BLOOD PRETREATMENT AND RENAL ALLOGRAFTING IN THE DOG

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CHAPTER 1

Introduction

25.108 Kidney transplants in human recipients have been registered throughout the world up to 1977 (118). Serological histocompatibility matching in related donor recipient combinations has a definite effect on the outcome of the renal graft (108, 119, 131). However, the relevance of histocompatibility matching in cadaver kidney transplants is less clear. Matching for human leukocyte antigens (HLA) can be performed by serological techniques. Lymphocytes of the donor and the prospective recipient are tested against a panel of specific antisera. Leukocyte antigens are recognized. The patterns formed by means of the antisera are controlled by loci of the major histocompatibility complex (MHC). HLA-A and HLA-B are serologically defined (SD) loci. Several international kidney exchange organisations perform prospective matching for HLA in order to improve outcome of renal cadaveric transplant survival. Identity for HLA has a beneficial effect on renal allografts according to many studies (5, 12a, 24, 38, 83, 98, 107, 109, 120, 123), although these finding are not confirmed by others (82, 112). Van Hooff et al. (54), Oliver et al. (92, 93) and van Rood et al (121) report that matching for HLA is particularly important in the presence of pre-formed antibodies and identity for HLA-B antigens is of greater importance than identity for HLA-A antigens. Recently Opelz et al. have reported the correlation between HLA matching and graft survival in different transplantation centers (104, 107). The relevance of histocompatibility matching was greatly affected by the quality of the transplantation center. Centers with an excellent one year transplant survival showed only some correlation between histocompatibility matching and transplant survival, whereas such correlation was highly significant in centers with a poor one year graft survival.

Matching for the serologically defined loci of the major histocompatibility complex (MHC) HLA-A and HLA-B has not improved one year kidney graft survival and this figure is still depressingly low (about 50%) (117, 118). Experimental and clinical observations stress the importance of matching for determinants of HLA-D, being another locus of the MHC, that can be recognized by mixed lymphocyte reaction (MLR) tests. (22, 55, 106).

Lymphocytes of the kidney donor and prospective recipient can be cultured in a medium (mixed lymphocyte culture). Lymphocytes of subjects non-identical for the lymphocyte defined (LD) HLA-D will be stimulated to grow, whereas cells of HLA-D identical subjects do not grow in mixed lymphocyte cultures. Since these tests take at least 5 days, they are too time consuming to be used for prospective matching in cadaver kidney transplantations. Serological techniques for matching for HLA-D will probably be available in the near future (122).

The presence of preformed lymphocytotoxic antibodies in the serum of the prospective kidney recipient leads to hyperacute graft rejection, when these antibodies are directed against the kidney donor (71, 110). Antibodies that are not directed against the kidney donor, when the crossmatch test between recipient's serum and donor's cells is negative, can also influence graft survival by causing accelerated rejection, according to many publications (7, 54, 84, 91, 92, 93, 95, 97, 107, 111, 117, 118, 121, 137). Observations of others do however not support these data (8, 9, 17, 36, 72). The fear for stimulation of antibody production kept nephrologists and transplantation physicians from treating their prospective kidney donors with blood transfusions.

Yet a deterioration of the renal transplant results was observed as reported by Terasaki et al., (138) and by van Hooff et al. (56), for which the change in blood transfusion policy was thought to be, at least partly, responsible.

Accelerated renal allograft rejection after multiple blood transfusions was not observed and even a paradoxical effect of blood transfusion was reported in early studies of human kidney grafts (27, 60, 81, 85). In 1973 a protective effect of pretransplant blood transfusions on the outcome of cadaver kidney transplants was reported by Opelz et al. in a retrospective study (97). These data heve been confirmed by his and other groups in retro- and prospective studies (3, 12a, 39, 42, 42a, 87, 99, 102, 105). The one year graft survival of non-transfused kidney graft recipients was very low (12a, 56). Still blood transfusions may lead to antibody production against a percentage of a leukocyte panel in many cases and fear for accelerated graft rejection still leads to the restriction of the number of blood transfusions given to future recipients and more often future recipients have not been transfused at all. And as long as there is still some skepticism about a general transfusion policy (4), in vivo and in vitro experiments will be necessary to elucidate the effect of blood transfusions on renal allograft survival in man.

Transplantation experiments in well controlled animal models have to be carried out to study the effect of blood transfusions on allograft survival and antibody production in order to design an optimal transfusion policy. Because of the experience in our laboratory with transplantation studies in DLA tissue typed beagles this model was also chosen for the present experiments. The aim of our investigation was therefore to study renal allograft survival after different blood pretreatment schemes in DLA tissue typed beagles.

In this thesis the results of kidney transplantation experiments, that have been performed in the Surgical Laboratory of the Erasmus University, Rotterdam, the Netherlands are reported.

Chapter 2 contains a survey of the literature on the effect of blood transfusions on kidney transplant survival in man. The relevance of histocompatibility matching and presensitization is also considered in this chapter.

In Chapter 3 experimental data on kidney transplantation after pretreatment with blood or other antigens in small rodents, dogs and rhesus monkeys, as presented in the literature, are summarized. Data on active enhancement will get special attention in the light of our experimental work.

In Chapter 4 experiments testing the effect of one i.v. transfusion of donor blood on renal allograft survival in DLA identical, one and two DLA haplotype different littermates are reported.

Chapter 5 contains the report of experiments in which graft protection by s.c. donor blood injections prior to transplantation has been tested in beagle littermates. (2 DLA haplotype different).

In Chapter 6 experimental data on renal allotransplant survival have been reported after multiple transfusions in the recipient with pooled blood from non related blood donors. The segregation of immune responsiveness has been studied in this model. DLA identical and non-identical littermates have been used.

Chapter 7 contains a report of the effect of blood transfusions from related blood donors (father or mother of kidney donor and recipient) on the survival of renal allografts of DLA identical littermates. Parental blood was used in order to prevent a positive crossmatch and to obtain enhancing antibodies against non DLA antigens.

Chapter 8 contains the experimental data on the effect of third party non related blood transfusions on renal allograft survival in immunosuppressed beagles.

Chapter 9 contains a general discussion about the findings presented in

the foregoing chapters and a summary in English and Dutch language concludes this thesis.

CHAPTER 2

Effect of blood transfusions on renal allograft survival in man

2.1. Introduction

Lymphocytotoxic antibodies, that can be detected in prospective kidney recipients will be a result of pregnancies, blood transfusions or former allotransplantations. Females which did not receive blood transfusions in the past have antibodies in 19% of the cases, whereas males have antibodies in only 4% of the cases (96). The difference is obviously the result of pregnancies and in males the positive cytotoxic antibodies must be a result of cross-reaction with other antigens or due to an error in technique. Prior to transplantation (after hemodialysis and transfusions) 30.4% of all female prospective kidney graft recipients are presensitized and 19.5% of all male prospective recipients have cytotoxic antibodies (103).

Although a clear correlation exists between duration of hemodialysis and the number of blood transfusions (73), only 20-25% of prospective recipients have cytotoxins regardless of the duration of hemodialysis as has been reported by Terasaki et al. (137). This percentage is relatively low because of loss of antibodies (94). Antibody levels can decrease after some time resulting in a so-called secondary unresponsiveness (37); also about one half of the patients do not produce antibodies at all during a period of one year of hemodialysis according to Opelz et al.(94). It is clear that the leukocytes in the transfused blood play a role in the degree of sensitization. Frozen blood which is depleted of leukocytes (42) and leukocyte poor blood give (21) less sensitization. Casely et al. (21) have detected antibodies in 76% of the patients that received whole blood during dialysis, whereas patients that have been treated with leukocyte poor blood did not produce cytotoxins at all (21). Frozen blood gives antibody production in 4.8% of the cases versus whole blood in 20%, as reported by Fuller et al. (42). To prevent the production of lymphocytotoxic antibodies, leukocyte poor or frozen blood will probably be the ideal treatment for patients awaiting a renal graft. Opelz et al. (99) report however that the beneficial effect on renal grafts, as can be induced by whole blood transfusions is not seen when frozen blood has been

used instead. Fuller et al. (42, 42a) and Briggs et al. (12a), on the other hand, observe an equal degree of graft protection by either whole or frozen blood.

No supportive data can be found for a policy as advised by Lucas et al. (76) of transplanting hemodialysis patients as early as possible regardless of the tissue match, in order to avoid sensitization by blood transfusions. On the other hand, blood transfusions are not altogether harmless. Although non-transfused kidney graft recipients have a greater graft failure rate, sensitization by blood transfusions will increase with the length of hemodialysis. Thus, the chance of antibodies against a high percentage of prospective kidney donors will increase. Patients will be lost for transplantation by a liberal blood transfusion policy (4, 42a).

2.2. Sensitization and renal allograft survival

Presence of lymphocytotoxins in the prospective recipient directed against the kidney donor transplantation antigens as estimated by a strong positive crossmatch test will give rise to hyperacute rejection of the allograft (71, 110). A weak positive crossmatch reaction can be caused by the presence of antibodies directed against non HLA antigens present on B lymphocytes which themselves may have an enhancing effect (32). These antibodies may have an analogy to antibodies directed towards immune response locus associated antigens (Ia) in mice.

Graft prognosis may be worse in the presence of cytotoxic antibodies, which are not directed against the kidney donor but instead are cytotoxic against a percentage of a panel of leukocyte donors, as has been reported in several publications (7, 54, 84, 91, 92, 93, 95, 97, 107, 111, 117, 118, 121, 137). Prospective recipients who did not produce lymphocytotoxic antibodies after more than one year duration of hemodialysis had a one year graft survival of 85%, whereas recipients who did not produce antibodies in less than one year of hemodialysis had a one year graft survival of 50% (94, 95).

The observation that the presence of cytotoxic antibodies influences graft prognosis has not been confirmed by other groups (8, 9, 12a, 17, 36, 73). Callender et al. (17) report the follow-up of 185 renal allograft recipients from a transplantation center, well-known for its good results (129, 130). Thirty-two patients who showed anti HLA antibodies against more than 15% of a panel were compared with the same number of patients, who did not have antibodies. The patients were matched as to sex, age of recipient,

time of transplantation and other factors. No difference between the two groups (positive antibodies: 78% one year graft survival, negative antibodies: 74% one year survival) was observed. In their communication there is no evidence that "responders" and "nonresponders" behave differently, as has been suggested by Opelz et al. (94). Ferguson et al. (36), report that division into responders and non-responders on the basis of the presence of lymphocytotoxic antibodies has little relation with the ultimate graft function. A sensitive crossmatch technique prevents the transplantation of a graft that can be rejected in an accelerated way. Graft prognosis will be the same regardless of whether antibodies are present as long as a sensitive crossmatching technique is used.

2.2.1. Sensitization and survival of related and non-related renal allografts

In the 13th report of the Human Renal Transplant Registry (1977) (118) a significantly shorter graft survival in presensitized recipients of cadaver grafts over non sensitized recipients has been reported. In recipients of a graft of a living related donor such difference has no statistical significance.

2.2.2. Relation between sensitization and sex

Beleil et al. (7) have reported that no difference in renal transplant survival could be found between female and male recipients, although preformed cytotoxins were twice as frequent among females. Graft survival of female recipients was not influenced by the presence of preformed antibodies. The graft survival time in male recipients with antibodies was shorter than in those without. Opelz et al. (103) report in their series that female recipients received an unexpectedly high number of blood transfusion prior to transplantation as compared to males.

2.2.3. Sensitization, histocompatibility matching and renal allograft survival

Matching for HLA antigens has a very great influence on survival of related donor kidneys (108, 119, 131). The effect of HLA matching on cadaver donor transplants has been studied by many authors. In the earliest reports on

this subject Patel et al. in 1968 (109), Morris et al. in 1968 (83), van Rood et al. in 1969 (120) and Batchelor et al. in 1969 (5) have observed a positive influence of matching for HLA on renal cadaveric graft survival. However, only small groups of patients were included in these investigations and a limited number of HLA antigens was known by that time. In confirmation with these early findings were the observations made by Festenstein et al. (38), Hors et al. (58) and Perkins et al. (114). In 1971 the correlation between HLA matching and renal cadaveric graft survival was critized by Mickey et al. (82) who did not find such a correlation in 487 patients in North America. But since that time the reports of van Hooff et al. (54) and Oliver et al (92, 93) in 1972 and of van Rood et al. (121) in 1973 and especially the very clear data of the combined France and London transplant groups presented by Dausset et al. (24) in 1974, and later of Sachs et al. (123), made the relevance of matching for HLA antigens very likely for cadaver renal transplants. Dausset et al. (24) reported a 2 year cadaver graft survival in recipients that had 4 HLA antigens in common with the kidney donor of 70% ± 7%. When 3 HLA antigens were common: 54% ± 7%, when 2 HLA antigens were common: 45% ± 3%, and when 1 or zero HLA antigen was common: 34% ± 4% 2 year graft survival was found respectively. Oliver et al. (92, 93), van Hooff et al. (54) and van Rood et al. (121) showed HLA matching only to be useful in the presence of cytotoxic antibodies whereas matching for HLA-B was more relevant than matching for HLA-A.

The North-American kidney graft survival data were examined by Opelz et al. (98) in 1974. Only a marginal influence of HLA-matching in 2172 cadaver graft recipients from eighty transplantation centers was found. In a recent survey by the same group (107) in 1977 a highly significant correlation between matching for HLA and renal graft survival in 4,851 cadaver donor grafts was reported. HLA-A locus derived antigens expressed a slightly stronger effect than did HLA-B locus derived antigens. The influence of HLA matching is most significant in transplantation centers having the poorest one year transplant survival (< 40%). However, in the presence of cytotoxic antibodies matching for HLA did not give a better correlation as could be expected from studies by others (54, 92, 93, 121). In another communication from Opelz et al. (103) in 1977 HLA matching was only shown to be of value in male recipients regardless of the presence of cytotoxic antibodies.

Although matching for HLA-A and HLA-B will probably improve the outcome of renal cadaver grafts, one year graft survival is still around 50% (118). This is the case probably because other loci of the major histocompatibility complex (MHC) and minor histocompatibility differences affect graft sur-

vival. The locus that is represented by the reaction in the mixed lymphocyte culture is called HLA-D. Identity for the HLA-D derived antigens between kidney transplant donor and recipient has a positive effect on renal graft survival. Matching for HLA-D by mixed lymphocyte reactions (MLR) will prolong cadaver kidney graft survival in human recipients already matched for serologically defined antigens (22,55,106). In dogs, such an improvement of allograft outcome could not be observed due to the high linkage disequilibrium between DLA-A, DLA-B and DLA-D (16).

In a recent communication (106) a good correlation was found for matching for HLA-D and kidney allograft prognosis in 131 human cadaver kidney recipients. The mixed lymphocyte culture is too time consuming to be used for prospective selection of cadaver graft recipients. Recognition of HLA-D determinants may be possible in the future by serological means since a linkage disequilibrium exsists between HLA-B and HLA-D (55) and detection of HLA-D antigens by means of B-cell sera will probably be feasible as well (122).

2.2.3. Blood transfusions and renal allograft survival

Blood transfusions are equated with immunization and accelerated graft rejection and no blood transfusions or leukocyte poor blood have been recommanded, although a paradoxical effect of pre-transplant blood transfusions was observed as early as 1966 (81). Instead of expected increase in numbers of rejection episodes or accelerated rejection after an increasing number of blood transfusions, a normal rejection reaction (or in some cases a so-called paradoxical effect) has been observed in human kidney graft recipients (27, 60, 81, 85). However, the number of recipients was small in those studies and the differences were not highly significant. In a retrospective survey of 148 kidney recipients in 1973 (97) Opelz et al. reported the striking effect of blood transfusions prior to transplantation on graft prognosis. After more than 10 blood transfusions the one year graft survival was 66% ± 7% for the group as a whole and 80% ± 8% for transfused recipients without lymphocytotoxins. The one year graft survival of non-transfused recipients was a mere 29% ± 6% in the same series. The deterioration of overall kidney transplant results, as has been reported by Terasaki et al. (138), was though to be a result of the changed blood transfusion policy for prospective kidney transplants. Since 1969 the number of blood transfusions was reduced in patients on hemodialysis in order to prevent antibody production.

The data of Opelz et al. (97) in 1973 stressing the benificial effect of blood transfusions on the outcome of renal grafts have more recently been confirmed in many studies (3, 12a, 39, 42, 42a, 56, 87, 99, 102, 105). Van Hooff et al. (56) reported a significant decrease in kidney graft survival when the number of blood transfusion given prior to transplantation was dropped. Until 1972 prospective recipients received a median number of blood transfusions of 20 to 30. Since 1972 this number has decreased to only three. In this study (56) an increased one year cadaver kidney graft survival was seen in recipients that received blood transfusions prior to transplantation. (see table below)

0 transfusion	17%	1 year surv. (18 pts)
1- 4 transfusions	67%	1 year surv. (33 pts)
11-20 transfusions	74%	1 year surv. (35 pts)
21-40 transfusions	89%	1 year surv. (30 pts)
> 40 transfusions	69%	1 year surv. (29 pts)
unknown number	83%	1 year surv. (6 pts)

from van Hooff, thesis (57).

Recipeints, which had received transfusions of frozen blood have been studied by Opelz et al. (99). Between them and the recipients which did not receive any bloodtransfusions at all no difference in outcome of kidney transplant was observed (28% ± 6%), whereas those recipients which were transfused with whole blood prior to transplantation had an one year graft survival of 51% ± 1%.

In recent publications by Fuller et al. (42, 42a) the one year graft survival of patients, transfused with frozen blood was as good as the graft survival of patieints who were transfused with whole blood. The stimulation for antibody production was very low after frozen blood transfusions. However, the deglycerolization procedure of the frozen blood before transfusion was important for the protective effect on renal allografts. Deglycerolization by agglomeration (DGA) resulted in a lower percentage of HLA lymphocytotoxins and a better 2 year graft survival, whereas deglycerolization by centrifugal washing (DGC) resulted in a higher percentage of antibodies and a lower graft survival (42, 42a) (see table). The presence of HLA antibodies also precluded or delayed renal allografting in transplant candidates. Fuller et al. (42a) report that following a dialysis period of nearly 8 months 44% of the sensitized patients underwent transplantation, whereas more than 90%

of the non-sensitized patients received an allograft within two months of their acceptance in the program.

	patients	antibodies	2 year graft survival
non-transfused	10	0%	25,0%
whole blood + frozen blood	80	20%	47,1%
frozen blood only	39	13,2%	47,6%
frozen blood (DGA)	21	4,8%	55,4%
frozen blood (DGC)	12	16,6%	31,8%

from: Fuller et al. Transpl. Proc. 9, 117, 1977 (42).

Also Briggs et al. (12a) reported an improvement of one year graft survival after transfusions of whole blood as well as of frozen-thawed blood. These data are very promising, especially since transfusions with frozen blood have also the advantage of not transferring hepatitis (59). In a recent communication about the results of 382 recipients of cadaver kidneys (102) in which the transfusion data had been collected prospectively, patients which did not receive blood transfusions had a inferior graft survival after 1 year (31% ± 4%) and after two years (21% ± 4%). 10 out of these non-transfused patients had preformed antibodies. 9 out of these 10 grafts failed within the first year. Recipients who had received 1-5 blood transfusions had a better one year graft survival: 57% for recipients with preformed antibodies and 54% for recipients without antibodies. Thus, the presence of antibodies did not effect graft survival in this group, but did influence the one year graft survival (37%) of those recipients who received more than 5 blood transfusions prior to grafting, whereas in the absence of antibodies the one year graft survival was 51%. Thus no further improvement by an increasing number of bloof transfusions could be seen. It will be evident that the graft survival data, as presented by van Hooff in the Netherlands (56) show a qualitative difference with those presented by Opelz in the U.S. (107). Many other factors must have an effect on renal graft survival. And difference in quality between various centers is such that well matched kidneys in poor centers (1 year graft survival $< 40\%$) have a worse prognosis than badly matched kidneys in a good transplantation center (1 year graft survival $> 40\%$) (104, 107).

2.4 Conclusion

The outcome of renal cadaver transplants is greatly influenced by matching for HLA antigens. One year graft survival is however still low (about 50%). Matching for the HLA-D locus by MLC or serological techniques will probably improve transplantation results in the near future. Since transplant survival shows a wide variation in different transplantation centers improvement in patient care in those centers with poor results will probably improve the overall transplantation results.

In the last few years a consistent decrease of graft survival has been observed, partly due to a diminished number of blood transfusions to the prospective graft recipients. In the past the policy of restricting blood transfusions has been advised in order to prevent the formation of lymphocytotoxic antibodies with the chance of accelerated graft rejection. Recently it became clear that preformed antibodies do not have a deleterious effect as long as crossmatch tests are being performed in an accurate way.

A beneficial effect on kidney transplants of pretransplant transfusions with whole blood has been reported by several groups. This effect is more pronounced when no antibodies have been formed, according to most reports. Increase of the number of blood transfusions does not further improve the benificial influence on renal allograft survival in man. Frozen blood may be used with an equal beneficial effect as has been shown by the promising data of two groups.

Although the protective effect of blood transfusions seems evident, particulary on poorly matched cadaver renal grafts, the risk of sensitization will always be present and some patients will be lost for transplantation by an universal transfusion policy. Further in vivo and in vitro experiments need to be carried out to study whether an improved outcome of the kidney transplants will compensate for the loss of potential kidney recipients.

CHAPTER 3

Effect of blood transfusions on renal allograft survival in experimental animals

Pretreatment with blood of the prospective kidney donor or of a third party blood donor with or without the addition of immunosuppressive treatment has been shown to have a beneficial effect on kidney grafts in rats (34, 35, 77) and in rare cases in rabbits (29), dogs (1, 48) and rhesus-monkeys (31).

Prolonged graft survival by preventing or delaying the allograft rejection after pretreatment with donor antigen has a long history. Flexner and Jobling (1907) (40) observed a prolonged survival of transplantable sarcoma in rats after pretreatment of these rats with the same tumor. Since that time much work has been carried out on immunological enhancement, as this phenomenon was called.

3.1. Immunological enhancement

Enhancement is an antibody dependent phenomenon, resulting in prolonged survival of an allogeneic graft (69). Immunological enhancement can become so pronounced, that indefinite prolongation of graft survival can be achieved without the use of immunosuppressive treatment. Many reviews on enhancement and many communications dealing with the possible mechanisms of enhancement have been published. (2, 6, 41, 69, 70, 74, 133, 136).

Immunological enhancement can be achieved by active conditioning of the recipient with the antigens of the prospective donor or donor strain (34, 35, 66, 77, 78, 88) or passive immunization with appropriate antibody raised by hyperimmunization of a recipient strain (i.e. antidonor serum) (33, 135). Also a combination of antibody and antigen can be used (70, 90, 135). Enhancement can be considered as the opposite to rejection. Enhancement as well as rejection of a graft is induced by antibodies against the antigens that determine histocompatibility between donor and recipient. The relevant antibodies in immunological enhancement are directed against alloantigens determined by the MHC by which donor and recipient differ. These antibodies can be identified by lymphocytotoxicity or haemagglutinating tests

in some species and possibly by a weak crossmatch test or positive B-lymphocyte crossmatch test in humans (32), or by blocking factor activity in mixed lymphocyte cultures. (116, 126, 127, 128). Further studies have been carried out to identify which antigens are concerned with the production of enhancing antibodies. In mice, antigens determined by the Immune region of the MHC (Ir) or Immune region associated antigens (Ia) are thought to be responsible for induction of enhancement (25, 61).

In the light of our studies on the effect of blood transfusions on renal allograft survival in DLA-tissue typed beagle littermates, we will focus in the next paragraph on the reports about experiments in rodents, which deal with active enhancement of renal allografts, particularly when whole blood has been used as antigen. Reports of succesful renal allograft protection by (donor) blood in larger experimental animals are rare. We shall therefore analyse in the following paragraph all transplantation experiments in dogs in which prolongation of kidney graft survival by immunological manupulations had been the main object of investigation.

3.2. Active immunological enhancement of allografts by donor blood in rodents

Active immunological enhancement of allografts by pretreatment with whole blood has been reported by several authors (34, 35, 66, 77, 78). Marquet et al. (77) have reported the effect of donor strain blood on renal and heart allograft survival in two rat models. In the BN to Wag/Rij model indefinite survival of heart allografts and prolonged survival of renal allografts could be achieved by one injection of donor strain blood one week prior to transplantation, whereas in the reversed model (Wag/Rij to BN rats) accelerated rejection was seen after the same pretreatment schedule. When immunosuppressive therapy had been given at the time of blood injection indefinite survival of heart allografts and prolonged survival of renal allografts was seen. Indefinite survival was also achieved in this model when complexes of donor antigen and anti-donor strain antibody were added to donor blood pretreatment as reported by Marquet et al. in a recent publication (80).

Immunosuppression that could prevent graft rejection in one model could also prevent immunological enhancement in another model (78). The antagonistic effect of immunosuppressive drugs on immunological

enhancement has also been reported by Myburgh et al. for liver allografts in baboons (88).

Fabre and Morris (34) were led to their studies in rats at that time by the observations of Hume, Michielsen, Dossetor and Morris (27, 60, 81, 85) describing a possible benificial effect of blood transfusions in human renal allograft recipients. Specific immunosuppression (enhancement or tolerance) of renal allografts by one i.v. injection of donor strain blood 1 week prior to grafting could easily been achieved in a "weak" rat model. The (DA x Lewis) F_1 to Lewis rat model represented the strongest incompatibility, characterized by a vigorous unmodified rejection and ready production of lymphocytotoxins. In this "strong" rat model immunological enhancement could only be achieved by multiple pretransplant blood injections.

Further studies by the same group (35) showed that donor serum could not elicit enhancement. It was concluded that the effect was due to the cellular elements of the blood. Passive transfer of serum of long surviving rats did not lead to enhancement, whereas passive enhancement by treatment with hyperimmune anti-donor serum is very well possible in the same experimental model (33).

From these and other investigations it is clear that a delicate balance exists between accelerated rejection and prolonged allograft survival after donor antigen (blood) pretreatment in rats due to the presence of enhancing and/or cytotoxic antibodies.

Prolongation of graft survival depends on histocompatibility between donor and recipient, dosage of antigen, timing of antigen injection and transplantation, route of injection and additional aspecific immunosuppressive treatment (78, 90).

3.3. Enhancement of renal allografts in the dog

In contrast with the easily obtainable prolongation of renal allograft survival by blood pretreatment in rodents, pretreatment with blood transfusions rarely leads to protection of renal allografts in dogs. In larger outbred experimental animals like dogs, reports of prolongation of allograft survival by pretransplant immunological manipulation are scarce and seldom well documented. In the light of our own experiments in DLA tissue typed dogs, which are presented in this thesis, reports of experiments in dogs concerning renal allograft survival after blood pretreatment will be reviewed more in detail.

Prolongation of renal allograft survival in non tissue typed mongrel dogs was achieved in 1964, by Halasz et al. (48). Two ml. of whole donor blood was injected subcutaneously in the prospective kidney recipient 10 and 5 days prior to renal grafting. This form of pretreatment resulted in a significantly better outcome of the graft when compared with renal allograft survival in non-pretreated mongrels. No antibodies were estimated prior to transplantation. These results are impressive, although a certain discrepancy in histocompatibility between donor and recipient combinations in the experimental and the control group cannot be excluded. Many authors have referred to this communication as an example of active enhancement in the dog. It is even more interesting that by the foregoing pretreatment scheme skin allografts could be protected as well (47), although skin grafts are supposed to be more immunogeneic than vascularized grafts.

Halasz et al. (49) also studied the effect of i.v. donor spleen cells on allograft survival in mongrel dogs. In their opinion large amounts of donor antigens were necessary to prolong renal graft survival. This observation seems in contrast with the low dosage used in earlier experiments (48). Pretreatment with high doses of spleen cells did not lead to prolongation of kidney graft survival, but when low doses of immunosuppressive drugs were added or when an antigen extract was used, prolongation of graft survival was achieved.

Linn et al. (75) reported in 1966 an experiment in which mongrel dogs were pretreated with spleen cells of the prospective donor two weeks prior to transplantation or post-transplantation. Renal allografting was performed by implanting the kidney in the recipient's neck. No nephrectomy was performed and no immunosuppressive therapy was given. The renal allograft survival was prolonged in the dogs that had received pretransplant antigen treatment compared with non-treated dogs and dogs that received the spleen cell solution after transplantation. The difference was however barely significant.

Calne et al. (18) used several different pretreatment schemes in canine renal allograft experiments. Mongrel dogs were used and third party blood transfusions, donor spleen cells, donor lymphe node cells, donor bone marrow and a semi-soluble antigen derived from donor spleen cells were used as pretreatment antigens. No influence on renal allograft survival in non related mongrel dogs could be observed, whether immunosuppressive drugs were given or not.

Calne et al. (18) suggested that the genetic differences between outbred dogs were too great to be overcome by the amount of antigeneic matter

available from one single donor. Another possibility could be that the right scheme had not been found or that immunological reactivity of dogs could not be compared with that of small rodents.

Zimmerman et al. (149) pretreated mongrel dogs by repeated i.v. injections of subcellular fragments of spleen cells, used as donor antigens over a two week period, without immunosuppression. A small but statistically insignificant renal graft prolongation had been observed. But when antigen dosage was increased or when the course of injections was continued longer than three weeks, effectiveness was lost or accelerated rejection was found. When small doses of immunosuppressive drugs were given postoperatively in this protocol a dramatically prolonged survival time of renal allografts was observed in this model by Wilson et al. (147, 148). When the solubilized antigen had been derived from donor lymphocytes still a considerable prolongation of renal graft survival was achieved in mongrel dogs (52, 53). The doses of immunosuppressive drugs alone did not lead to prolongation of graft survival. Patterns of antibody production were related to duration of pretreatment and dosage of antigen and only cytotoxic antibodies were measured.

Currier and Pierce (23) reported the effect of donor blood transfusions on antibody production and renal allograft survival in mongrel dogs. Transfusions were given three and two weeks prior to grafting and low immunosuppressive treatment postoperatively. Cytotoxic antibodies were measured immediately before transplantation. After transfusion with whole blood 5 out of 10 recipients produced antibodies and 4 out of 10 recipients showed accelerated rejection. The longest survival was 16 days. When, however, leukocyte poor blood had been transfused no lymphocytotoxic antibodies could be detected and the longest graft survival was ten months.

Jeekel et al. (63) carried out experiments in DLA tissue typed beagle littermates in our laboratory in order to achieve active and passive enhancement of renal allografts. For each set of experiments two DLA identical littermates were selected. One recipient had been immunized by weekly s.c. injections of peripheral lymphocytes of the prospective kidney donor to raise anti-donor serum. The other recipient was treated with the anti-donor serum. The immunization scheme had been adapted from immunization schemes used in enhancement experiments in mice and rats (62, 64). Kidneys of a 1 haplotype different littermate donor were grafted in the two recipients. The kidneys in the hyperimmunized recipients were rejected in an accelerated way. Out of the 6 dogs that had been treated with antidonor serum 2 had a prolonged survival of 70 and 250 days without histological

signs of rejection, whereas control DLA 1 haplotype different kidney transplants had a mean survival of 18,5 days (10 to 44 days) (145). Passive enhancement of renal allograft was thus achieved, although these results could not be reproduced in later experiments.

In the same dog model i.v. injection of bone marrow cells of the prospective kidney donor did not increase the prolongation of renal allograft survival which had already been obtained by a short course of horse anti dog lymphocyte serum (65). Thus combination of ALS and donor bone marrow cells did not produce enhancement of renal allografts as described by the group of Monaco (20).

Storb et al. (134) studied the effect of blood transfusions on bone marrow grafting between histocompatible canine siblings. 100 ml donor blood given ten days prior to whole body radiation gave accelerated rejection of DLA identical bone marrow grafts when compared with engraftment in non-transfused dogs.

In a recent communication Abouna et al. (1) have reported that prolongation of renal graft survival resulted, when recipient mongrel dogs had been pretreated with multiple transfusions from a pool of non-related blood donors in combination with immunosuppressive treatment.

We may conclude that attempts to achieve prolongation of graft survival by blood pretreatment in dogs were rarely succesful and not very well documented. Renal graft prolongation was successful when third party blood was combined with postoperative immunosuppression. Definite active enhancement as can easily be achieved in other species has only been reported in a few cases, in which soluble antigens were used in combination with immunosuppressive treatment. Further experiments must be carried out in outbred animal models to study the effect of blood transfusions on kidney graft survival in man.

3.4. Blood transfusions and prolongation of renal allograft survival in rhesus monkeys

Recently observations on renal allograft prolongation after blood transfusions in rhesus monkeys have been reported by van Es et al. (31). The renal allograft survival in SD and LD typed rhesus monkeys was studied after five transfusions of 20 ml fresh blood from third party blood donors were given in biweekly intervals. Donor and recipient were matched for two or three antigens of the RhLA-A or -B locus. Grafting was performed 11 to 23 days after the last transfusion. A low dosage of immunosuppressive treatment was given. The mean survival time of the transfused monkeys was significantly longer

(MST = 48,9 ± 25,3 days) than the mean survival time of nontransfused animals with (MST: 11,0 ± 1,4 days) or without immunosuppression (MST: 11,4 ± 3,5 days). The shortest interval between last transfusion and transplantation correlated with the longest graft survival. The degree of histocompatibility between blood donors and kidney donors did not show a correlation with graft survival. Lymphocytotoxins were produced in all transfused animals but no clear influence of antibody production was observed. Two recipients showed positive crossmatch test prior to transplantation, without a hyperacute or accelerated rejection. Their prolonged graft survival can possibly be a result of antibodies against I_a or B cell antigens. Van Es et al. (31) provide us with an excellent preclinical model to study the effect of blood transfusions on kidney transplant survival in man. They suggest that an nonspecific immunological mechanism is responsible for the graft protective action of pretransplant blood transfusions.

3.5. Effect of blood pretreatment on renal allograft survival in DLA tissue typed beagle littermates (Introduction to the experiments)

In the following five chapters our experiments with renal allograft transplantation in beagle littermates after blood pretreatment will be reported. Some of the work has been published or has been submitted for publication. The beagles have been typed for the major histocompatibility complex (SD) antigens in all cases and by mixed lymphocyte defined (LD) antigens in some cases.

Beagle dogs were chosen as experimental animals because of the experience with this well tested model in our laboratory. The short generation time and the litter size make the dog very well apt for histocompatibility research as has been shown in earlier communications (15, 139, 140, 142, 146). Data on graft survival have become available for skin, kidney, heart, liver, small bowel, pancreas, lung and bone marrow transplants in this model (11, 44, 45, 46, 67, 113, 139, 141, 143, 144, 145). In all cases an influence of histocompatibility matching has been observed (143). Attempts to prolong renal allograft survival by pretreatment with donor antigen have been unsuccessful thus far in our hands. Reports of prolonged graft survival by specific immunosuppression by donor antigens, without the use of aspecific immunosuppression, are rare in larger (outbred) experimental animals.However, we were tempted to test the effect of blood transfusions in our model without the use of immunosuppressive therapy, since we considered the immunogeneic barrier between littermates low enough to obtain immunological enhancement. Azathioprine and prednisone were given only in the last protocol (Chapter 8).

CHAPTER 4

First experiment

THE EFFECT OF DONOR BLOOD ON RENAL ALLOGRAFT SURVIVAL IN DL-A TISSUE TYPED BEAGLE LITTERMATES

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Summary

A single transfusion of 200 ml of donor blood 14 days before renal transplantation in prospectively DL-A tissue typed beagle littermates appeared to have an effect on graft survival. Seventeen per cent of the recipients did respond to the transfusion with formation of lymphocytotoxic and haemagglutinating antibodies. These "responder dogs" rejected kidney grafts in an accelerated way compared with the "nonresponders" and with the nontreated control dogs. Responsiveness appeared to occur in pairs of littermates, which suggests that responding potency is genetically determined. There was histological evidence of acute arteritis in the renal grafts of responders, whereas cell-mediated rejection was noted in nonresponders.

Introduction

Human kidney graft recipients will in most cases have received several blood transfusions before the time of transplantation, which often leads to formation of leukocyte antibodies (9, 16, 17). Different effects of these transfusions on the survival of the grafts have been noted. Accelerated kidney allograft rejection after blood transfusions has been described in patients with circulatory lymphocytotoxic antibodies which react with donor lymphocytes (12). On the other hand, it has been suggested in other publications (4, 15) that recipients of renal grafts who had received many blood transfusions before kidney transplantation had a similar or even better course than patients that only received a few transfusions. Recently Opelz et al. (16) reported that an increased number of pretransplant blood transfusions leads to prolonged kidney graft survival.

A beneficial effect of pretreatment with donor blood on allograft survival has been reported in different experimental animals. In rats, prolonged survival of cardiac, renal, and skin grafts by pretreatment with whole blood has been described (6, 7, 11, 13). In dogs a small dosage of blood injected s.c. had a specific immunosuppressive activity (8). In this species a major histocompatibility complex has been described which resembles the human HL-A complex and which has been labeled DL-A (21). Storb et al. (19) described a deleterious effect on graft survival of a single transfusion with donor blood before bone marrow transplantation in DL-A tissue typed dogs.

The effect of one transfusion of donor blood on the survival of renal allografts in host-donor combinations with different DL-A relationships is the subject of this communication.

Materials and methods

Prospectively tissue typed beagles from an outbred colony were used. Tissue typing was done using a one-stage microcytotoxicity test, which has been reported previously (20, 21). The production and evaluation of test sera and the way DL-A specificities are recognized by these sera have been described elsewhere (22, 23).

On the basis of the different segregation patterns of the typing sera in beagle families, three experimental groups were formed: group 1, two DL-A haplotype differences between donor and recipient; group 2, one DL-A haplo-

type difference between donor and recipient; group 3, no DL-A haplotype difference between donor and recipient.

Donor blood (200 ml) was collected in citrate and immediately transfused to the future recipient 14 days before kidney transplantation (day - 14). Lymphocytotoxic titers and haemagglutinating titers (19) were measured on days - 6 and - 1. Renal grafts were exchanged in pairs. The donor kidney was transplanted to the contralateral iliac fossa of the recipient following a standard procedure as described elsewhere (26). Contralateral nephrectomy was performed at the time of transplantation.

Histological examination of the graft was done when the recipient died or when the serum creatinine levels rose above 1,000 μ moles/liter. In that case animals were killed by an overdose of Pentothal. All animals included in this series of experiments died exclusively from rejection. No immunosuppressive therapy was given. Survival data were analysed statistically with the Student-Welch test ($\bar{X}$ stands for mean survival time).

Results

The survival times of the individual nonpretreated recipients of kidney allografts are given in Table 1 as reported by Westbroek et al. (26). Statistical analysis showed a significant difference between the mean survival times of groups 1 and 3 ($P < 0.001$) and groups 2 and 3 ($P < 0.005$), but no significant difference between groups 1 and 2 ($0.10 > P < 0.20$).

The survival times of the individual recipients of kidney allografts after pretreatment with a single transfusion of donor blood (200 ml) 14 days before transplantation are given in Table 2.

Table 1. Survival times of individual kidney allografts in nonpretreated littermate beagles (controls)[a]

Group No.	Survival times (days)	$\bar{X}$	SD
1	9, 9, 11, 11, 12, 12, 12, 14, 14, 23, 30, 31	15.6	7.8
2	10, 11, 11, 13, 17, 20, 31, 39, 42, 44	23.8	13.8
3	26, 31, 33, 35, 35, 41, 41, 42, 48, 59, 60, 65, 254[b]	43.0	12.5

[a] Survival times (days) for groups 1, 2 and 3 (see Materials and Methods for explanation of groups).
[b] This graft was excluded from the statistical analysis.

Table 2. Survival times of individual kidney allografts in littermate beagles after a transfusion with donor blood[a].

Group 1				Group 2				Group 3			
Day of rejection	Litter[b]	Antibody at day[c]		Day of rejection	Litter[b]	Antibody at day[c]		Day of rejection	Litter[b]	Antibody at day[c]	
		-6	-1			-6	-1			-6	-1
8	a^1	+	-	3	a^1	+	+	8	a^1	+	+
10	b^1	-	-	7	a^2	+	+	12	a^2	+	+
11	b^2	-	-	11	b^1	-	-	17	b^1	-	-
12	c^1	-	-	11	b^2	-	-	20	b^2	-	-
13	a^2	-	-	11	c	-	-	31	c^1	-	-
18	d^1	-	-	12	d^1	-	-	62	d	-	-
19	e	-	-	18	d^2	-	-	$>300^d$	c^2	-	-
20	f^1	-	-	22	e	-	-	$>300^d$	e	-	-
25	d^2	-	-	43	f^1	-	-				
26	f^2	-	-	68	f^2	-	-				
46	c^2	-	-								
$\bar{X} = 18.9$				$\bar{X} = 20.6$				$\bar{X} = (56.8)$			

[a] Survival time (days) for groups 1, 2 and 3 (see Materials and Methods).
[b] Littermates are denoted with similar letters.
[c] Haemagglutination and microcytotoxicity tests were done 6 days and 1 day before transplantation.
[d] Without any sign of rejection.

The slightly longer mean survival time of renal grafts in pretreated recipients (18.9 days) compared to control dogs in group 1 (15.6 days) was not statistically significant. In groups 2 and 3, pretreated and control dogs also rejected in approximately the same period. In group 3 two out of the eight pretreated dogs survived longer than 350 days without any sign of rejection.

It can be seen in Table 2 that only 5 out of the 29 dogs that received transplants in the three experimental groups had positive lymphocytotoxic and haemagglutination titers on days - 6 and -- 1, varying from 1 : 32 to 1 : 256. They have been labeled "responders" (11, 14), whereas the other 24 "nonresponders" did not develop antibody titers.

The five responders showed accelerated graft rejection (3-12 days) when compared with the individual survival times of control dogs and pretreated dogs with negative titers. Nonresponder dogs did not reject their renal grafts before the 12th day. There was no clear correlation between sex or DL-A haplotype difference of donor and recipient and the number of responders. The responders of group 2 were littermates, which was also the case in group 3. It appeared that the individual pairs that exchanged grafts showed similar rejection patterns (Table 2). No significant difference between the mean survival time of nonresponders with transplants and control dogs could

be shown. The prolonged survival times of immunised nonresponders might reach significant levels in a larger material instead of the suggestive indication found in the present experiments.

Histology. In the three experimental groups the difference in the histological picture between the responders and the nonresponders was similar in that the responders showed early severe vasculitis with thrombosis and tissue destruction, whereas in the nonresponders the vasculitis was in all cases much less marked and the cellular infiltration more prominent. This difference between responders and nonresponders was most marked in group 3 (no haplotype difference).

Discussion

The effect of blood transfusion before kidney transplantation on the production of lymphocytotoxic antibodies and the survival of the graft has been the subject of several recent investigations (16, 17). Except for a well known deleterious effect of blood transfusions, especially in the case of preformed antibodies with antidonor activity, a beneficial effect has been described (17).

In this study DL-A tissue typed beagle littermates were used. The effect of the prospective typing on the survival of small bowel, cardiac, pancreas, and kidney allografts has been described elsewhere (2, 3, 24-26). A significant difference of survival times of identical and nonidentical kidney allografts has been previously reported (26). Two different rejection types were documented, depending on the DL-A haplotype difference. In identical combinations a cell-mediated rejection was obvious in post-mortem biopsies. In the nonidentical group, and especially in the group with a two-haplotype difference, a humoral rejection type with acute arteritis was predominantly present.

After one blood transfusion with 200 ml of fresh donor blood 14 days before transplantation, 17% of the dogs produced lymphocytotoxic and haemagglutinating antibodies against their donors. In one of these five responder dogs positive antibody titers were only found on day – 6. No attempts were made to study the specificity of these antibodies. Four responder dogs consisted of two pairs. This finding, that responsiveness occured in two pairs of littermates and that comparable rejection patterns could be noted in many other pairs, would be compatible with speculations that responding potency is genetically determined. Obviously no conclusions can be reached yet on whether this presumed genetic control of responsiveness is achieved by one (11) or more mendelian systems (1).

Accelerated rejection occurred in the responder group, although hyperacute rejection was not seen. The nonresponders did not show any accelerated rejection.

In the experimental group with a two-haplotype difference a prolonged survival time was noted compared with the nontreated group, although the difference did not reach conventional significance levels. Prolonged survivals were also noted in two recipients that did not respond with antibody formation to blood transfusions of DL-A-identical donors. The possibility that these two identical donor-recipient pairs were in fact monozygotic twins cannot be excluded, inasmuch as both were of identical sex. The occurrence of monozygotic twins in dogs has only been described once (5), however, and according to our experience is highly infrequent.

Histologically there were clear signs of severe vasculitis in all responders except one that survived for 3 days. The nonresponders did not show the expected humoral rejection type with vasculitis, but a predominantly cell-mediated rejection was found instead. Thus, in the present experiments two effects of a pretransplant donor blood transfusion were noted. First, in a few dogs transfusions led to production of antibodies and a slightly accelerated rejection with histological evidence for an unusual severe vasculitis. Second, in the majority of the dogs antibody production did not occur, nor was there any deleterious effect of blood transfusion on graft survival, whereas the rejection pattern appeared to be of a cellular rather than of a humoral type.

In the present study responders were identified by serological methods, which might be an unsatisfactory way to demonstrate such a complicated phenomenon. Moreover, these serological methods may lack adequate sensitivity to demonstrate all types of antibodies, and it may well be that so-called nonresponders do form specific antibodies, in which case the normal and prolonged survivals might be caused by enhancing antibodies. Enhancement of renal allografts in dogs has been studied before with moderate success (10).

The fact that those dogs in the present experiments that responded to a blood transfusion with antibody formation also displayed a special histological type of rejection, which was found to be caused by antibodies, does suggest that indeed these dogs were responders.

It remains unclear whether unresponsiveness is genetically determined or specifically induced by donor-specific blood transfusions. Future experiments will be aimed at the question of whether the unresponsiveness found in the present studies is genetically determined or not. The dog

will be an excellent model for such studies because of its short generation time and large litter size. If it again can be shown in further studies that under certain conditions blood transfusions can induce nonresponsiveness in the host, then the identification of these conditions becomes of prime importance to clinical allografting.

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CHAPTER 5

Second experiment

EFFECT OF SUBCUTANEOUS INJECTIONS OF KIDNEY DONOR BLOOD ON RENAL ALLOGRAFT SURVIVAL IN DLA TYPED DOGS

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Summary

Renal allografting was carried out between donor-recipient pairs of beagle littermates that were mismatched for 2 DLA haplotypes. No immunosuppressive treatment was given. Two subcutaneous injections of whole blood from the prospective kidney donor 5 and 10 days prior to transplantation resulted in accelerated rejection of the kidney graft when compared to graft survival in non-pretreated dogs and dogs that were pretreated with one intravenous transfusion of 200 ml donor blood 14 days before transplantation. A positive crossmatch correlated with accelerated graft rejection in most cases, although accelerated graft rejection was also observed in the presence of negative crossmatches. Results obtained by others in non-tissue typed dogs indicating the induction of active enhancement of renal allografts by the subcutaneous injection of donor blood could not be reproduced.

The results stress the relevance of DLA tissue typing in testing tentative active enhancement protocols in this preclinical animal model.

Introduction

Retrospective analyses have shown that blood transfusions prolong the survival of a subsequent human kidney graft (1, 8, 10, 14, 15, 25, 26, 27, 28). This observation has been confirmed by prospective studies in larger experimental animals. Third party blood transfusions combined with immunosuppressive treatment increased kidney graft survival in rhesus monkeys (5) and dogs (Obertop et al., chapter 8). In rodents, enhanced graft survival can be obtained by giving organ donor blood without immunosuppressive drugs (6, 7, 19, 21, 22). Dogs have been used in the extrapolation of results obtained in rodents to men. Enhanced renal allograft survival by donor blood pretreatment without the help of immunosuppression was described by Halasz et al. (11) in this species. At that time histocompatibility testing had not been developed yet in the dog. The aim of this investigation was to repeat the early experiments of Halasz et al. (11) in tissue typed dogs. Failure to prolong graft survival in tissue typed dogs by subcutaneous injections of blood from the kidney donor suggests that the early results are probably due to chance host-donor histocompatibility and not to an enhancing or tolerazing effect of the injected donor blood.

Materials and methods

Prospectively DLA typed beagles from the colony of the Centraal Proefdierenbedrijf TNO, Austerlitz, The Netherlands (Dr. J. van Vliet) were used. Typing was performed in a one or two stage microlymphocytotoxicity test using sera and techniques previously described (32, 33, 34). Donor and recipient were littermates of the same sex and were 2 DLA haplotypes mismatched. In the experimental group (Group 1) 2 ml of fresh whole blood from the prospective kidney donor was injected subcutaneously 10 and 5 days prior to renal allografting, as reported by Halasz et al. (11). No anticoagulant was used.

Kidneys were exchanged between donor and recipient and transplanted in the left or right iliac fossa as previously described (35). A bilateral nephrectomy was performed at the time of grafting. Recipients recieved no immunosuppressive treatment. Histological confirmation of rejection was obtained when animals died or were killed when the serum creatinine level rose above 1,000 μ moles/liter. In this series, no dogs died from technical failures. The mean survival time (MST) of kidney allografts in recipients inject-

ed s.c. with donor blood was compared with the MST of kidney allografts in two other groups of dogs receiving a kidney from a 2 DLA haplotype mismatched sibling. One group (Group 2) received one intravenous transfusion of 100 ml of donor blood 14 days before transplantation and one group (Group 3) received no blood before transplantation. The results of these two groups have been previously reported (24, 35). Crossmatches were performed in the one and two stage microlymphocytotoxicity test (29) and in the one stage test in Group 2.

Results

Renal allograft survival was not prolonged by two subcutaneous injections of 2 ml of donor blood. In contrast, the mean survival time of Group 1 (s.c. blood) was significantly shorter when compared with the MST of Group 2 (i.v. blood) ($p < .005$) and the MST of Group 3 (no blood) ($p < .005$, Table 1). The difference in MST between Group 2 and 3 is not significant ($p > .1$). In three dogs in Group 1 (s.c. blood) and one dog in Group 2 (i.v. blood) antibody production against the kidney donor was detected prior to transplantation. A positive crossmatch was found in some of the recipients that had decreased graft survival but not in all. Exclusion of dogs with antibodies did not change the relevance of the observations. No clear-cut correlation between DLA inheritance and antibody production was seen. Two of the dogs with antibodies were littermates whereas the other two had littermates who did not produce antibodies.

Discussion

It has previously been shown that transfusions with whole blood do not protect a renal graft against rejection in dogs (2, 4, 24) and have a deleterious effect on bone marrow grafting in canine siblings (30). In rodents, however, prolonged kidney and heart survival can be obtained after pretreatment with blood from the organ donor without immunosuppression (6, 7, 19, 21, 22) although the effect differs according to the donor recipient combination and depends on dosage and timing of the injections. Accelerated graft rejection has also been observed after donor blood injections in rats (22). Other protocols which were effective in prolonging allograft survival in rats, such as pretreatment with anti-donor sera (17) or administration of

anti-lymphocyte serum and donor bone marrow (23) were not effective in tissue typed dogs (16, 18). In other publications the succesful induction of active enhancement in dogs with (3, 12, 13, 36, 37) or without (11, 38) immunosuppression has been reported. However, tissue typing was not employed and in view of the irreproducibility of some of these positive results the occurrence of chance donor recipient compatibility in the experimental groups alledgedly showing enhancement cannot be excluded.

Table 1. Survival of individual kidney allografts.

Group 1 (2 ml donor blood day - 10 and - 5)			Group 2 (200 ml donor blood day - 14)			Group 3 Control
day of rejection	litter*	antibody	day of rejection	litter*	antibody	day of rejection
3	a^1	-	8	e	+	9
4	b^1	+	10	f^1	-	9
5	a^2	-	11	f^2	-	11
6	c^1	-	12	g^1	-	11
7	a^3	+	13	h^1	-	12
9	c^3	-	18	h^2	-	12
10	d^1	-	19	i	-	12
10	d^2	-	20	j^1	-	14
11	b^2	+	25	h^3	-	14
28	c^3	-	26	j^2	-	23
			46	g^2	-	30
						31
m.s.t. 9.3			m.s.t. 18.9			m.s.t. 15.7
$p < .005$**			$p > .1$**			
$p < .005$**						

* Littermates are denoted with similar letters.
** Wilcoxon rank sum test.

The report of Halasz et al., (11) describing prolonged kidney graft survival in dogs after subcutaneous donor blood injections is unique because of the use of the s.c. route of giving donor antigen and because it has been referred to as a clear example of active enhancement in the dog. Other investigators have stressed that the route of injection is important in obtaining enhancement of an allograft (9, 13) and the most effective route is probably intravenous (31). Repeating the Halasz protocol in DLA tissue typed beagle littermates with a defined incompatibility between donor and recipient did not lead to prolongation of kidney graft survival. Accelerated re-

jection after s.c. donor blood injections was observed when compared with the non-pretreated or i.v. pretreated controls. This confirms that route of injection as well as dosage and timing of donor antigen pretreatment is of importance in altering kidney graft survival. In addition, these data show again that histocompatibility matching is an important variable in a preclinical model as the dog and that two DLA haplotypes mismatched siblings can be used as a standard histocompatibility difference. The use of random donor recipient pairs with gross anatomical differences will not guarantee DLA differences (39).

Hyperacute rejection (20) was not found in this series; all dogs produced urine immediately after transplantation. A positive crossmatch between recipient serum and donor lymphocytes predicted decreased graft survival in three out of four dogs, whereas accelerated rejections were seen in other cases despite negative crossmatches. This probably indicates a lack of sensitivity of the crossmatch procedure as it is currently performed in the dog.

In the present study we were again unable to demonstrate the applicability of a so-called active enhancement protocol to the outbred tissue typed dog (2, 16, 18). The graft prolongation that can be obtained by third party blood transfusions combined with immunosuppression is probably not based on active enhancement (Obertop, et al., chapter 8). A further exploration of the effect and mechanism of third party blood transfusions in a tissue typed dog model might be more rewarding and have a higher immediate clinical relevance than additional studies of tentative active enhancement protocols.

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CHAPTER 6

Third experiment

THE EFFECT OF PRIOR THIRD PARTY BLOOD TRANSFUSIONS ON CANINE RENAL ALLOGRAFT SURVIVAL

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Summary

The relationships between immune reactivity after blood transfusions, subsequent kidney allograft survival and donor selection were studied in dogs. Animals with a high as well as a low serological immune reactivity towards antigens contained in blood transfusion were observed.

Genetic control of this reactivity or a linkage of this property to DLA sex or red cell markers inheritance was not apparent in the four beagles families studied. The two recipients with the lowest immune reactivity scores were also found to be the longest survivors after a DLA mismatched kidney graft. Seven other recipients with higher scores rejected their DLA mismatched kidneys as rapidly as did untransfused animals. Kidney graft survival was decreased in some recipients of DLA identical kidneys ($n = 5$) presumably through sensitization for minor histocompatibility antigens. A normal or an increased survival time of DLA identical kidneys was found in the remaining animals ($n = 6$). The majority of these recipients appeared to have a higher than average reactivity in two stage microcytotoxicicty testing. This might have been due to the presence of enhancing antibodies. Further studies in preclinical animal models are needed to define the optimal transfusion policy for human patients awaiting a kidney graft.

Introduction

Several retrospective analyses of kidney transplantation in man have been made to determine the possible influence of prior blood transfusions on the survival of a kidney allograft and the efficacy of host/donor matching. The results indicated that (1) a positive cross match between kidney donor lymphocytes and a recipient's pregraft serum correlated positively with hyperacute rejection (15, 26); (2) conflicting observations were made concerning a negative cross match. Several authors found a better graft survival in patients that had received blood transfusions prior to kidney grafting (10, 13, 23, 24). Others reported that kidney graft recipients with lymphocytotoxic alloantibodies prior to grafting experienced a shorter average graft survival than recipients who did not produce antibodies following blood transfusion (19, 24, 27, 28, 29, 32). This observation could not always be confirmed in later studies (2, 3, 7, 8, 10, 16, 20). A beneficial effect of matching for HLA antigens (especially HLA-B antigens) on kidney graft survival was demonstrated in transfused patients with antibodies (12, 22, 29). This observation was also not always reproducible (3, 8). The variation in results obtained are a reflection of the difficulties inherent in an analysis of complex clinical situations such as human kidney transplantation with many uncontrollable variables. A consequence of the conflicting results on the relationships between transfusions, kidney graft survival and donor selection is that a uniform "transfusion" policy cannot be established for human patients, who will require a kidney graft in the future. Too many blood transfusions might lead to a high degree of sensitization of the recipient, with a positive crossmatch to the majority of random unrelated kidney donors. However, too few blood transfusions might contribute to an overall decrease in average kidney graft survival, since patients who did receive blood transfusions prior to grafting appeared to experience a better survival than those patients who did not.

A reliable preclinical animal model is evidently required to study this complex situation. Studies in inbred rats have shown that transfusion of donor blood can result in prolongation as well as a shortening of kidney graft survival depending, among other things, on the rat strains used as donors and recipients (17). Attempts to extrapolate the conditions for successful rat blood and kidney transplants to outbred species of laboratory animals have so far failed. Studies in rhesus monkeys were recently reported; in this species prior blood transfusions did have a significant effect on kidney allograft survival (9).

The present report deals with an ongoing analysis of the stated problem in the dog. This animal model has the advantages of ready availability, simple identification and high frequency of littermate donors, identical for the Major Histocompatibility Complex (MHC), and the accumulation of previous experience in kidney grafting in this model under various conditions (5, 14, 21, 36, 37).

The aims of the current study were:

a. to describe the specific immune reactivity of dogs after multiple third party transfusions (i.e. not autologous and not from the kidney donor);
b. to investigate the possibility of a straight forward genetic control of such specific allogenetic immune reactivity;
c. to determine the possible effects of prior third party blood transfusions on the survival of MHC identical or mismatched kidney allografts in non-immunosuppressed recipients.

Material and methods

Four beagle families (a total of 24 offspring) were purchased from the Central Institute for the Breeding of Laboratory Animals (TNO, Zeist, The Netherlands). All the animals and their parents (whenever available) were typed for the genetic markers indicated below and all received blood transfusions. Only the offspring were used as kidney recipients and/or donors. Seven mongrel dogs from four different families were used as blood donors. They were selected on the criteria: 1) DLA-SD different from the beagles, in an attempt to avoid cross immunization with the kidney donor; and 2) DEA 1.1. and 1.2. negative, to prevent the occurrence of transfusion reactions after multiple transfusions (31).

Genetic markers

The Dog Major Histocompatibility Complex (DLA)

Serologically defined (SD) antigens were determined by employing a microcytotoxicity test, in which more than 70 antisera recognizing the specificities 1, 2, 3, 7, 8, 9, 10 of the DLA-A series, 4, 5, 6, 13, R16 and R20 of the DLA-B series and 11, 12, R15 of the DLA-C series (30, 33, 34) were used. Lymphocyte defined (LD) antigens determine the reactivity in mixed lym-

phocyte cultures (MLC). So-called LD typing cells were not used in this study. In 3 of the 4 families one way mixed lymphocyte cultures using a technique described elsewhere (6, 11), were performed.

Dog Erythrocyte Antigens (DEA)

The antigens belonging to the systems 1, 2, 3, 4, 5, 6, 7 and 8 were determined using methods and reagents previously described (4, 31).

Canine Secretory Allo-antigens (CSA)

The A, X and Y antigens were determined through the kind cooperation of Dr. A. Zweibaum, using methods described earlier by this group (38).

Blood transfusions

Each beagle received a 100 ml transfusion which represented a pool of citrated blood from seven different donors on days 1, 7 and 14. Serum samples were obtained on day 21 and prior to each transfusion. Samples were inactivated for 30 min at 56°C and stored at -20°C until testing.

The humoral immune response of the transfused animals

Cell suspensions of the following animals were used to screen the sera of the transfused animals for antibody activity in the one and two stage modification of the microlymphocytotoxic assay (1).

I. blood donors,
II. family members (including the kidney donor),
III. the leukocyte panel, a group of twenty unrelated dogs of various (for the majority, undefined) breeds.

All of the currently known DLA groups are represented in the panel. All sera were tested undiluted; for cell suspensions of groups I and II, three serum dilutions (1 : 2, 1 : 4, and 1 : 8) were also included. Each serum was given a reactivity score. This score was obtained by considering all lymphocytotoxic

reactions as positive where more than 25% of the lymphocytes were stained. Each positive reaction was multiplied by the reciprocal of the serum dilution. All scores of positive reactions per serum were added, divided by the maximum obtainable score and multiplied by a hundred. When undiluted sera were tested, the strength of the reaction was scored as 4, 6 or 8 depending on the per centage of cells killed (25-50, 50-75, and 75-100 respectively). Here the antibody score was obtained by multiplying the positive reactions by their strength factor, adding all of these multiplications for each serum and dividing it by the maximum obtainable score, and multiplying it by 100. This scoring system was chosen to facilitate comparisons in immune reactivity among different animals in this and future experiments.

Renal allograft

The transplant was performed between days 21 and 30 of the protocol following a previously described standard procedure (36), which includes a bilateral nephrectomy of the recipient on the day of allografting. Donor kidneys came from littermate donors that were DLA identical (n = 14), 2 DLA haplotype different (n = 8) or 1 DLA haplotype (n = 2) different.

Postoperative treatment

Antibodies and parenteral fluids were given during the first five post operative days following a standard, previously reported protocol (36). Immunosuppressive treatment was not given. Recipients were observed until 200 days after grafting, until they died or were killed when serum creatinine levels rose above 1,000 μ mol/1. An autopsy was routinely performed and fresh specimens of the of the kidney graft were obtained for histopathological studies.

Results

The immune reactivity of the transplanted dogs

The results obtained are summarized per immunized family in reactivity scores in Tables 1 and 2.

Table 1. Reactivity score[a] or sera from transfused dogs

		microlymphocytotoxicity							
		one stage				two stage			
Family	Dog	0[b]	7	14	21	0	7	14	21
		•[c]	•	•	•	•	•	•	•
Sire	D 976	•	•	•	•	•	•	•	•
		0[d]	50	92	85	0	33	58	85
Dame	886	•	•	•	•	•	•	•	•
		0	0	0	0	0	0	0	6
offspring	D4373	0	0	0	4	0	5	5	11
		0	2	7	3	0	10	14	49
	D4374	0	•	41	65	0	•	57	57
1		0	16	17	10	0	22	34	30
	4375	0	78	38	64	0	79	68	64
		0	0	0	4	0	4	4	4
	4376	0	14	3	4	0	26	19	30
		0	1	3	0	0	1	0	15
	4377	0	18	8	0	0	39	44	25
		0	8	20	19	0	15	34	63
Sire	D2277	•	•	•	•	•	•	•	•
		0	•	0	5	0	•	0	9
Dame	814	•	•	•	•	•	•	•	•
		0	1	1	24	0	10	10	70
offspring	D4385	0	53	31	39	0	56	30	79
		0	0	2	8	0	0	11	67
	D4386	0	0	25	65	0	51	31	70
2		0	0	0	7	0	0	10	50
	D4387	0	5	29	38	0	25	90	58
		0	0	0	21	0	0	16	74
	4389	0	15	43	59	0	54	96	66
		0	0	2	5	0	0	6	31
	4390	0	0	19	46	0	18	15	68
		0	0	0	1	0	0	1	16
	4391	0	0	0	3	0	3	9	46
		0	0	2	19	0	24	15	56
	4393	0	18	5	28	0	44	18	62

[a] see methods for computations
[b] day as related to first transfusion
[c] • = not done
[d] upper row gives scorer with cells of transfusion donors, lower row gives scores with cells of leukocyte panel.

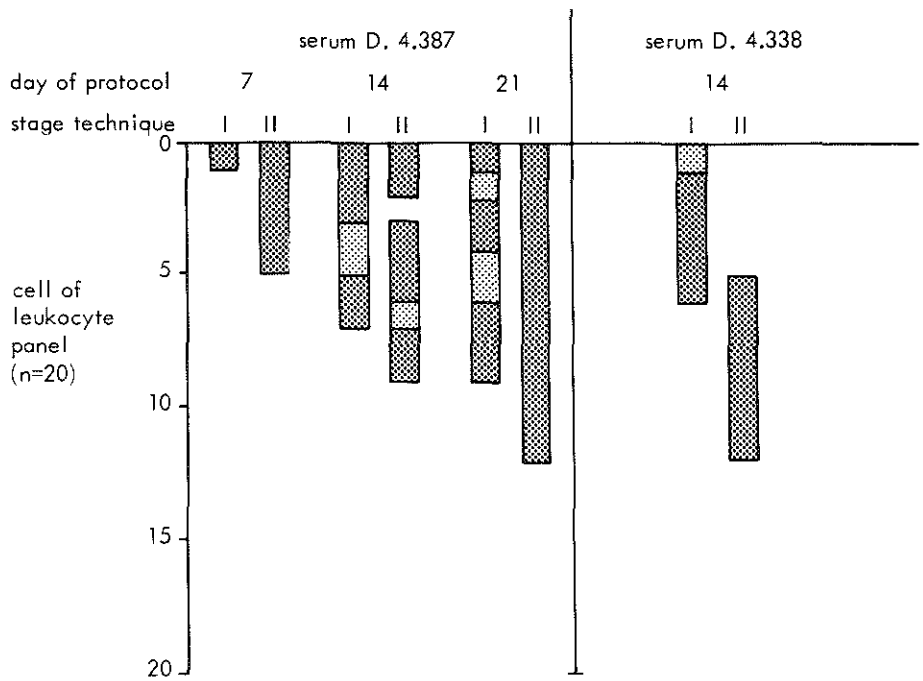
Table 2. Reactivity score[a] of sera from transfused dogs

		microlymphocytotoxicity							
		one stage				two stage			
Family	Dog	0[b]	7	14	21	0	7	14	21
		0	1	1	20	0	•	3	17
Sire	R DA3	•[c]	•	•	•	•	•	•	•
		0[d]	5	23	30	0	9	32	36
Dame	9201	•	•	•	•	•	•	•	•
		0	0	3	22	0	0	6	42
offspring	D4240	0	14	•	89	0	48	•	71
		0	1	0	1	0	0	5	7
3	D4241	0	21	48	5	0	13	10	24
		0	6	1	14	0	8	39	92
	D4242	0	59	35	95	0	10	51	95
		0	0	0	0	0	0	0	0
	D4243	0	•	•	39	0	•	•	14
		0	•	4	0	0	0	2	1
Sire	153	•	•	•	•	•	•	•	•
		•	•	•	•	•	•	•	•
Dame	930	•	•	•	•	•	•	•	•
		0	2	3	5	0	9	18	32
offspring	D4335	0	0	28	61	0	23	16	38
		0	15	15	10	0	50	66	60
	D4336	0	9	49	64	0	71	57	61
4		0	1	0	3	0	4	2	3
	D4337	0	•	•	•	•	3	•	•
		0	1	1	4	0	6	19	23
	D4338	0	4	28	55	0	15	28	22
		0	13	47	60	0	58	77	85
	D4339	0	0	89	86	0	68	66	76
		0	13	47	60	0	58	75	85
	D4340	•	•	•	•	•	•	•	•
		0	6	12	35	0	35	22	49
	4341	•	•	•	•	•	•	•	•
		0	10	22	41	0	26	70	92
	4342	•	•	•	•	•	•	•	•

[a] see methods for computation
[b] day as related to first transfusion
[c] • = not done
[d] upper row gives scores with cells of transfusion donors, lower row gives scores with cells of leukocyte panel.

Before transfusion no antibodies were found. After transfusion antibodies were found in all dogs. No prozones were noted in the titrations. Reactivity scores were generally higher in two-stage tests than in one-stage tests. This is in accordance with the observed higher sentitivity of the two-stage test (1). However, the results obtained with the cells of the leukocyte panel indicated that different specificities are sometimes determined by the two methods. An example of both (i.e. difference in sensitivity as well as specificity) is given in Figure 1. In sera taken early in the immunization period, suggestive evidence for DLA specificity was found in the reactivity patterns observed against cells of the leukocyte panel using both serological techniques. The specificities found corresponded with those present on the cells of the transfusion donors. Family studies to confirm the DLA specificity of the produced reagents were not performed. Sera taken late in the immunization period, showed high frequencies of positive

Figure 1. Reactivity of dogs after blood transfusions to cells of leukocyte panel in one (I) and two (II) stage microcytotoxicity.



Positive results indicated as bars of large black dots; weakly positive results as small black dots. The other results were negative.

results. A meaningful specificity analysis is not possible without the use of extensive absorptions. Such investigations were not performed in this study.

In one-stage tests the reactivity of the sera is more pronounced against the cells of the leukocyte panel than against those of the blood transfusion donors. No such difference is found in two-stage tests, except just before the second transfusion. Reactivity scores of below 10 against the transfusion donors and below 20 against the leukocyte panel for all sera per animal were arbitrarily taken as cut-off points, to indicate low responders. If a higher reactivity score was found, an animal was designated as high responder. More high responders were found in the two-stage test. For each method for responsiveness determination a separate analysis of the genetic data and the kidney graft survival was made.

Genetic control of immune reactivity after blood transfusions

In the limited material studied, the segregation of high or low responsiveness to allogenetic blood tranfusions was compared to the segregation patterns of the markers listed in the Method section, including sex. An example of such a family study is given in table 3. Neither one-nor two-stage responsiveness appeared to be correlated with DLA, sex or DEA 1, 4, or 5 inheritance. The segregation patterns for other DEA groups and CSA were not informative in the families studied.

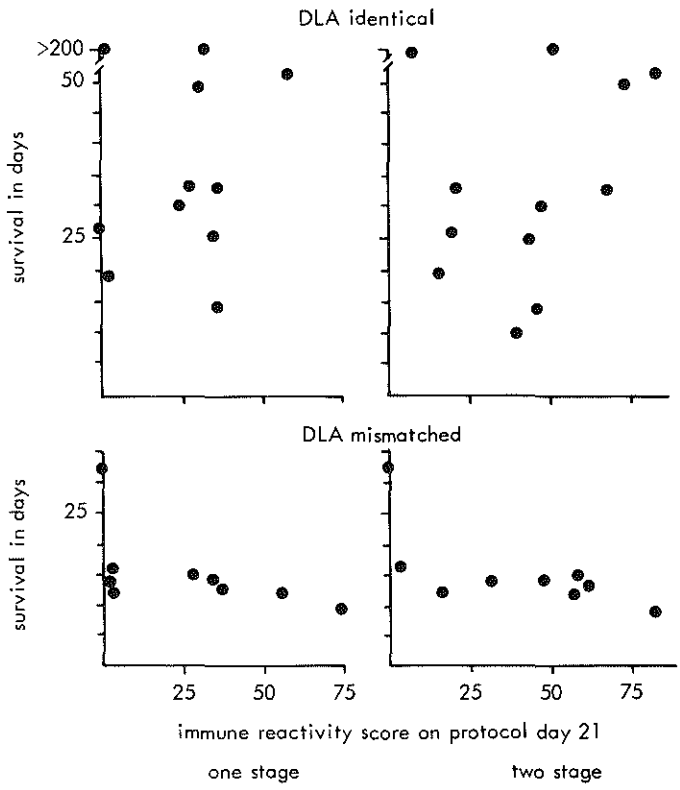
Table 3. Dog family study of immune reactivity after blood transfusions and different genetic markers

			immune reactivity				
	dogs	DLA haplotypes	I stage	II stage	sex	DEA	CSA
parents	♂	AB	not tested	not tested	♂	not tested	
	♀	CD	high	high	♀	not tested	
offspring	1	AC	low	low	♂	4,5,6,7	AY
	2	BC	high	high	♂	1.1,4,5,6,7	AY
	3	AC	high	high	♀	1.1,4, 6.7	AY
	4	BC	low	high	♀	1.1,4,5,6.7	AY
	5	AC	low	high	♀	1.1,4, 6,7	AY

Immune responsiveness after blood transfusion, donor selection and kidney graft survival

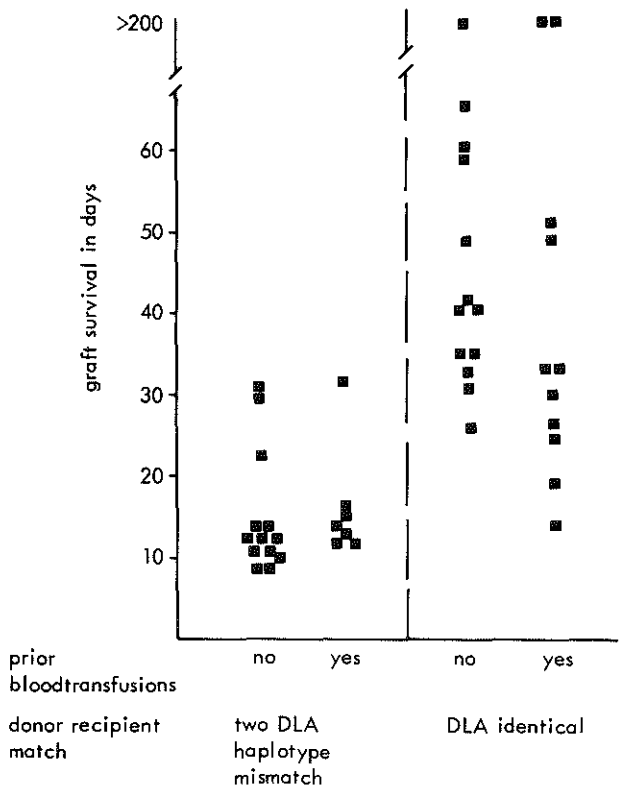
The relationship between immune reactivity scores and graft survival times are given in Fig. 2. Four animals are not included in this table because of (a) technical failure of the kidney graft (2x), (b) a positive cross match between donor and recipient, resulting in accelerated rejection (one animal that rejected the kidney within 5 days) and (c) a DLA recombination which occurred in one of the families (one animal that received a long surviving, LD identical, SD mismatched graft; this will be reported in detail with results of other DLA recombinants in the future).

Figure 2. Kidney graft survival with and without prior blood transfusions.



The results in Fig. 2 for the mismatched in one- and two-stage testing indicate that the same survival was found in recipients with widely different reactivity scores. In both tests two animals with the lowest scores are the longest survivors (35 and 16 days). The recipients of DLA matched kidneys seem to show a longer survival with higher reactivity scores in two-stage testing. This observation has a statistical significance level of just below $p = 0.05$ (see table 4). A comparison with survival times of untransfused animals is given in Fig. 3 The results in the DLA mismatched animals are not altered by transfusions. The survivals of DLA identical kidneys show approximately the same medium survival time. However, a much wider range in results can be observed in the transfused group.

Figure 3. Immune reactivity scores and kidney graft survival.



See method section for computation of reactivity scores.

Table 4. Immune reactivity scores in two-stage testing and DLA identical kidney graft survival

	Reactivity score in II stage testing		
	> 50	< 50	
survival ⩾ medium survival time (33 days)	4	2	6
survival < medium survival time	0	5	5
	4	7	11

P = 0.045
(Fisher exact test)

Discussion

The results obtained indicate that nonresponders against multiple allogeneic blood transfusions were not found. By an artificially chosen division point, animals could be divided into low and high responders. The percentage of high responders seems to be elevated in beagle dogs, in comparison to the results obtained in human patients, awaiting a kidney allograft. This difference might be due to a lower immune reactivity in the human patients with end stage renal failure than in the healthy dogs. Another possibility is the use of multiple (7) donors in one transfusion and the twice repeated administration of blood products from the same donors. This immunization scheme was chosen to ensure that each recipient of blood transfusion received the same antigenic mixture in the same sequence. Perhaps higher reactivity scores were obtained by the application of this unusual transfusion regimen. This possibility will be subjected to further study.

A search for a possible genetic control of immune reactivity after allogeneic blood transfusions is a gigantic task. A multitude of antigens is transfused in different dosages and a multitude of assays can be applied to screen the recipient's immune system for reactivity. Consistent simple genetic patterns of inheritance have been obtained in studies of the immune response in which a low-limiting-dose of a simple antigen was used for immunization and one type of antibody was used to measure reactivity (18). The majority of such investigations showed that the genetic control of the immune response to such antigens was limited to the chromosomal region coding for the MHC (for dogs, see ref. 34). If one chooses to neglect the existence of all of the extra complicating variables in blood trans-

fusions and kidney grafting in comparison to the experiments with "simplified" antigen-antibody responses, one might be tempted to expect a similar simple genetic control of immune reactivity towards all allogeneic tissues. In such a model a high or a low immune reactivity after blood transfusions would be predictive for a subsequent short or long kidney allograft survival. Such a working hypothesis can be rejected on the basis of the data obtained in the present study. The segregation patterns found in the dog families for high and low immune reactivity after blood transfusions did not correlate with the inheritance patterns of any of the other markers investigated (DLA, sex, DEA 1, 4 or 5). This excludes a simple relationship between any of these markers and an "allogeneic response locus".

Similar results were previously obtained in studies of blood transfusions given *after* a kidney allograft. Antibodies were found in most of the long and in some of the short term surviving kidney graft recipients (5). A positive crossmatch between donor lymphocytes and recipient serum was found once in this series of experiments and was again as in previous human (15,25) and dog (21) experiences correlated with an extremely short graft survival. The kidney graft data for animals with a negative crossmatch, as listed in table 4, provide suggestive evidence for a variable significance of the intensity of immune reactivity after blood transfusion. A very *low* reactivity might be beneficial to survival in the case of a DLA mismatched kidney; however, the number of animals with such scores is too low for a reliable analysis. High reactivity in two-stage testing appears to be more frequent in recipients of longer surviving DLA identical kidneys (table 4). In two-stage microcytotoxicity testing more and sometimes different (see fig. 1) types of antibodies are found than in one-stage testing. Therefore high reactivity scores in two stage testing might be correlated to the presence of enhancing antibodies, only detectable in the two-stage and not in the one stage technique.

A wider range of results around the medium DLA identical kidney graft survival time has been found in the present study, when compared to the survival times of similarly transplanted but untransfused beagles (36). This indicates the presence of two opposing tendencies; one towards prolonged, one towards shortened kidney graft survival in the transfused dogs. The tendency to a shorter survival of DLA identical grafts might be explained by the sentitization of the recipient prior to kidney grafting against minor histocompatibility antigens. Evidence has been obtained suggesting that some of these antigens are controlled by the sex chromosomes (35).

The tendency of a longer survival of DLA identical grafts might be caused by the induction of enhancing antibodies. The assumption of this balance between sensitization and enhancement will be tested in further dog kidney allografts.

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CHAPTER 7

Fourth experiment

THE EFFECT OF PRIOR PARENTAL BLOOD TRANSFUSIONS ON THE SURVIVAL OF RENAL ALLOGRAFTS FROM A DLA IDENTICAL SIBLING

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Summary

Renal allografting was performed between DLA identical beagle littermates without immunosuppressive treatment. One transfusion of 200 ml of parental blood was given 14 days prior to transplantation. A parent of the kidney donor and recipient was chosen as blood donor to induce the formation of antibodies against non DLA antigens that might enhance renal graft survival. Kidney graft survival times of transfused dogs were compared with the survival times of DLA identical non-transfused littermated. Blood transfusions did not have a significant influence on the median graft survival time. Antibodies against the kidney donor lymphocytes were not demonstrated after blood transfusions. However, antibodies were induced in three out of the six animals tested as shown by the reactivity of the sera of these animals against a lymphocyte panel. Antibodies occurred in animals with long as well as short surviving grafts.

Introduction

Blood transfusions were reported to have a beneficial effect on the outcome of renal transplants in some human patients (10, 12, 19). However, blood transfusions will lead to antibody formation in a considerable number of patients increasing the chances of a positive crossmatch between recipient's serum and donor lymphocytes. This is a contraindication to transplantation because of the high risk of the occurrence of hyperacute rejection of the graft (14). Even when lymphocytotoxic antibodies induced by blood transfusions are not directed against the kidney donor antigens, the outcome of the graft can be poor (11, 18, 20, 21), although this observation could not be confirmed in some studies (3, 5, 6). However, at least some patients will be lost for transplantation after sensitization by blood transfusions and a recent quantitative analysis of the consequences of deliberately transfusing blood for kidney transplantation showed that at best this would lead to a marginal increase in average kidney graft survival time (2). Kidney transplantation in preclinical animal models is necessary to elucidate the mechanism and possible benefits of the effects of blood transfusions on renal allograft survival. Recently such a model was described in rhesus monkeys (9). In this species a detailled knowledge of genetic determinants of the major histocompatibility complex (MHC) has been obtained (1). The limited availability of unrelated and in particular related rhesus monkeys for kidney graft experiments led us to the development of a dog model where these limitations do not prevail. The dog major histocompatibility complex has been extensively analyzed in the past (26). Gene products of various loci within and close to the MHC can be recognized (LD, SD, Ir, PGM_3). However, so-called Ia antigens which might be of relevance to graft prolongation (7) have not been identified yet in this species. In a dog model, efforts to prolong renal graft survival by kidney donor blood pretreatment, have been unsuccessful thus far (17). Antibodies were formed in 17% of the cases and led to accelerated, though not hyperacute, graft rejection. Multiple transfusions with pooled blood from non related mongrel dog donors gave rise to antibody production in all cases. A clear-cut predictable effect on graft survival was not noted. Suggestive evidence for deleterious as well as protective effects of prior blood transfusions on the kidney graft were found (Bull et al., Chapter 6). The aim of the present investigation was to find a method to separate deleterious effects of blood transfusions from protective effects by a judicious selection of blood and kidney donor. The working hypothesis was that in the dog antibodies with enhancing properties are directed

against non-DLA antigens, i.e., antigens whose genetic control is on chromosomes distinct from the one carrying the dog MHC DLA. Evidence for enhancing properties of such antibodies has been obtained in rodents (7, 13). Graft destructive antibodies would be reactive with antigens of MHC loci. This hypothesis led to the selection of a blood donor that would stimulate antibody formation against DLA as well as against non DLA antigens in the hope that a better antibody induction against minor histocompatibility antigens would occur when a major histo-incompatibility was concurrently present. A DLA identical sibling was chosen as kidney donor antigens. The results obtained thus far have failed to fulfill the aim of the investigation. The conditions required for a predictable beneficial effect of pre-transplant blood transfusions remain to be defined.

Materials and methods

Dogs, histocompatibility testing, kidney graft procedure

Prospectively tissue typed beagles of the Centraal Proefdierenbedrijf TNO, Austerlitz, The Netherlands (Dr. J. van Vliet) were used.

Histocompatibility testing was performed by serology as well as mixed lymphocyte reaction as previously reported (4, 23, 24, 25, 26). Identity for the LD antigens was defined as the complete absence of any stimulation in the mixed lymphocyte reaction in the presence of a positive control.

Kidney grafting and recipient nephrectomies were performed at the same operation. Operative procedure and postoperative care were described elsewhere (27).

Experimental design

Six groups of three SD as well as LD identical littermates of the same sex were selected. One dog out of each triplet was transfused with 200 ml of blood of a parent 14 days prior to renal allografting. One dog was used as a kidney donor for one transfused and one non-transfused DLA identical littermate. When this was technically feasible, one kidney of the non-transfused dog was transplanted in the kidney donor. No immunosuppressive treatment was given. Histological confirmation of rejection was obtained when the animal died or was killed when serum creatinine levels rose above 1,000 μmoles/liter.

Antibody screening

Sera of transfused dogs were screened for antibody activity immediately before grafting in the one and two stage modification of the microlymphocytotoxicity test (23) against cell suspensions of blood donors, kidney donors and a leukocyte panel of 18 unrelated mongrel dogs. The cell panel was selected to provide a representation of the currently defined DLA antigens. An immune reactivity score against the cell panel was computed as described elsewhere (Bull, et al., Chapter 6). Briefly it consists of expressing the antibody reactivity of a serum as a percentage of the maximum possible antibody response

Exclusions

Two dogs died because of technical failures and were excluded from the analysis.

Results

Donor recipient pairs and their survival times are given in table 1.

Table 1. Parental blood transfusions and subsequent kidney graft survival.

	Graft survival time in days		
Blood donor	Kidney donor (non-transfused)[a]	Kidney recipient (transfused)[a]	Kidney recipient (non transfused)[a]
P	– ♂	18 ♂	100 ♂
P	19 ♂	27 ♂	71 ♂
P	– ♂	28 ♂	100 ♂
P	– ♂	49 ♂	44 ♂
M	54 ♀	100 ♀	46 ♀
M	t.f. ♂	t.f. ♂	32 ♂

P = Father of the three identical siblings
M = Mother of the three identical siblings
[a] = See materials and methods
t.f. = Technical failure

Transfused dogs have a shorter survival time as compared with non-transfused dogs. The difference is not statistically significant in a Wilcoxon rank

sum test ($.05 < p < .1$). Positive cross match tests or hyperacute graft rejections were not seen. In transfused as well as non-transfused dogs a wide range in survival times was observed similar to one found previously in DLA identical donor recipient pairs (27). In three out of five triplets the transfused dogs rejected the kidney earlier than the non transfused dogs. Two out of the three untransfused dogs did not reject their graft at all. In one triplet graft survival of transfused and non-transfused littermates showed little difference (49 and 44 days respectively). In one other triplet the transfused dog did not reject his graft whereas the non transfused dog died 46 days after transplantation. The results of the screening for lymphocytotoxic antibodies are shown in table 2.

Table 2 Lymphocytotoxic antibodies in transfused recipients prior to grafting

Survival time in days	against panel		against blood donor		against kidney donor
	1 stage	2 stage	1 stage	2 stage	1 and 2 stage
18	25	25	–	+++	–
27	0	0	–	–	–
28	0	0	–	–	–
49	6	22	–	+	–
100	0	6	–	–	–
t.f.	7	14	–	–	–

Discussion

A transfusion with parental blood to the propective recipient of an SD and LD identical littermate kidney graft did not prolong the median graft survival time of transfused over non-transfused dogs. It was evident even from this small number that no consistent effect of one parental blood transfusion could be observed. In the past a similar observation was made when one blood transfusion of the kidney donor was given (17). The present study with parental blood was performed with the hope that the addition of DLA differences to non-DLA differences between blood donor and kidney graft recipient might lead to a better production of anti-non-DLA antibodies. Some rare antibodies against non-DLA antigens are reactive in microlymphocytotoxicity tests (26). The well known difficulties in establishing reliable serological techniques for minor histocompatibility antigens, leave the possibility open that non-DLA antibodies against kidney donor

antigens were produced, notwithstanding a negative cross match (15). If they were produced, no in vivo effect was demonstrated as evidenced by the absence of a change in the median survival times of the transplanted kidney. The antibodies found by one and two stage lymphocytotoxicity tests, were apparently not indicative for the formation of enhancing antibodies, since antibody production occurred in some of the long as well as the short surviving animals.

The result contradicts the working hypothesis that the careful selection of the blood donor will allow a separation of the deleterious from the protective effect of blood transfusions. Similarly, in human patients no effects of blood transfusions were noted on graft survival, if the donor was related to the recipient (22). In dogs different experimental circumstances as another transfusion schedule or the use of immunosuppression in the kidney graft recipients can be tested to confirm this first impression. The prolonged graft survival in unimmunosuppressed recipients of DLA identical kidneys has been noted previously (17, 27). Attempts are being made to elucidate the mechanism. The occurrence of monozygotic twins has been excluded as a cause for this phenomenon in the past (26). From this and other experiments it is clear that a predictable prolongation of renal allograft survival in dogs is hard to obtain (Bull et al., Chapter 6). Recent evidence was obtained in dog studies that in immunosuppressed recipients beneficial effects of blood transfusions on kidney graft survival are more easily demonstrated and can be predicted prospectively (Obertop et al., Chapter 8). More information is required on the interaction between minor and major histocompatibility, immunosuppression and blood transfusions before appropriate guidelines for a reliable induction of kidney graft protection by blood transfusions can be given.

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CHAPTER 8

Fifth experiment

PROLONGATION OF RENAL ALLOGRAFT SURVIVAL IN DLA TISSUE TYPED BEAGLES AFTER THIRD PARTY BLOOD TRANSFUSIONS AND IMMUNOSUPPRESSIVE TREATMENT

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Summary

Significant prolongation of survival of non-related DLA mismatched renal allografts has been obtained in beagle recipients receiving three blood transfusions from non-related donors prior to kidney transplantation and a low dosage of immunosuppression after transplantation. Non-transfused DLA identical or DLA 1 haplotype different littermates of the transfused dogs were used as controls. Lymphocytotoxic antibodies were formed after the blood transfusions. A quantitative immune reactivity score correlated with graft survival. Low scores prior to transplantation were found in five transfused dogs that did not reject their allografts. High scores prior to transplantation were found in four animals rejecting their graft and in one dog that survived after an abortive rejection episode. The great similarities between the results obtained in this animal model and the observations made in human transplant patients indicate that this model can be utilized for a further analysis of the possibilities of blood transfusions in protecting subsequent renal allografts from immunological rejection.

Introduction

When blood transfusions are given to human patients with end stage renal failure, different effects can be observed on a subsequent cadaver kidney allograft, depending on the recipient's response to the blood transfusion.

If antibodies are produced directed against the kidney donor, as shown by a positive crossmatch between donor's lymphocytes and recipient's serum, a high frequency of hyperacute graft rejection can be expected (9, 15).

If antibodies are produced, that are not directed against the kidney donor's lymphocytes but against third party lymphocytes, a shorter average survival time of the kidney graft is noted in comparison to patients without antibodies (7, 12, 13, 16, 17, 26).

If no antibodies are detected after blood transfusions in future kidney graft recipients, a better allograft survival is observed in those patients as compared to patients without preformed antibodies who did not receive blood transfusions (2, 8, 13, 14).

The first two observations have led to a policy of withholding blood transfusions from prospective kidney graft recipients to increase the chance of graft survival (10). However, in recent reviews figures for kidney graft survival were reported to show a downward trend (8, 20). This was at least, in part, attributed to the reluctance to transfuse potential kidney graft recipients, whereby less patients would be transplanted in the category where blood transfusions had a beneficial effect on renal graft survival.

In view of the high failure rate of human kidney grafts and the morbidity of clinical immunosuppression the development of clinically applicable methods which will lead to the prolongation of kidney graft survival is highly desirable. The inability to predict whether blood transfusions will lead to a beneficial or detrimental effect on subsequent kidney grafts indicates the need for studies in an animal model resembling the human situation. In such a model the conditions might be worked out, in which the transfusions of blood before kidney transplantation have a consistent and predictable effect on graft survival.

This report deals with a study on the effect of pretransplant blood transfusions on renal allograft survival in tissue typed dogs. The results obtained show a great similarity to the human data and appear to be a good starting point for futher evaluation of the immunosuppressive quality of pretransplant blood transfusions.

Materials and methods

Kidney graft recipients

Ten pairs of tissue typed beagle littermates of the Centraal Proefdierenbedrijf TNO, Austerlitz, The Netherlands, were used as recipients. Eight pairs were DLA-identical littermates and 2 were 1 haplotype different, 4 pairs were males, 4 pairs were females and 2 pairs were of different sex.

Kidney graft donors

Ten male mongrel dogs of unspecified breeds were used as kidney graft donors.

Blood donors

Seven non-related tissue typed mongrel dogs were used as blood donors. The donors were selected for negativity for DEA 1-1 and DEA 1-2 to avoid transfusion reactions (19).

Histocompatibility testing

Histocompatibility testing was performed by serological methods as previously described (18, 21, 22).

Blood transfusions

One dog of each pair received three 100 ml blood transfusions with weekly intervals from three different blood donors out of the pool of seven dogs. The littermate did not receive blood transfusions and served as a control.

Immune reactivity testing

One and two stage microlymphocytotoxicity tests (1) were performed before each blood transfusion and before transplantation. Sera were tested

against lymphocytes of the kidney donor, blood donors and a panel of 18 dogs of various - for the majority undefined - breeds. This panel was selected to present all the currently known DLA antigens (23). An immune reactivity score against this panel was computed and expressed as the percentage of the maximal obtainable score, including strength and titer of the reaction as weighing factors.

Kidney transplantation

Both kidneys from one mongrel donor were transplanted two weeks after the last blood transfusion in the left or right iliac fossa of a transfused and a non-transfused beagle littermate of a pair according to the technique described before (24). Bilateral nephrectomy was performed at the time of transplantation and one kidney of the non-transfused beagle was grafted in the mongrel kidney donor, when technically feasible.

Immunosuppression

Azathioprine (2 mg/kg body weight) and Prednisone (1 mg/kg body weight) was given daily per i.v. injection to all transplanted dogs postoperatively until they died because of graft rejection or until the 60th postoperative day when the experiment was terminated.

Postoperative control

Serum creatinine levels were estimated at least bi-weekly and histological confirmation of allograft rejection was obtained in all cases when dogs died before tje 60th postoperative day or when dogs were killed, when serum creatinine levels rose above 1.000 μmol/l. The Wilcoxon test was used for statistical evaluation of graft survival and immune reactivity data.

Results

Survival

After three blood transfusions, nine out of ten renal allografts survived longer

than the comparable allografts from the same donor transplanted in non-transfused dogs (table 1). One transfused animal and his non-transfused littermate were both long survivors. Graft survival times of the transfused dogs showed statistically significant prolongation as compared with non-transfused controls ($P < 0{,}01$) (figure 1). Mongrel dogs receiving kidneys from non-transfused beagles showed no difference in graft survival as compared with non-transfused beagles receiving mongrel kidneys (figure 1). All dogs received the same immunosuppressive regimen. Random dog kidney grafts from DLA mismatched donors without immunosuppressive

Figure 1. Renal allograft survival in transfused (TR) and non-transfused (NTR) beagle littermates and mongrel dogs (M) with immunosuppression and mongrel dogs without immunosuppression (C).

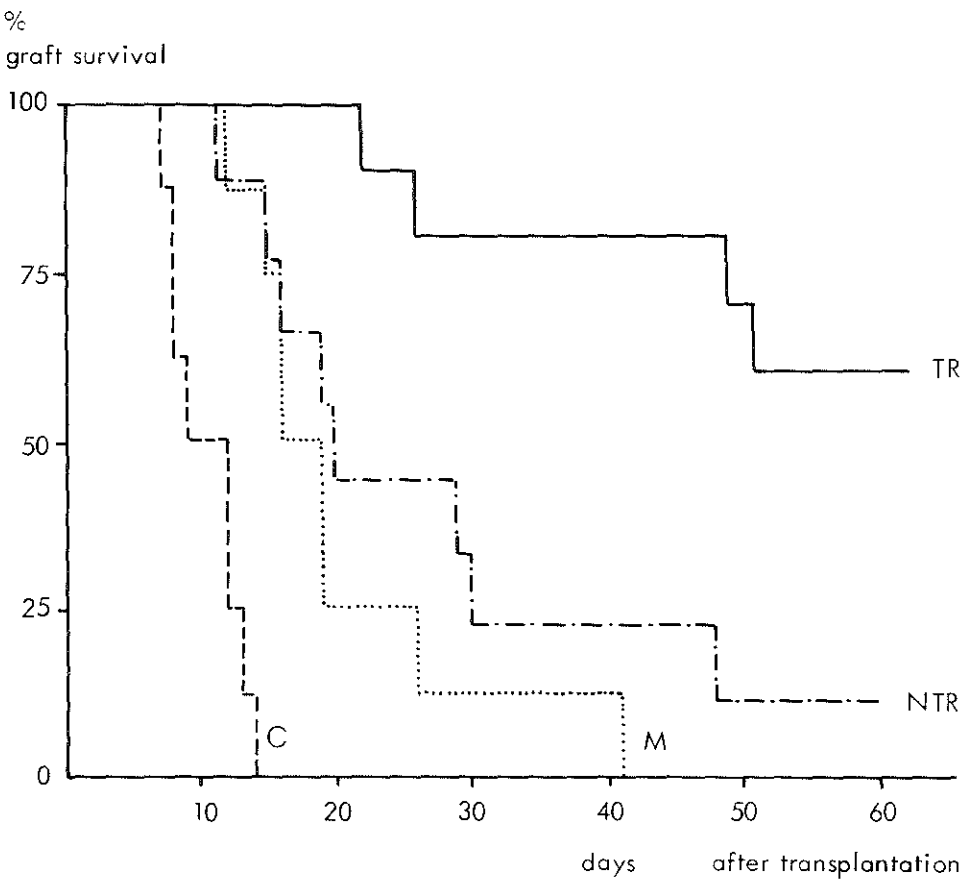


Table 1 DLA typing and renal allograft survival times.

Mongrel blooddonors	transfused beagles			non-transfused beagles			mongrel kidney donor (recipient)	
	nr	DLA typing	survival days	nr	DLA typing	survival days	nr	survival days
A. B. C	D6510 ♂	2, 5, 11/7, 4, —	101[a]	D6514 ♂	2, 5, 11/7, 4, —	11	RH115 ♂	16
D, E, F	6814 ♀	9, 4, 12/3, –, —	51	6817 ♀	9, 4, 12/3, —, —	48	RH116 ♂	26
G, A, B	6815 ♀	9, 4, 12/9, 4, 12	49	6816 ♀	9, 4, 12/9, 4, 12	29	RH117 ♂	–
C, D, E	D 716 ♂	1, 13, —/2, 5, 11	63[b]	D 717 ♂	1, 13, —/2, 5, 11	30	RH118 ♂	16
F, G, A	D6711 ♂	2, 5, 11/3, –, R15	26	6715 ♀	2, 5, 11/3, —, R15	–	RH119 ♂	–
B, C, D	D6812 ♂	9, 4, 12/9, 4, 12	> 60	D6813 ♂	9, 4, 12/3, —, —	16	RH120 ♂	15
E, F, G	D6710 ♂	9, 6, 12/7, –, —,	> 60	D6714 ♂	9, 6, 12/7, —, —	20	RH121 ♂	12
A, B, C	6516 ♀	9, 6, 12/7, 4, —	21	D6511 ♂	9, 6, 12/7, 4, —	15	RH124 ♂	41
B, E, F	6214 ♀	–, 4, —/7, –, —	> 60	6215 ♀	–, 4, —/7, —, —	> 60	RH123 ♂	19
G, A, B	6252 ♀	3, –;R15/2, 5, 11	> 60	6253 ♀	9, 4, 12/3, —, R15	19	RH133 ♂	19

[a] = graft rejection after discontinuation of immunosuppression
[b] = sepsis

Table 2 Immune reactivity scores of transfused beagles before transfusions and transplantation and their relation to transplant survival.

Beagle	Immune reactivity score[a]								
	1st transfusion		2nd transfusion		3rd transfusion		transplantation		
	1 stage	2 stage	1 stage	2 stage	1 stage	2 stage	1 stage	2 stage	survival in days
6214	3	3	4	37	1	12	1	5	>60
6252	5	0	n.t.	n.t.	n.t.	n.t.	5	7	>60
D6812	n.t.	n.t.	7	12	14	51	10	5	>60
D6510	3	3	4	37	1	12	1	5	101[b]
D 716	5	0	n.t.	n.t.	n.t.	n.t.	5	7	63[b]
D6710	3	7	43	51	32	60	18	40	>60[c]
6814	3	4	4	8	5	12	11	40	51
6815	3	5	17	47	11	51	11	36	49
D6711	4	4	5	29	29	37	17	54	26
6516	4	3	n.t.	n.t.	20	28	n.t.	n.t.	21

n.t. = not tested
[a] = see materials and methods
[b] = no rejection
[c] = abortive rejection episode

treatment survive significantly shorter (figure 1) (Bijnen, thesis, 1978).

Seven beagles that survived more than 60 days after transplantation, were called long survivors (six transfused and one non-transfused). One dog died at day 63 because of sepsis and another dog died at day 101 because of graft rejection two weeks after discontinuation of the immunosuppression. In five dogs immunosuppressive treatment was gradually diminished over a four week period. One dog died of graft rejection two weeks after immunosuppression was discontinued, whereas no signs of graft rejection were recognized in the other four dogs (three transfused and one non-transfused) more than four weeks after finishing immunosuppression.

Rejection

Four out of ten transfused dogs and only one out of ten non-transfused dogs did not show any significant elevation of serum creatinine levels 60 days after transplantation. In the other dogs three different rejection patterns could be distinguished by observing serum creatinine levels.

Type 1: acute rejection

Fast elevation of serum creatinine from normal to 1,000 μmol/l in about one week, beginning 1-2 weeks after transplantation. Two transfused beagles, five non-transfused and six mongrels showed this type of rejection (fig. 2a beagle 6514).

Type 2: chronic rejection

Gradual rise of serum creatinine from normal to 1,000 μmol/l in two weeks, beginning 2-5 weeks after transplantation. Two transfused beagles, three non-transfused beagles and two mongrels showed this type of graft rejection, as depicted in figure 2b for beagles 6815 and 6816.

Type 3: abortive rejection episode

One dog (D 6710) showed a transient rise of serum creatinine at the time his non-transfused control (D 6714) rejected his graft (fig. 2c).

Figure 2a. Posttransplant serum creatinine levels of a transfused beagle and DLA identical non-transfused control.

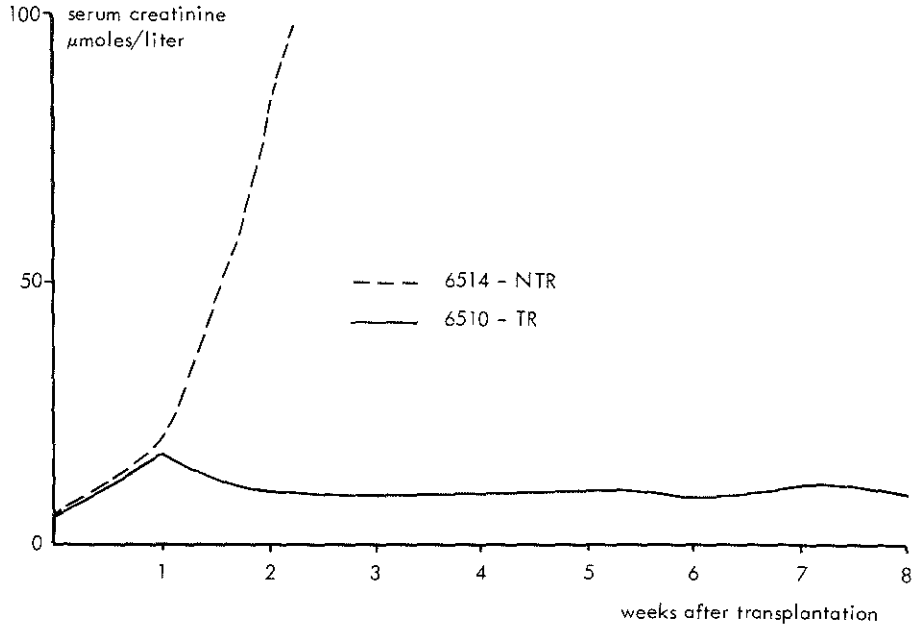


Figure 2b. Posttransplant serum creatinine levels of a transfused beagle and DLA identical non-transfused control.

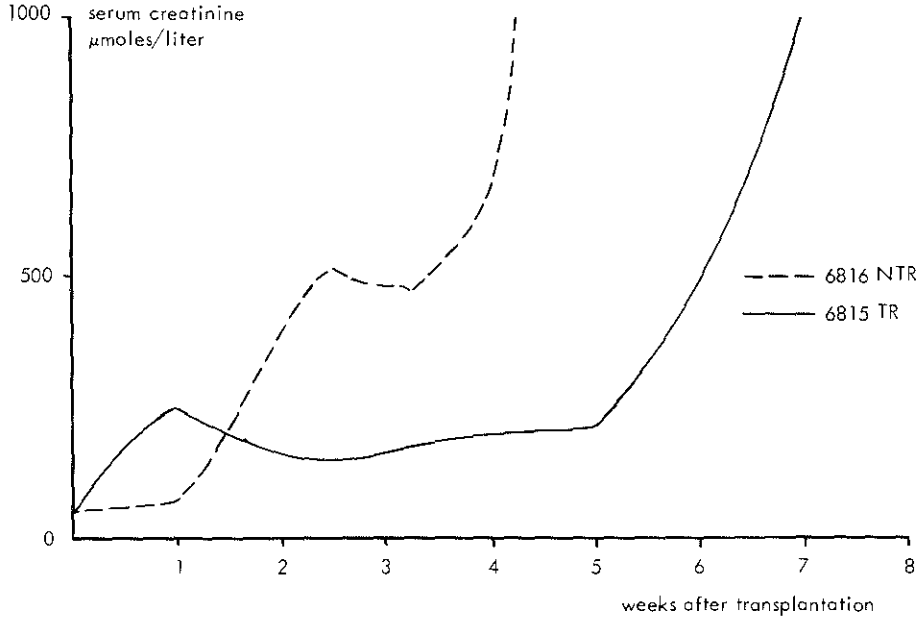
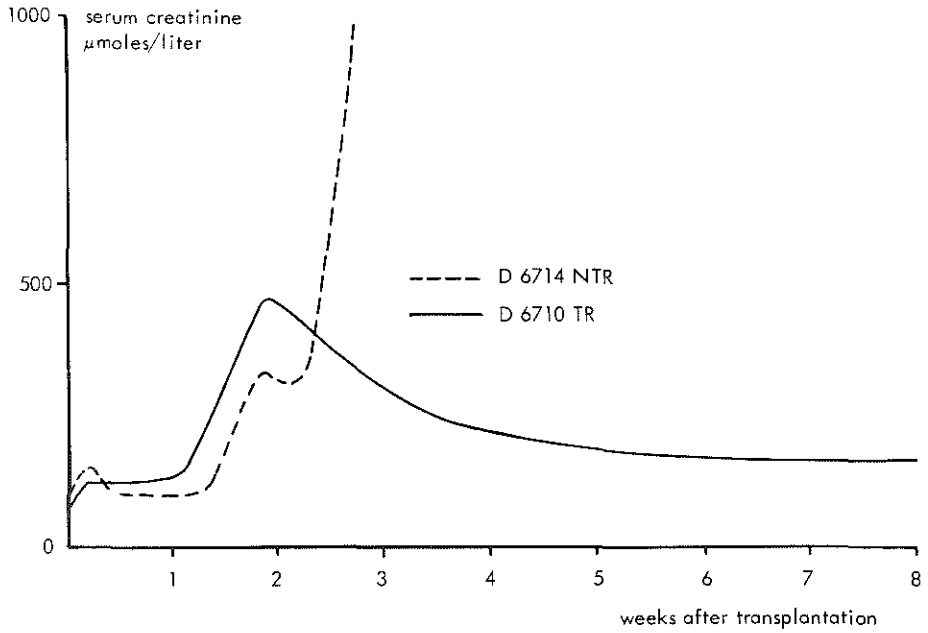


Figure 2c. Posttransplant serum creatinine levels of a transfused beagle and DLA identical non-transfused control.



Histology

Histological signs of graft rejection were observed in rejected kidneys. Mononuclear cellular infiltrates and vasculitis were found to some degree in all postmortem biopsies of the renal allografts rejected before day 60. Differences in histological pictures were mainly quantitative. Mononuclear infiltrates were small and patchy in three out of four rejected kidneys of transfused recipients whereas extensive diffuse mononuclear infiltrates were present in all but one rejected kidneys of non-transfused beagles or mongrels. Vasculitis and ischaemic changes were present in all rejected kidneys. No clear correlation between type of clinical rejection and histology was seen.

Immune reactivity

Allogeneic immunization and pregnancy could be excluded in all beagles used in these studies and no antibodies were detected prior to blood trans-

fusions. All beagles produced cytotoxic antibodies after blood transfusions. No antibodies were found against kidney donor lymphocytes (i.e. all cross-match tests were negative). The immune reactivity score computed against a panel of 18 dogs was higher for the two stage modification of the lymphocytotoxicity test, which is in accordance with the higher sensitivity of the two stage test. Several patterns in the development of immune reactivity in time were observed. In some transfused beagles the peak of antibody production was reached early in the course of blood transfusions (table 2 e.g. dogs D 6510 and 6812), whereas in others the peak was reached at a later stage and the immune reactivity score was still high immediately before transplantation (table 2 e.g. dogs D 6711, 6815, 6814). A high immune reactivity score before transplantation correlated with allograft rejection in four transfused dogs and an abortive rejection episode in one, whereas low scores correlated with lack of rejection. The difference was statistically significant ($P < 0.005$). (table 3).

Table 3. Immune reactivity scores of transfused beagles immediately before transplantation and their relation with graft rejection.

	graft rejection	no graft rejection
1 stage immune reactivity score	11, 11, 17, 18[b], 20[a]	1, 1, 5, 5, 10
2 stage immune reactivity score	20[a], 36, 40, 40[b], 54	5, 5, 5, 7, 7

[a] = 14 days prior to transplantation
[b] = abortive rejection episode

Score is significantly higher in recipients showing allograft rejection ($p < 0.005$ in Wilcoxon test for 1 and 2 stage modification of lymphocytotoxicity test)

Discussion

Transfusions of non-related third party blood had a beneficial effect on canine renal allografts in this experiment, when recipients were treated postoperatively with a low dosage of immunosuppressive drugs. The occurrence of chance histocompatibility in the transfused group was excluded by using DLA tissue typed sibling pairs as transfused and non-transfused recipients of kidneys from the same donors. Out of ten transfused dogs only four rejected their kidneys within 60 days; two in an "acute" and two in a "chronic" way. The

"acute" type of graft rejection was mainly seen in non-transfused beagle or mongrel kidney graft recipients. Two different types of graft rejection have been reported before in DLA tissue beagle littermates (24).
The immunosuppresive regimen used led to a significant prolongation of allograft survival by itself. The difference with other reports (5, 6, 25) in which a similar immunosuppresive protocol has been reported as ineffective, can be explained by the use of i.v. instead of i.m. or s.c. injections of the immunosuppressive drugs in this study. The suggestion in this study that immunosuppressive treatment is necessary to elicit the protective effect of pretransplant blood transfusions, is supported by previous experiments in tissue typed beagle littermates in our laboratory. None or only minimal allograft protection could be achieved after blood transfusions if postoperative immunosuppression was not used. In other studies in non-tissue typed dogs, prolongation of allograft survival could also only be achieved when kidney donor antigen pretreatment was combined with postoperative immunosuppression (5, 6, 25).
Recently renal allograft protection by pretransplant blood transfusions was successful too in rhesus monkeys when immunosuppressive treatment was used postoperatively (3). However, the beneficial effect of the pretransplant blood transfusion scheme used in our present study has not yet been tested without immunosuppression. It is possible that earlier transfusion experiments without addition of immunosuppression failed because the dose of transfused blood was too small, the same blood donor was used more than once, or the duration of the immunization scheme was too short (11).
In transfused dogs a high immune reactivity score before transplantation correlated with graft rejection. No accelerated graft rejection has, however, been observed and even dogs that rejected their kidney did so later than their non-transfused littermates. Low immune reactivity before transplantation occurred in transfused beagles that did not show any sign of graft rejection. If immune reactivity has a causal relationship to allograft rejection, it might be a useful parameter in the selection of the best moment for kidney transplantation in experimental animals or man.
Low immune reactivity before transplantation would be useful and could be obtained by an increase of the interval between blood transfusions, the use of leukocyte poor or frozen blood (4) and the use of immunosuppressive treatment during blood transfusions.
These speculations are in contrast with the findings of Van Es et al., which suggest that a short interval between transfusion and grafting is optimal to obtain the best protective effect of pretransplant blood transfusion on kidney grafts in rhesus monkeys (3).

The mechanism of prolongation of kidney graft survival by third-party blood transfusions is unknown and further animal studies are required to elucidate the mechanism. It is evident that the immune reactivity is altered by blood transfusions. Graft destruction as well as protection might be induced in the recipient. The use of aspecific immunosuppression might lead to suppression of the destructive properties, while permitting full expression of the protective effect. What component in the blood induces the protective effect is unknown, although there is some evidence that erythrocyte transfusions will suffice to induce this effect (4). What the effector mechanism in the host is or whether a histocompatibility difference between blood donor and kidney recipient is required remains unknown. Enhancing antibodies are however unlikely to be induced by third-party blood transfusions since blood donors and kidney donors do not have all transplantation antigens in common that are necessary to induce specific enhancing antibodies. Our findings are in favour of a non-specific immunosuppressive effect being aroused by third party blood transfusions. This effect can be observed when cytotoxic antibodies are low and immediate graft rejection is suppressed by immunosuppressive drugs. One of the possible mechanisms for this effect might be the induction of suppressor lymphocytes in the host. The data of this communication mirror the clinical observations. An excellent animal model is therefore available to study the mechanism of the protective effect of blood transfusions on renal allografts and to identify the most effective way to induce graft protection by blood transfusions.

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CHAPTER 9

General discussion and conclusions

In the previous chapters of this thesis our studies on the influence of blood transfusions on renal allograft survival in DLA tissue typed beagles have been reported. The aim of these investigations has been threefold.
First, development of a model in an outbred laboratory animal to study the relevance of blood transfusions in prospective kidney recipient on renal allograft survival.
Second, study of possible correlation between pretransplant immune reactivity after blood transfusions and graft survival, searching for predictive criteria for graft survival in transfused future allograft recipients.
Third, identication of the mechanism of the beneficial effect of blood transfusions on renal allograft survival.

A beneficial effect of pretransplant blood transfusions on renal allograft prognosis in man has been reported since 1973 (4, 12a, 39, 42, 42a, 56, 57, 71, 79, 87, 96, 97, 99, 102, 105). By 1966 a paradoxical effect after immunization had been observed since the expected deleterious effect of multiple pretransplant blood transfusions on the renal allograft was not seen (27, 60, 81, 85). However, the influence of pretransplant blood transfusions on graft survival can be overshadowed by many other conditions in the clinical situation. HLA compatibility (5, 24, 38, 54, 55, 57, 58, 82, 83, 92, 93, 98, 103, 107, 112, 114, 117, 118, 120, 121, 123), the presence of preformed cytotoxic antibodies (42a, 51, 54, 57, 89, 92, 93, 99, 11, 117, 118, 121, 137) and the quality of postoperative patient care (100, 104, 107) have their impact on graft survival.

Although critically ill patients, older patients and hyperimmunized patients are accepted for renal grafting at this time, the gradual decline of one year renal allograft survival, with the slight increase in one year patient survival cannot be explained by these factors (56, 138). A decrease in the number of blood transfusions in prospective renal transplant recipients is probably responsible for deterioration in overall one year graft survival results.

Sensitization by transfusions of whole blood has always thought to be dis-

advantageous to prospective recipients since the presence of antibodies was correlated with accelerated or even hyperacute graft rejection (76). Because there is a correlation between duration of hemodialysis (73), number of blood transfusions, and cytotoxic antibody production (115), a reduced pretransplant hemodialysis period has been advised and transplantations are being carried out after only few or no previous blood transfusions. The protective effect of blood transfusions on human renal transplants has been pointed out by Opelz et al. in several (most of them retrospective) studies (96, 97, 99, 102, 105). Yet some questions have to be answered before an universal blood transfusion policy will be accepted by every transplantation physician or nephrologist (4). Experiments in laboratory animals are a proper way to test the effect of pretransplant bloodtransfusion in well controlled protocols.

In small rodents, blood transfusions can prolong renal allograft survival indefinitely, in some donor recipient strain combinations (34, 35, 77). Donor blood has always been used as pretreatment in these studies and active immunological enhancement is thought to be the mechanism (see ref. 136 for review).

Reports on prolongation of renal allografts by donor antigen pretreatment without additional immunosuppressive therapy in dogs are scarce (75, 149).

Only a few investigators report successful renal graft enhancement in dogs by pretreatment with subcellular donor antigen fragments in combination with suboptimal doses of immunosuppressive drugs (52, 53, 147, 148).

Some data has recently become available on renal graft prolongation by third party blood pretreatment and post-operative immunosuppressive treatment in rhesus monkeys (31) and prelimary data in mongrel dogs (1). As of yet, no studies on dogs have been published in which intravenous donor blood pretreatment *without* immunosuppression was succesful in prolongation of graft survival, but no histocompatibility matching was or could be performed.

9.1. Blood transfusions and renal allograft survival in DLA tissue typed beagles

Because of the availibility of beagle dogs in our laboratory and the extensive experience by earlier transplantation and genetic studies in this animal (14, 15, 16, 44, 45, 46, 63, 65, 67, 139, 140, 141, 142, 143, 144, 145, 146) DLA tissue typed beagles have also been used in our experimental protocols. Litter size and shortness of generation time make the dog applicable for studies in which the effect of histocompatibility on allograft survival is tested (140). Well controlled series of renal allografting without the use of immuno-

suppressive treatment are possible in DLA tissue typed siblings. The effect of matching for loci of the major histocompatibility complex on renal allograft survival has proved to be relevant in related and unrelated donor recipient pairs. (140, 143, 144, 145, 146). Since matching for DLA in beagle littermates donor recipient combinations could be performed in our laboratory immunosuppression was thought to be superfluous in testing the effect of blood transfusions on graft survival. In one of our experiments we have attempted to achieve prolongation of renal graft survival by one transfusion (200 ml) of donor blood prior to grafting in DLA identical, DLA 1 and 2 haplotype different donor recipient combinations (chapter 4). A significant influence of histocompatibility matching was found as having been shown before in untreated beagle littermates (145). 17% of the dogs responded with antibody formation before transplantation and kidneys were rejected in an accelerated way by "responders" although no hyperacute rejection was seen. "Non-responsiveness" after one blood transfusion correlated with a somewhat longer mean survival time in the non-identical groups as compared with non-transfused controls, but no statistical significance could be determined. This negative result confirms the difficulty in obtaining active enhancement of renal graft without immunosuppressive therapy in dogs.

Other experiments have been described that were initiated by the findings of Halasz et al. (48) in 1964. The authors found significant prolongation of renal allograft survival in dogs by two s.c. injections of donor blood prior to transplantation without further treatment. The results were somewhat in contradiction with other reports in which massive quantities of donor antigens were used (49). The publication has been referred to as an example of active immunological enhancement in the dog. Halasz's protocol has been repeated by us in DLA 2 haplotype different beagle littermates. In the study of Halasz et al. (48) no tissue-typing was feasible and a chance histocompatibility matching in the pretreated groups was theoretically possible. In our study prolongation of renal graft survival was not observed after s.c. blood injections. The grafts were rejected in an accelerated way and the mean graft survival time was shorter as compared with animals that had been pretreated with one i.v. donor blood transfusion and with non-pretreated controls. (Chapter 5). Timing of transplantation, dosage of antigen and route of injection are probably important in the balance between accelerated rejection and graft enhancement, as has been previously stressed. (6).

No protective effect (active enhancement?) on renal allografts could be obtained in our protocols by pretreatment with donor blood. Increase of the volume of the transfused donor blood was not feasible and multiple

donor blood injections would probably give rise to antibody production and a higher percentage of dogs rejecting their graft in an accelerated fashion, as described by others (23).

Three *third party* blood transfusions were given in another study (Chapter 6) in which pooled blood of seven unrelated mongrel blood donors was used to transfuse four beagle families (Chapter 6). No effect of blood transfusions on renal allograft survival was obtained and the effect of immune reactivity, as measured by lymphocytotoxicity testing, on allograft survival after blood transfusion will be discussed in the next paragraph. No hyperacute rejection was seen although high levels of cytotoxic antibodies were present in some graft recipients prior to transplantation. However, hyperacute rejection of renal allografts has not been observed by us in dogs, as has been reported in man (71) and rabbits (28).

Furthermore, we have described an experiment in which we used one parental blood transfusion to pretreat the beagle recipient of a DLA identical littermate renal allograft in order to induce enhancing antibodies and to prevent positive crossmatching. A DLA identical sibling was used as a non-transfused control kidney recipient. (Chapter 7). The wide variation in graft survival times of the DLA identical kidneys in transfused and non-transfused littermates suggests both cytotoxic and enhancing effects of blood transfusions. But no correlation between blood transfusions and graft survival could be shown. Addition of postoperative immunosuppressive treatment may strengthen the weak effect of parental blood transfusions by inhibiting the rejection governed by minor histo-incompatibilities. This was however not tested.

In our last set of experiments a low dosage of i.v. injected immunosuppressive drugs was given postoperatively. (Chapter 8). Results as reported by others in so-called active enhancement studies in dogs (52, 53, 147, 148) and blood transfusions experiments in rhesus monkeys (31) and dogs (1) seemed to suggest the importance of immunosuppression. Three *third party* blood transfusions (from different blood donors) were given to prospective recipient dogs. Kidneys from mongrel dogs were grafted in transfused beagles and non transfused DLA identical or DLA 1 haplotype different littermates in pairs. Significant prolongation of graft survival could be observed after blood transfusions and 50% of transfused renal allograft recipients did not show any clinical sign of rejection. The aim of our studies as pointed out in the beginning of this chapter, has thus been reached since a protocol for testing the effect of blood transfusions in the dog has been obtained and will be helpful in further experiments.

9.2. Antibody production and renal allograft survival

The presence of preformed antibodies in the prospective human graft recipient is regarded as a poor prognostic sign for the outcome of the graft. When antibodies are directed against the kidney donor's transplantation antigens, hyperacute rejection will occur in man in most cases. But even in the absence of a positive crossmatch between recipient's serum and donor's cells a deleterious effect on renal allograft has been described in many communications (7, 51, 89, 92, 93, 117, 118, 121). More recently however excellent outcome of renal grafts has been reported in the presence of preformed cytotoxic antibodies (8, 9, 12a, 17, 36). Improvement of crossmatching by more sensitive techniques will prevent renal allografting in a recipient that is presensitezed against the kidney donor (17, 26).

Antibodies are produced after pregnancies, former allotransplantations, and blood transfusions. Females have a higher percentage of preformed antibodies although a one year graft survival seems not to be affected and HLA compatibility is less relevant in females than in males (7, 103). Antibodies induced by a former kidney transplant seem to have a deleterious effect on second graft survival (51, 101). The duration of hemodialysis period correlates with the number of blood transfusions (73) and antibody production (91) Fifty percent of the hemodialysis patients does not form antibodies at any time during hemodialysis, according to Opelz et al. (94, 96). Other investigators report a gradual increase of the percentage of sensitized patients after increase of the number of blood transfusions (115). Unresponsiveness after multiple small blood transfusions in human volunteers was only 20% and half of these non-responders had lost antibodies formed during the transfusion period and were thus, secondary unresponsive (37). The loss of antibodies during hemodialysis and after a previous renal transplant has also been reported by others (51, 94). Regardless of whether unresponsiveness of a prospective kidney recipient is a genetically determined condition or has been induced by blood transfusion, renal allograft survival is probably superior in non-responders (3, 42a, 86, 97, 102, 105), although it has been reported that transfused patients with cytotoxic antibodies have a better outcome of their graft than non-transfused patients (12a, 42a, 97, 102, 105). Presence of antibodies after blood transfusions may always be deleterious for the patients. Many prospective recipients will be excluded from renal transplantation because of a positive crossmatch test (4, 42a) and patients with HLA lymphocytotoxins against a high percentage of a leukocyte panel may reject their graft in an accelerated way in spite of a negative crossmatch (89). Use

of leukocyte poor (21) and frozen blood (12a, 42, 42a) does not induce antibody production since leukocytes and probably platelets are the antigeneic substances in human blood. In a report by Fuller et al. the beneficial effect of frozen blood on human renal allografting in the absence of cytotoxic antibody production has been reported (42, 42a). Absence of cytotoxic antibodies after blood transfusions will thus be beneficial for the transplant candidate.

Blood transfusions may also produce non cytotoxic antibodies or enhancing antibodies. Anti B-cell antibodies, that can be detected by B-cell crossmatch tests (32), may have an enhancing effect and may correlate with blocking factor activity in mixed lymphocyte reactions or other in vitro analogues of the cellular rejection reaction (13). Blocking factor activity has been reported in human sera of transfused patients and renal allograft recipients. A correlation between blocking factor activity in MLR and graft prognosis has been reported (116, 126, 127, 128), although these data are not confirmed by other studies in man and experimental animals (50, 72, 106).

Enhancing antisera can be produced by immunization by donor antigens in rodents (33, 41, 62). Active enhancement has been suggested to be the mechanism of prolonged kidney graft survival in rats after blood transfusions (34, 35, 77, 78), although passive transfer of serum of long surviving transfused rats did not give graft protection in non-transfused rats (35). Enhancing or blocking antibodies has been reported once in dogs (63), but passive transfer of serum of long surviving recipient dogs was without effect on allograft survival (1).

In our experiments in tissue typed beagles, (Chapter 4 and 5) the presence of positive crossmatches as detected by one and two stage modifications of the microlymphocytotoxicity tests led to accelerated graft rejection in most cases, although accelerated graft rejections were also seen when the crossmatches were negative. After one donor blood transfusion, the percentage of responder dogs was low and no significant prolonged survival in non-responder dogs was seen (Chapter 4). Responsiveness seemed to be dependent on the number of blood transfusions, since dogs that received multiple transfusions of non-related donor blood produced cytotoxic antibodies and no non-responsiveness could be observed (Chapter 7 and 8).

After subcutaneous injections of very small doses of donor blood in DLA non-identical littermates, antibodies could be detected in 30% and accelerated graft rejection was the case in almost all s.c. injected animals regardless of the presence of antibodies (Chapter 5). The absence of antibodies in the other

70% may be explained by the used techniques, that are possibly too insensitive to detect all cytotoxic antibodies.

The difference in graft survival between DLA non-identical littermates treated with i.v. and s.c. blood can be due to the difference in the route of injection, the dosage of antigen, and the number and timing of the injections (Chapters 4 and 5). Analysis of these factors seperately in DLA tissue typed beagles would be too laborious and it suffices to say that cytotoxic antibodies were formed both by s.c. as by i.v. pretreatment with donor blood without prolongation of renal allograft survival.

A simple inheritance pattern of immune responsiveness was looked for in another study (Chapter 6). Four beagle families were transfused with pooled blood of non-related third party blood donors and segregation of high and low responsiveness was studied. No simple relationship between a hypothetical immune response locus and other genetic markers could be observed and there is little evidence of responsiveness being a genetically determined condition in the beagle dog. All dogs produced alloantibodies after three third party blood transfusions and graft survival was not greatly influenced by the degree of immune reactivity against a panel. The height of antibody production can be the result of an unusual transfusion schedule. Pooled blood had been used, and the same dogs were used as blood donors more than once in the same recipients (Chapter 6).

In an attempt to stimulate enhancing antibody production without the chance of inducing a positive crossmatch test, beagle littermates were pretreated with one blood transfusion from one of the parents of the kidney donor and his DLA identical recipient, whereas a third DLA identical littermate was used as a non-transfused control recipient (Chapter 7). No antibodies against the kidney donor were found in this protocol and antibodies against blood donor and a random lymphocyte panel correlated with a short allograft survival in one dog, whereas another dog with a low immune reactivity after one parental blood transfusion survived indefinitely as did two non-transfused animals.

A strong predictive value of antibody production as expressed as immune reactivity, computed against a random lymphocyte panel, could be observed in our last study (Chapter 8) when three *third party non-pooled* blood transfusions prior to grafting were combined with immunosuppressive therapy given postoperatively. All animals produced antibodies when tested against the panel and the level of immune reactivity was predictive for renal graft success. High immune reactivity correlated with graft rejection or an abortive rejection episode in one dog, but no

hyperacute or even accelerated rejection was observed and all transfused dogs survived as long or even longer than the non-transfused DLA tissue typed littermate controls. No graft rejection was seen when immune reactivity immediately before transplantation was low. The two stage lymphocytotoxicity test used for the estimation of immune reactivity is more sensitive than the one stage test. Our data confirms clinical observations that third party blood transfusions given prior to transplantation can give renal graft protection in the absence of cytotoxic antibodies. In the presence of antibodies no accelerated rejection is expected as long as crossmatching is negative. Immune reactivity only immediately before transplantation correlates with renal allograft prognosis in transfused dogs and the effect of the length of interval between last transfusion and transplantation will be tested in future experiments.

9.3. Mechanism of the effect of pretransplant blood transfusions on renal allografts

Three possible mechanisms will be discussed in the next paragraph.

1. Blood transfusions lead to selection of prospective graft recipients on basis of responsiveness which is a genetically defined condition related to allograft rejection.
2. Blood transfusions induce specific immunosuppression of the allograft rejection reaction.
3. Blood transfusions induce aspecific immunosuppression of the allograft rejection reaction.

9.3.1. Unresponsiveness as a genetically defined mechanism

Unresponsiveness after multiple stimuli by blood transfusions with whole blood has been reported in man and the genetical determination of responsiveness has been suggested in man (57) as in mice (10).

The increase in the number of blood transfusions results however in decrease of unresponsive patients (37, 115) and beagle dogs in our studies. And some of the so-called immune unresponsiveness may be secondary since transient elevations of antibody titers have been reported in man (37, 94, 96). The

presence or absence and quality of antibodies in man and experimental animals depends greatly on the sensivity of the techniques used (26). When responsiveness on non-related blood transfusions was studied in beagle families segregation patterns of high and low responsiveness did not correlate with simple inheritance patterns and a relation of an immune response locus with other genetic markers seems unlikely at least in the dog. Our experiments do not support the theory of a genetical determination of immune responsiveness in the dog.

9.3.2. Blood transfusions induce specific immunosuppression

Antibodies are formed by blood transfusion and are detected in lymphocytotoxicity tests. Leukocytes and platelets are responsible for antibody formation directed against products of the major histocompatibility complex. When cytotoxic antibodies are directed against transplantation antigens of the kidney donor, they can give rise to allograft rejection in cooperation with cellular mechanisms. Non-cytotoxic antibodies could have a protective effect on the renal allograft by moderating the rejection mechanism on a peripheral or central level. These antibodies could block the afferent limb of graft rejection reaction by preventing initial graft recognition and no cytotoxic antibody production will follow, or these non-cytotoxic antibodies combined with antigen in antigen antibody complexes could produce a central inhibition of immunocompetent cells. Antibodies or antigen antibody complexes also could block the efferent limb of the graft rejection reaction, possibly by occupying the antigen receptor sites. A correlation between blocking activity in vitro and immunological enhancement has been reported in man (13). As a rule blocking antibodies are not detected by lymphocytotoxicity tests. Although a correlation between cytotoxic and blocking antibody activity has been found (127, 128), and sera of transfused patients or renal allograft recipients can show blocking factor activity in vitro models of cellular rejection like mixed lymphocyte cultures (116, 127, 128). Blocking factor activity of sera of these patients correlates with excellent outcome of renal allografts in man. However, this correlation could not be confirmed by others in man as well as in experimental animals (72, 106). Blocking antibodies are possibly directed against HLA-D determinants and may be equal to B-cell antibodies that can possibly be detected in human recipients by a weak positive crossmatch (32) and analogy with anti Ia antibodies in mice has been suggested (25, 61). Since cytotoxic and blocking antibodies will be induced by the same stimulus the ba-

lance between graft protection and rejection will be delicate after blood transfusions. It is possible that blocking antibodies can only have their protective effect in the absence of cytotoxic antibodies as suggested for beagle dog recipients in our studies. Kidney graft survival after blood transfusions in dogs, with low or absent immune reactivity, is longer as compared with non-transfused dogs, whereas both have no cytotoxic antibodies. The protective effect is not due to the mere absence of cytotoxic antibodies but a definite result of an induced specific protective mechanism. Presence of blocking antibodies has not been tested in our dogs yet and results of our experiments are not very much in favor of a specific immunosuppressive action since a strong protective effect could be seen after only three transfusion of third party donor blood. Blocking or enhancing antibodies have to be directed against donor transplantation antigens and probably against HLA-D derived antigens.

Therefore kidney donor blood will be necessary to elicit specific antibodies because of the polymorphism of the HLA-D locus. Some clinical observations are in favor of an increased graft protection after an increasing number of transfusion (96) others are not (57).

9.3.3. Blood transfusions induce aspecific immunosuppression

Three transfusions of non related blood gave a moderation of allograft rejection in 60% of the transfused beagle dogs in our experiments (Chapter 8). Transfusions of frozen blood without leukocytes in man gave as good graft protection as whole blood whereas practically no cytotoxic antibodies could be detected as reported by Fuller et al. (42, 42a). It is attractive to speculate on the existence of an aspecific immunosuppressive mechanism independent of transplantation antigens. However no such aspecific immunosuppressive mechanism is evident, although the immunosuppressive activity of polysacharides as described by Calne et al. (19) and the protective effect of leukocyte poor blood may be based on the same unknown mechanism. Stimulation of suppressor T cells derived from the spleen may be responsible for inhibiting graft rejection in an aspecific immunosuppressive action (12, 43).

In conclusion, we can say that a significant prolongation of allograft survival in DLA tissue typed beagles was achieved after three *third party* blood transfusions prior to transplantation, when immunosuppressive treatment

was given post-operatively. *Pooled third party* blood transfusions did not protect renal allografts from rejection without additional immunosuppression, possibly because of the DLA lymphocytotoxins that were produced against a high percentage of a panel, probably by iterative transfusions of the same blood donors.

Attempts to obtain active enhancement of renal allografts by i.v. and s.c. injections of blood of the prospective kidney donor or of one of the parents of the prospective kidney donor were unsuccesful, although no immunosuppressive treatment was given to those dogs.

DLA antibodies detected after i.v. or s.c. injections of donor blood led to accelerated graft rejection, but no hyperacute graft rejection was observed. Accelerated graft rejection was also found after blood pretreatment in spite of a negative crossmatch. The technique used may be too insensitive to detect DLA antibodies in all cases.

High and low immune reactivity against the leukocytes of a random dog panel was observed in the sera of beagle families that had been treated with pooled third party blood transfusions. Inheritance of "responsiveness" was studied and no simple inheritance pattern could be recognized.

Immune reactivity prior to kidney transplantation had a highly significant value in the prediction of graft rejection in beagles that had received three third party blood transfusions before grafting and immunesuppressive treatment after grafting. Low immune reactivity correlated with prolonged graft survival and high immune reactivity correlated with graft rejection. No support could be found in our experiments for unresponsiveness as a genetically defined phenomenon. All dogs seemed to be able to respond with alloantibody production after blood transfusions and secondary unresponsiveness could be found since high immune reactivity after blood transfusions disappeared before transplantation. Active enhancement was probably not the mechanism responsible for graft protection in our experiments. It seems unlikely, in the light of the polymorphism of DLA, that a strong graft protection could be obtained in 60% of the recipient dogs after only three third party (non-related) blood transfusions since in active enhancement antibodies elicited by the transplantation antigens of the kidney donor play a role. Also in those experiments, in which active immunological enhancement was attempted by pretreatment with donor or parental blood, no prolongation of renal allograft survival was seen. Therefore, an aspecific protective mechanism seems to be most likely, by which pretransplant blood transfusions can mitigate the graft rejection reaction.

Blood transfusions are helpful in protection of the future allografts in

experimental animals as well as in man, but the induction of lymphocytotoxic antibodies remains a serious side-effect of blood transfusions since these antibodies may lead to hyperacute or accelerated graft rejection or at its least to exclusion or delay of renal allografting in transplant candidates because of positive crossmatch tests.

Therefore, blood transfusions have to ideally be carried out without the chance of inducing cytotoxic antibodies. This may be achieved by the use of frozen-thawed blood, leukocyte poor blood, the use of immunosuppression during transfusion or increase of the interval between transfusions. When antibodies can still be detected after pretransplant blood transfusions, it seems advisable to perform kidney transplantation at the moment that the immunereactivity of the patient serum against a leukocyte panel is low or absent.

Before a general blood transfusion policy can be designed more research in laboratory animals will be necessary. Because of its striking similarity to the human situation our experimental protocol in DLA tissue typed beagles as presented in this thesis, can be used in further transfusion and transplantation studies.

SUMMARY

In this thesis studies on the effect of pretransplant blood transfusions on renal allograft survival in the dog are reported. These studies were initiated by the fact that blood transfusions given to patients with end stage renal failure are reported to have a protective effect on the future renal allograft. Deterioration of worldwide kidney transplant survival in recent years may be caused by the policy to avoid blood transfusions to prospective kidney graft recipients because of the chance of sensitization that may lead to hyperacute or accelerated graft rejection.

After an introduction to this thesis in Chapter 1, the literature on the effect of blood transfusions on cytotoxic antibody production and renal allograft survival in mean is discussed in Chapter 2.

Although many observations in human renal allograft recipients are in favor of the benificial effect of pretransplant blood transfusions on the kidney graft, many factors can obscure this effect and no parameters are available to predict the outcome of a renal allograft after blood transfusions. Transfusion and transplantation experiments are necessary to elucidate the mechanism of the protective effect of blood transfusions and to develop a transfusion policy in prospective human kidney recipients.

In Chapter 3 a review is given of the literature on experiments in laboratory animals in which the effect of pretransplant blood transfusions on renal allograft survival was tested. The attention is focused on the reports of experiments in the dog. In this animal prolongation of renal allograft survival by prior blood transfusions is hard to obtain in contrast with the findings in similar experiments in small rodents.

The essential part of this thesis is our own experimental work, reported in the Chapters 4, 5, 6, 7, and 8. Studies on the effect of pretransplant blood transfusions on renal allograft survival in DLA tissue typed beagles were performed in the Laboratory for Experimental Surgery of the Erasmus University, Rotterdam, the Netherlands. The five chapters are written in the form of scientific papers. Chapter 4 and 5 have been published in Transplantation. Chapters 6, 7 and 8 have been submitted for publication.

In Chapter 4 (first experiment) the effect of donor blood pretreatment on cytotoxic antibody production and renal allograft survival in DLA identical and non-identical beagle littermates is reported. Antibody production and accelerated graft rejection was found in 17% of the dogs. Non-responders did not show a significant prolongation of renal allograft

survival. Genetical determination of responsiveness is suggested in this chapter.

In Chapter 5 (second experiment) the report of an experiment in 2 DLA haplotype different beagle littermates is given. Future renal allograft recipients were pretreated with s.c. injections of donor blood. Accelerated graft rejection was found, which is in contrast with earlier findings by others, reporting active enhancement of canine renal allografts in a similar experimental protocol. The importance of tissue typing in these types of studies is stressed.

In Chapter 6 (third experiment) transfusion and kidney transplantation experiments are described in beagle families. Dogs received transfusions of pooled non-related blood and low and high immune reactivity tested against a random dog leukocyte panel was recognized. No simple inheritance pattern of responsiveness was found. Renal allograft survival was not greatly affected by the pretransplant blood transfusions and immune reactivity correlated with the outcome of the graft only in some animals.

The experiment reported in Chapter 7 (fourth experiment) deals with the effect of one pretransplant transfusion of parental blood on the survival of DLA identical sibling renal grafts. Parental blood was used to induce non-DLA (enhancing?) antibodies. No accelerated graft rejection was seen neither was a definite prolongation of renal graft survival.

In the experiment described in Chapter 8 (fifth experiment) immunosuppressive treatment was given postoperatively and third party blood was transfused three times prior to grafting of a non-related, non-identical kidney in a beagle recipient. A non-transfused DLA identical or 1 haplotype different littermate served as control. Prolongation of allograft survival was highly significant and 50% of the transfused recipients did not show any sign of graft rejection. Immune reactivity prior to transplantation showed a highly significant correlation with kidney graft prognosis.

In Chapter 9 the foregoing experiments are discussed and hypotheses for the mechanism of the protective action of pretransplant blood transfusions are given. It is concluded that pretransplant blood transfusions can protect canine renal allografts from immunological rejection. Blood transfusions have ideally to be carried out without the chance of inducing cytotoxic antibodies. When antibodies can still be detected, after pretransplant blood transfusions, it seems advisable to perform kidney transplantation at the moment that the immune reactivity of the recipient is low. An excellent model has been obtained in the dog to develop a blood transfusion policy, in order to prevent renal allografts from immunological rejection in man.

SAMENVATTING

In dit proefschift worden experimenten beschreven, die betrekking hebben op het effect van bloedtransfusies op de niertransplantaat overleving bij de hond. De uitvoering van deze experimenten werd gestimuleerd door het feit dat bloedtransfusies gegeven aan patiënten met een terminale nierinsufficientie een beschermend effect op het toekomstige niertransplantaat zouden hebben. Een achteruitgang van niertransplantaat overleving in de laatste jaren is wellicht te wijten aan het beleid om bij toekomstige niertransplantaat ontvangers geen bloedtransfusies te geven in verband met de kans op sensibilisatie, waarna hyperacute of versnelde transplantaat afstotingen mogelijk zijn.

In hoofdstuk 1 wordt een inleiding tot dit proefschrift gegeven. Hierna volgt een overzicht van de literatuur betreffende het effect van bloedtransfusies op de vorming van cytotoxische antistoffen en niertransplantaat overleving in hoofdstuk 2.

Hoewel een aantal studies wijzen op een gunstig effect van voorafgaande bloedtransfusies op het niertransplantaat, kunnen vele factoren dit effect maskeren. Er zijn geen parameters, die het succes van een niertransplantatie na bloedtransfusies kunnen voorspellen. Transfusie en transplantatie experimenten zijn noodzakelijk om het mechanisme van het beschermde effect van bloedtransfusies op te helderen en om een bloedtransfusie beleid te ontwikkelen voor toekomstige ontvangers van een niertransplantaat.

Hoofdstuk 3 bevat een samenvatting van de publicaties van experimenten in proefdieren, waar het effect van voorafgaande bloedtransfusies op niertransplantaat overleving werd onderzocht. In het bijzonder wordt de aandacht gevestigd op publicaties van niertransplantatie experimenten in de hond. In dit proefdier wordt verlenging van de transplantaat overleving na voorafgaande bloedtransfusies slechts met moeite verkregen, in tegenstelling tot soortgelijke experimenten in kleine knaagdieren.

Het belangrijkste gedeelte van dit proefschrift wordt gevormd door ons eigen experimentele werk, dat wordt beschreven in de hoofdstukken 4, 5, 6, 7 en 8. Het onderzoek naar het effect van bloedtransfusies voor transplantatie op de overleving van niertransplantaten in getypeerde beagles werd verricht in het Laboratorium voor Experimentele Chirurgie van de Erasmus Universiteit te Rotterdam.

De vijf hoofdstukken zijn geschreven als wetenschappelijke publicaties. Hoofdstuk 4 en 5 werden gepubliceerd in Transplantation. De hoofdstukken 6, 7 en 8 werden recent ter publikatie aangeboden.

In hoofdstuk 4 (eerste experiment) wordt het effect van voorbehandeling met donorbloed beschreven, op niertransplantaties tussen DLA identieke en niet-identieke beagle "littermates". Productie van antistoffen en versnelde transplantaat afstoting waren het gevolg in 17% van de honden. "Non-responders" vertoonde geen significant verlengde niertransplantaat overleving. Een genetische controle van responderschap wordt in dit hoofdstuk gesuggereerd.

In hoofdstuk 5 (tweede experiment) wordt verslag gedaan van een experiment in 2 DLA haplotypen verschillende beagle "littermates". Toekomstige niertransplantaat ontvangers werden behandeld met subcutane injecties met donorbloed. Versnelde transplantaat afstoting was het gevolg, in tegenstelling tot vroegere observaties door anderen, die actieve enhancement van niertransplantaten in de hond vonden in een soortgelijk experimenteel protocol. Het belang van weefseltypering in dergelijke studies wordt benadrukt

In hoofdstuk 6 (derde experiment) worden transfusie en transplantatie experimenten beschreven in beagle families. De honden ontvingen voor transplantatie transfusies uit een bloed "pool" van niet verwante bloeddonoren. Een "hoge" en "lage" immuunresponse tegen een panel van hondenleucocyten werd gevonden. Een eenvoudig systeem van overerving van het "responderschap" werd niet gevonden. De niertransplantaat overleving werd nauwelijks beïnvloed door de bloedtransfusies voor transplantatie. De immuunresponse voor transplantatie correleerde met transplantaat overleving in enkele honden.

In het volgende experiment in hoofdstuk 7 (vierde experiment) wordt het effect beschreven van een transfusie met bloed van één van de ouders, voor transplantatie, op de overleving van een DLA-identiek verwant niertransplantaat. Ouderlijk bloed werd gebruikt om non-DLA antistoffen op te wekken, die enhancement zouden kunnen bewerkstelligen. Geen versnelde afstoting werd gezien, evenmin een duidelijk verlengde transplantaat overleving.

In het onderzoek dat wordt beschreven in hoofdstuk 8 (vijfde experiment) werd immunosuppressive behandeling gegeven na transplantatie en drie maal werd onverwant bloed getransfundeerd voor transplantatie van een niet verwante, niet identieke nier. Een niet getransfundeerde DLA identieke of 1 haplotype verschillende "littermate" diende als controle ontvanger. Duidelijk significante verlenging van niertransplantaat overleving werd

gezien en 50% van de getransfundeerde beagles vertoonden geen enkel teken van transplantaat afstoting. De immuunresponse voor transplantatie correleerde goed met de niertransplantaat overleving.

In hoofdstuk 9 worden de voorafgaande experimenten besproken en hypothesen voor het mechanisme van de beschermende werking van bloedtransfusies op een niertransplantaat worden vermeld.

Dit proefschrift wordt besloten met de conclusie dat bloedtransfusies voor de transplantatie een beschermende werking hebben tegen de immunologische afstotingsreactie van niertransplantaten bij de hond. De productie van cytotoxische antilichamen na bloedtransfusies dient voorkomen te worden en wanneer antilichamen toch aanwezig zijn verdient het aanbeveling om de niertransplantatie te verrichten wanneer de immuunresponse van de ontvanger laag is. We hebben een bruikbaar diermodel verkregen dat voor verdere onderzoeken gebruikt kan worden, die er op gericht zullen zijn een bloedtransfusie beleid te ontwikkelen waardoor niertransplantaten tegen afstoting beschermd kunnen worden.

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Curriculum Vitae

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