.

Dohlman: The results of Owens et al. (1948) provide some statistical data; in their series vascularization worsened the prognosis. This is my own impression too. Even if one excludes chemical burns, dry eyes and other situations with long-standing epithelial involvement and considers only cases with stromal vascularization for other reasons, prognosis is still worse than after grafting into an avascular cornea.

Lachmann: Is the relationship between the development of corneal lymphatic vessels and the allograft reaction any closer than that between vascularization and rejection?

Maumenee: We don't know, because we have no way of demonstrating corneal lymphatic vessels in the patient.

Silverstein: There are techniques but they have not been well applied. A student of mine is studying this now. A paper by Collins (1966) suggests that when neo-vascularization occurs in the rabbit cornea there is a concomitant ingrowth of lymphatics, but this was not a clean experimental study and the question needs reinvestigating.

Polack: Faure et al. (1970) have shown that the lymphatics follow vascularization in intralamellar transplants that are rejecting. I have also seen this in rejecting allografts (Fig. 1).

Dohlman: Professor Brent asked how any graft can survive if it is in contact with blood vessels. Isn't the answer that the endothelium is disconnected from the vessels because of the re-formation of an intact Descemet's membrane,
FIG. 1 (Polack). Lymphatic channel found in a vascularized (rejected) corneal graft in a rabbit. Silver nitrate. × 280.

which should not allow lymphocytes to penetrate? In late immune reactions any lymphocytes attaching to the endothelium must derive from the uvea via the aqueous, musn’t they?

Maumenee: One can have epithelial or stromal rejections, however.

Brent: Could Dr Silverstein confirm my impression that corneal transplants in presensitized rabbits are destroyed?

Silverstein: No. Under certain conditions there is both afferent and efferent privilege, as I shall describe (pp. 105-120).

Maumenee: We showed in rabbits (Kornblueth et al. 1949) that if a skin graft was placed on a recipient before the corneal transplant, no clear grafts were obtained. If skin was grafted two weeks after corneal grafting there was a high percentage of rejection, about 80%. If skin was transplanted three months after corneal grafting very few corneal grafts were rejected although the animals were sensitized to the donor. The point is that rejection requires both arms of the immune response—first, the sensitized animal and, second, effector cells reaching the graft. Even with host vessels adjacent to the area of wound healing of the graft, unless there is an irritation to initiate an inflammatory response, there is usually not enough stimulus to cause rejection.

Brent: Steroids have two actions which are of course closely connected, an anti-inflammatory and an immunosuppressive action. It is reasonable to argue that the immunosuppressive action is essentially an anti-inflammatory one, but alternatively it could be that locally applied steroids are acting in a more
DISCUSSION

general anti-inflammatory sense without necessarily being immunosuppressive.

Rice: Dr Maumenee mentioned the recurrence of what one feels clinically is an allograft reaction. We feel too that there is a close analogy between the studies in rabbits by Dr Khodadoust and Dr Silverstein (1969a, b) and what is seen clinically. However, our impression is that many patients respond quickly to an increase or re-introduction of steroid therapy but once the patient has been weaned off the steroids and has retained a clear graft it is unusual for that sequence to recur. Many patients, particularly certain types who are at risk, go through the sequence, but only once.

Jones: Our general management is to treat patients with intensive topical steroids and not always with systematic steroids. The response has been similar to that outlined by Dr Maumenee, although frequently we have had to keep patients on large doses of steroid for three, six or even nine months, because the graft has looked as though it would not recover in the first month or two and has only cleared on continuing therapy.

Maumenee: I have seen an allograft reaction occur for the first time 10 or 15 years after grafting, so it appears that—at least in the cornea—the identity of the donor material persists for as long as 15 years. This is a discouraging prospect for kidney and heart transplants, for patients will need to be immunosuppressed for that length of time.

Billingham: When this delayed reactivity occurs, is it always associated with the presence of blood vessels close to the graft or do you see it when the host area is devoid of vessels?

Maumenee: I have seen a reaction when the whole host area is clear of vessels. Two other points are relevant. A graft may be surrounded by clear host cornea, perhaps with one or two small blood vessels. A response occurs, and keratic precipitates are found on the back of the graft but not on the adjacent host tissue. Secondly, in the patient in whom two Descemet's membranes remained (see p. 11), a positive aqueous ray developed and cells were found in the pocket between the two membranes. Keratic precipitates appeared on the donor cornea only. The adjacent recipient cornea was completely free of cells and the aqueous in the normal chamber was also clear. This demonstrates how specific this reaction is to the donor tissue.

Silverstein: How does this phenomenon affect your argument and that of Dr Polack on the uveal origin of those inflammatory cells?

Maumenee: I think the cells can come from blood vessels, as they did in the vascularized recipient cornea in this case, or from the uveal tract. They do not come from any one place.

Jones: We initially thought that in certain cases the reaction was mediated by blood vessels in the cornea and in others by cells from vessels in the uveal tract.