

The Effect of Blood Transfusions on Rat Corneal Graft Survival

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To determine whether a preoperative blood transfusion could influence corneal graft rejection, full-thickness 3 mm orthotopic corneal grafts were performed in Dark Agouti (DA) rats from syngeneic (DA) or allogeneic (Brown Norway and Lewis) inbred donors and in Lewis rats from syngeneic (Lewis) or allogeneic (DA) donors. Rats with syngeneic grafts showed no signs of rejection. The corneal buttons remained clear indefinitely, although vessels grew up to the wound. All allogeneic grafts in nontransfused rats rejected between 6 and 25 d (the interval depended on the strain combination). The median survival of BN to DA grafts was 9 d, and the median survival of Lewis to DA grafts was 7 d. Therefore, both can be described as high responder strain combinations. The DA to Lewis grafts survived longer, with a median survival of 15 d. This was described as a low responder strain combination. A single preoperative transfusion of blood from the same strain as the corneal graft donor prolonged the survival of corneal allografts in the two high responder combinations, an effect called active enhancement. However, a blood transfusion did not enhance the survival of allografts in the Lewis rat. Corneal grafts from animals of a third party strain were not enhanced but were rejected, following the same time course as nontransfused controls. Thus, preoperative donor-specific blood transfusion specifically delayed the immune response to corneal allografts in some strain combinations but not in others. This may limit the clinical application of this technique. *Invest Ophthalmol Vis Sci* 33:1974-1978, 1992

End stage corneal disease is a major cause of blindness.¹ The only current treatments are keratoprosthesis² or keratoplasty. Corneal grafts remain clear in 80-90% of patients operated on for dystrophic conditions,³ but the risk of failure increases substantially if there has been previous inflammation or vascularization.⁴ The most common cause of failure is rejection,⁵ which occurs when the recipient recognizes foreign glycoprotein determinants on the graft and mounts an immunologically mediated response.⁶

There are several potential ways of overcoming the problem of graft rejection. Reducing the disparity between donor and recipient by matching the major histocompatibility antigens can significantly decrease the rejection rate, particularly in high risk cases.⁷⁻⁹ However, there inevitably will be some difficulty obtaining well-matched donor material even in centers with eye banks. Considering the class I gene complex alone, the polymorphism is so great that there is less than a 1:40,000 chance of a perfect match between

random individuals. Partial matching may not be adequate to prevent rejection, and experimental studies have shown that even minor transplantation antigen disparity is enough to provoke corneal graft rejection in a rat model.¹⁰

An alternative approach is to suppress the immune response. This can be achieved nonspecifically by using steroids. Systemic steroids may cause several important side effects, and their use for preventing corneal graft rejection is limited. Topical steroids are widely used and are effective. Despite their use, however, many grafts in inflamed corneas fail.¹¹ The possibility of specifically suppressing the immune system so it fails to respond to the graft transplantation antigens but reacts normally against all other antigens is an exciting, novel approach that has been used in other experimental organ grafts with success.¹²

Induced immunologic unresponsiveness to foreign cells was recognized over 30 years ago by Kaliss,¹³ who showed that animals treated with antibodies against donor tumor cells displayed enhanced growth of the transplanted tumors. The effect was donor specific for the tumor and was called "passive enhancement." Treatment of rat kidney graft recipients with anti-donor antibody was shown to lead to the prolonged survival of normal tissue grafts. This prolonged survival of the transplanted organ also was termed "passive enhancement."¹⁴

Pretreatment of a recipient with donor antigens

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also can induce allograft acceptance if the recipient is allowed time to mount an immune response prior to grafting. This effect is called active enhancement. Thus, a donor-specific blood transfusion will prolong renal allograft survival indefinitely in a number of rat strain combinations.¹⁵ The possibility that allografts of cornea also could be actively enhanced was investigated in the present study. Recipients of corneal allografts were transfused prior to transplantation with blood from the corneal donor strain or an unrelated strain, and the survival of the corneal grafts was assessed.

Material and Methods

Animals

Inbred male DA (RT1^a), Lewis (RT1^l) and BN (RT1ⁿ) rats aged 8–12 weeks were obtained from Manchester University Medical School Animal Unit. All the experiments in this study conformed to the ARVO Resolution on the Use of Animals in Research.

Blood Transfusion

Blood was obtained from anesthetized Lewis or BN donors by terminal cardiac puncture with a heparinized syringe. Each recipient rat was transfused intravenously through the tail vein with 1 ml of whole blood within 10 min of collection.

Corneal Transplantation

Donor rats were killed with ether anesthesia, and a full-thickness 3 mm button was trephined. Recipient animals had their right pupils dilated with atropine 1% and phenylephrine 10%. They were anesthetized in ether, and topical benoxinate drops were instilled before a 3mm central full-thickness corneal button was removed. The donor graft was secured with a continuous monofilament 10/0 nylon suture. Six to eight

bites were placed at a depth of 3/4 of the corneal thickness, and a small air bubble was introduced at the end of the procedure to reform the anterior chamber before a single drop of chloramphenicol was instilled.

Experimental Design

DA rats were allocated randomly to seven experimental groups (Table 1) and received syngeneic (DA) or allogeneic (BN or Lewis) 3 mm orthotopic penetrating corneal grafts. Seven days before grafting, some groups were transfused with 1 ml of whole blood from BN or Lewis donors (Table 1).

Three groups of Lewis rats also were grafted, with syngeneic Lewis or allogeneic (DA) corneae, and one group was transfused with 1 ml of whole blood from a DA donor a week prior to grafting. All grafts were performed by one ophthalmic surgeon.

Grafted eyes were examined on alternate days in a masked fashion by slit-lamp examination until rejection occurred or for at least 90 days postgrafting. The grafts were scored using a system based on a previously described method.¹⁶ Clarity, vascularization, and edema were assessed (Table 2). The maximum score achieved by syngeneic grafts was 3. Rejection of the allogeneic grafts was recorded as the day when the combined score reached 6 or more. This provides a clear distinction between rejected and nonrejected grafts.

The delays from time of transplant to the time of rejection were compared using the Mann-Whitney test.

Results

One hundred and sixty two penetrating grafts were performed. Seven were excluded from the study within five d because of infection, and 20 were technical failures as a result of wound dehiscence, iris prolapse, or severe cataract. The remaining 135 animals were analyzed as follows.

Table 1. The allocation of 135 recipients to 10 experimental groups

<i>Experimental group</i>	<i>Recipient strain</i>	<i>Blood donor strain</i>	<i>Corneal donor strain</i>
Nontransfused animals			
Syngeneic graft	DA	None	DA n = 8
Allogeneic graft	DA	None	Lewis n = 30
Allogeneic graft	DA	None	BN n = 14
Syngeneic graft	Lewis	None	Lewis n = 6
Allogeneic graft	Lewis	None	DA n = 14
Donor-specific transfusion			
Allogeneic graft	DA	Lewis	Lewis n = 17
Allogeneic graft	DA	BN	BN n = 11
Allogeneic graft	Lewis	DA	DA n = 14
Third-party transfusion			
Allogeneic graft	DA	BN	Lewis n = 14
Allogeneic graft	DA	Lewis	BN n = 7

Table 2. The grading of the grafted eyes according to clarity, vascularization, or edema

Score*	Clarity	Vascularization	Edema
1.	Faint haze	Onto recipient cornea	Faint epithelial
2.	Moderate: pupil detail observed	Up to wound	Established epithelial
3.	Severe: pupil just visible	Onto graft button	Epithelial with stromal thickening
4.	No detail of iris	Complete cover of button	Severe

* When combined score totaled 6 or more the graft was recorded as rejected.

Nontransfused Animals

- Syngeneic DA to DA corneal grafts remained clear indefinitely with no sign of rejection, although blood vessels grew up to the wound edge.
- Allogeneic Lewis to DA grafts showed a transient edema that cleared in all cases by day 3. Between 6 and 17 d, all the grafts became hazy (ie, grade 2–4), vessels grew to the wound and subsequently encroached onto the graft (ie, grade 2–4), and moderate to severe edema ensued (grade 2–4). By 7 d, 50% of the grafts had rejected (Fig. 1).
- Allogeneic BN to DA grafts behaved in a manner similar to that of allogeneic Lewis grafts. They rejected between 6 and 14 d, with 50% rejection occurring by day 9 (Fig. 2).
- Syngeneic Lewis to Lewis grafts remained clear in all cases despite vessels that encroached the wound edge.
- Allogeneic DA to Lewis grafts rejected in a slower tempo, with all grafts rejecting between 10 and 25 d, with a median rejection time of 15 d.

Donor-Specific Transfusion

- DA rats given a Lewis blood transfusion followed by a Lewis corneal graft rejected in significantly

delayed tempo ($P < 0.01$, Mann-Whitney test), and two did not reject after 100 d (Fig. 1a).

- DA rats given a BN blood transfusion followed by a BN corneal graft also showed delayed rejection ($P = 0.05$, Mann-Whitney test) compared to nontransfused controls (Fig. 2a). Although rejection was delayed, there were no long-term surviving grafts in this group.
- Allogeneic DA grafts in DA transfused Lewis recipients rejected in all cases between 11 and 20 d in a tempo similar to that of nontransfused animals. This was not significantly different from the nontransfused controls ($P > 0.1$). There were no long-term survivors, and the median rejection was 14 d.

Third Party Transfusion

- Lewis grafts in DA rats that had a prior BN blood transfusion all rejected by day 15, with no statistically significant delay in rejection ($P > 0.1$; Fig. 1b).
- BN grafts in DA recipient rats with a Lewis blood transfusion likewise showed no difference ($P > 0.1$) in the incidence or tempo of rejection from the nontransfused controls (Fig. 2b).

Because there was no enhancement with donor-

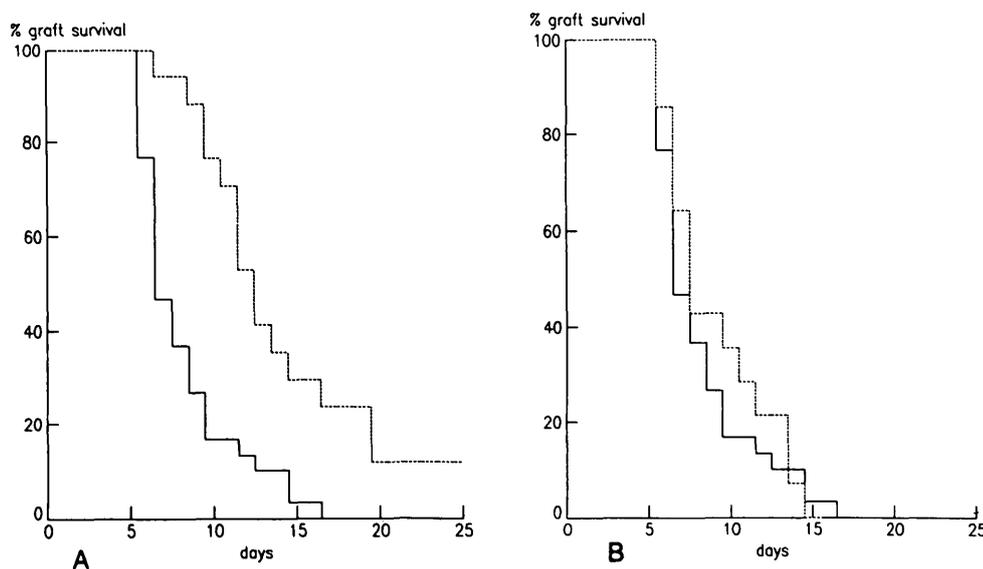
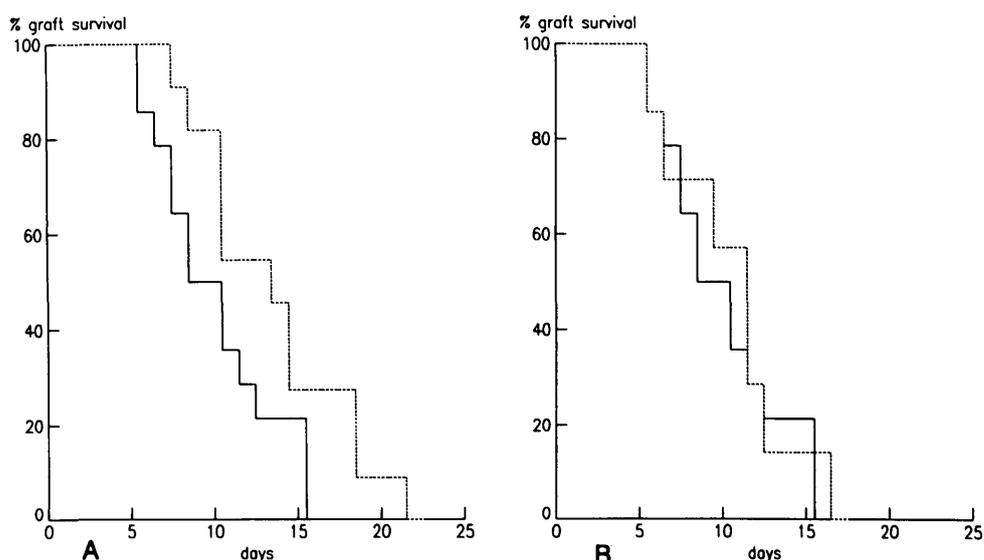


Fig. 1. Survival of Lewis corneal transplants in DA recipients. (A) Comparison of graft survival in untreated ($n = 30$, solid line) and donor specifically (Lewis) transfused ($n = 17$, dotted line) recipients. (B) Comparison of graft survival in untreated ($n = 30$, solid line) and third-party (BN) transfused ($n = 14$, dotted line) recipients.

Fig. 2. Survival of BN corneal transplants in DA recipients. **(A)** Comparison of graft survival in untreated ($n = 14$, solid line) and donor specifically (BN) transfused ($n = 11$, dotted line) recipients. **(B)** Comparison of graft survival in untreated ($n = 14$, solid line) and third-party (Lewis) transfused ($n = 7$, dotted line) recipients.



specific blood transfusion in the Lewis rat, third party transfusions were not performed.

The different numbers in each group reflect the use of some animals for *in vitro* studies after rejection had occurred. The data on rejection times was included for analysis and allows a more accurate assessment of graft survival.

Discussion

Corneal grafts from Lewis or BN donors to untreated DA recipients were rejected after 6–17 d in all cases (Figs. 1a, 2a). Because the response was rapid and complete, these strain combinations are “high responders.” In contrast, Lewis rats rejected DA corneal allografts less quickly, between 10–25 d, and are called low responders. Graft failure seems to be an immunologically mediated process because grafts within the same strain—which bear identical transplantation antigens—syngeneic grafts—do not reject in the DA or Lewis rats.

Sensitization to transplantation antigens, which sometimes occurs with a blood transfusion,¹⁵ might be expected to lead to accelerated graft rejection. However, under certain circumstances, pretreatment of a recipient with donor antigens can lead to enhanced graft survival because specific immunosuppression is induced. The ability of blood transfusions to enhance graft survival is used clinically in renal transplantation and is called “the transfusion effect.” In the DA rat, a transfusion of Lewis rat blood leads to indefinite survival of an orthotopic Lewis kidney graft. Experimental heart and skin transplants, however, do not demonstrate prolonged survival in the same strain combinations, but adjuvant immunosuppression may unmask the transfusion effect in some skin graft models.¹⁷ Our data indicate that corneal grafting falls somewhere between these two extremes, with a signifi-

cant enhancement of graft survival in 2 high responder strain combinations (Figs. 1a and 2a). The nature of this transfusion effect is unknown. However, it appears to be donor specific, because third party transfusions do not lead to a significant increase in corneal graft survival (Figs. 1b and 2b). This implicates a specific recognition process rather than a non-specific side effect of transfusion.

Four potential mechanisms could explain these findings: (1) delay in rejection that allows alteration of the graft itself (“modulation”) so that it does not express major histocompatibility antigens; (2) formation of blocking antidonor antibodies; (3) suppression of rejection by suppressor cells; or (4) elimination or inactivation of graft-reactive clones of lymphocytes. Modulation of the graft is unlikely because, based on clinical studies, humans can reject corneal grafts after rejection-free survival of many years.¹⁸ This would imply that long-term surviving corneal grafts retain their ability to express the donor major histocompatibility antigens.

The second possibility—that blocking antibodies are elicited by the blood transfusion and react with donor antigens on the corneal transplant to prevent recognition by the recipient’s lymphocytes—is supported by the findings of Chandler and coworkers. They soaked donor corneae with antidonor antibodies prior to grafting in rabbits and produced a modest increase in survival of grafts.¹⁹

The third possibility—cell-mediated suppression—seems to be important in prolonging kidney graft survival, particularly as blood transfusions may paradoxically prime the recipient’s cytotoxic cells despite enabling enhanced graft survival.¹⁵ However, recent evidence from our laboratory suggests that allospecific suppressor cells do not play a role in corneal graft enhancement (manuscript in preparation). Thus,

prolonging of survival of kidney and corneal grafts by donor-specific blood transfusion appears to depend on different mechanisms. Clonal inactivation or deletion is the fourth possible mechanism. In models of kidney and skin graft enhancement, cells that react to the graft are selectively removed from the circulation and are destroyed.¹² Further investigations are in progress to determine which of these possible mechanisms of specific immunosuppression is responsible for the prolonged survival of corneal transplants in recipients pretreated with donor antigens.

The potential clinical application is limited for several reasons. First, the transfusion effect is much weaker for corneal than kidney allograft enhancement. Second, because subcutaneous injection of even small amounts of blood sensitizes the recipient, such animals must be eliminated from a study of enhancement and a careful technique for transfusion is essential. Despite this, the phenomenon does not occur in all strains of rats, and a recent study in outbred rabbits not only failed to enhance survival of corneal grafts but induced accelerated rejection by donor-specific blood transfusion. Even adjuvant cyclosporine treatment failed to unmask a transfusion effect.²⁰ The factors that lead to sensitization as opposed to tolerance are poorly understood. Finally, the mechanism of enhancement remains an enigma after 50 years of investigation. These reasons and the small danger of the transmission of disease suggest that, at present, the use of unmodified blood transfusion to enhance human corneal graft survival is not indicated. However, further experiments are in progress to investigate whether the effect can be improved.

Key words: corneal graft, blood transfusion, enhancement

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