THE EFFECT OF BLOOD TRANSFUSION AND IMMUNOSUPPRESSION ON ORGAN GRAFT SURVIVAL

A study in dogs en rats

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PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN
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To my mother,
in memory of my father.

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"There is no quiet place in the white man's cities. No place to hear the leaves of spring or the rustle of insect wings. But perhaps because I am savage and do not understand — the clatter only seems to insult the ears. And what is there to life if a man cannot hear the lovely cry of the whippoorwill or the arguments of the frog around the pond at night."

from a letter
by Chief Sealth of the Duwanish Tribe
in Washington
to President Franklin Pierce in 1855

CyA : cyclosporin A
Aza/Pred : azathioprine and prednisolone

CONTENTS

PREFACE

Since the first preliminary report by Opelz et al. (1973a) on the beneficial effect of blood transfusions, it has gradually become evident that blood transfusions do have such an effect on renal allograft survival. Nevertheless, some physicians are still reluctant to adopt a deliberate blood transfusion regimen for fear of sensitization. The development of cytotoxic antibodies may delay transplantation for an indefinite time for some patients.

This possible danger of sensitization initially gave rise to the policy of avoiding blood transfusions in kidney transplant recipients and it took many years of study, experimentation and discussion before the present situation of general acceptance was established.

With respect to the clinical application of a deliberate blood transfusion policy, several questions remain to be answered regarding the optimal number and the timing of transfusions, the antigenic composition of the transfusate and the influence of immunosuppressive therapy on the expression of the blood transfusion effect. The effect of blood transfusions on kidney graft survival has only been studied in recipients treated with azathioprine and prednisolone for postoperative immunosuppression. It still has to be investigated if a beneficial effect of pretransplant transfusions would be demonstrable without conventional immunosuppression or in combination with postoperative administration of the novel immunosuppressant cyclosporin A.

Cyclosporin A was recently discovered in the Sandoz Laboratories during a screening program for antimycotics; its powerful immunosuppressive properties were described by Borel et al. (1976). It has been tested in a variety of experimental animal transplantation models. Preliminary data are available on its clinical effectiveness and side effects. Some of these side effects are serious and it is still too early to be sufficiently informed about the long-term effects of the drug.

Further questions awaiting evaluation pertain to the optimal dose of the drug, its potency to prevent rejection in sensitized recipients, the possible interference with other modes of treatment, and the possibilities of conversion to other immunosuppressants.

It was the objective of the present studies to elucidate the items mentioned above.

CHAPTER 1

GENERAL INTRODUCTION

Kidney transplantation is the present treatment of choice for end-stage renal failure. It enables patients to lead a relatively normal life, although the quality of that life is threatened by the possible side effects of immunosuppressive therapy.
Renal transplantation has shown a great increase because a major shift has been made to cadaveric transplantation.
By now, over 50,000 kidney transplantations have been performed in man (Rapaport, 1981). The supply of cadaver donors has increased due in part to the acceptance of the criterion of brain death and the improved results of cadaveric transplantation. In recent years, patient survival following cadaveric kidney transplantation has improved, which can be explained by a greater willingness to abandon an allograft and to consider a return to dialysis and subsequent retransplantation (Morris, 1981a; Starzl, 1978). Cadaveric kidney graft survival at one year is more or less presently fixed at about 50 to 70 percent in the large transplantation centres (13th Report of the Human Transplant Registry, 1977; Starzl, 1978); only some centres show a better graft survival (Alexandre, 1978; Mc Geown et al., 1980; Lameijer et al., 1978).
Rejection is by far the most frequent cause of graft loss today. Despite great advances in surgical technology and in the development of a reasonably effective immunosuppression regimen during the last 20 years, clinical kidney transplantation seems to have arrived at a relatively flat plateau of achievement, although there is good evidence that this procedure is safer than it formerly was (Rapaport, 1978; Russell, 1981). Yet, it should be stressed that considerable progress has been made in the area of transplantation biology and clinical transplantation (Hamburger, 1979). A few examples will be cited.
It has been recognized that suppressor cells play an important immunoregulatory role in transplantation; most of these suppressor cells are T lymphocytes (Brent et al., 1980). Both the T cells and the antibody producing B cells are required for a complete immunologic response. Early in the response to a graft, T cell function seems to predominate. T lymphocytes have been further divided into subpopulations such as helper T cells, cytotoxic T cells and suppressor T cells. The latter can be defined as cells which suppress the reactivity of other lymphocytes such as cytotoxic T cells which contribute to the rejection of a graft. Especially the suppressor cells seem to be important in successful take of the graft and they probably play a role in the beneficial blood transfusion effect.
Matching for HLA-DR antigens seems far more important for organ graft prognosis in cadaveric kidney grafting than matching for HLA-A and -B antigens (Morris, 1981a).

The novel immunosuppressant Cyclosporin A (CyA) is another most promising development in present clinical transplantation (Calne, 1980b; Starzl, 1981a). Monoclonal antibodies may soon find their way to the clinic, although it still has to be seen if their application offers better results than those obtained with conventional antilymphocyte sera (Balner and Marquet, 1981).

At present, the three mainstays in clinical kidney transplantation are the administration of pretransplant blood transfusions, nonspecific pharmacological immunosuppression and histocompatibility matching. These topics will be considered in more detail in this introduction. Ample attention will be given to CyA as an introduction to the experiments using this novel immunosuppressive agent.

1.1. BLOOD TRANSFUSION

Much has been written during the last ten years about the effect of blood administration to dialysis patients on subsequent kidney transplant survival. Dialysis patients were formerly transfused to correct for their anemia. In chronic severe uremia, a normochromic normocytic anemia which persists after institution of dialysis is observed. This anemia has been attributed to a shortened red blood cell survival time, diminished or no production of erythropoietin and a decrease in response to the available erythropoietin. Dialysis can partially compensate for the first and third factors by removing toxic substances. Despite intensive medical treatment, blood or red cell transfusions are often necessary to bring the very severe anemia to an acceptable level. In the sixties, this was practiced almost universally. However, multiple transfusions and in particular the leukocytes can result in the formation of cytotoxic antibodies against donor histocompatibility antigens.

Sensitization of the recipient is determined by a lymphocytotoxic crossmatch test between recipient serum and donor lymphocytes. In the 1960s, it was reported that a positive crossmatch test coincided with hyperacute graft rejection (Kissmeyer-Nielsen et al., 1966; Patel and Terasaki, 1969). In the former study, the recipients were probably hyperimmunized by multiple transfusions. These observations generally led to great restraint in the administration of blood to prospective kidney graft recipients. At about the same time, Dossetor et al. (1967) found that patients with longer surviving grafts had received significantly more blood transfusions. When Opelz et al. (1973a) reported on the beneficial effect of pretransplant blood transfusions on subsequent renal allograft survival, their publication was met with considerable skepticism. Yet, during the following years, many more centres reported a beneficial effect of pretransplant transfusions and it gradually became clear that witholding blood from transplantation candidates resulted in an extremely low kidney graft survival. Several articles in recent years have reviewed the retrospective data that in the majority supported the transfusion effect (Opelz and Terasaki, 1977; van Es and Balner, 1979; Solheim, 1979; Opelz et al., 1979a).

In the meantime, much experimental work had been done in animal models, which unequivocally gave evidence of the existence of a transfusion effect (van Es et al., 1977; Obertop et al., 1978b; Fabre et al., 1978; Marquet and van Bekkum, 1973).

During the last three years, several prospective studies from single centres (Persijn et al., 1979; Williams et al., 1979) or multicentre analyses (Spees et al., 1980; Opelz and Terasaki, 1980a) have confirmed the earlier retrospective data.

The existence of a beneficial blood transfusion effect is now generally accepted, although some controversies still remain (Opelz et al., 1981a). These controversies pertain mainly to the policy that should be followed to obtain an optimal transfusion effect. In the first place, it is desirable to know the minimal number of transfusions that is effective in prolonging kidney graft survival; secondly and related to the first item, it is essential to have information on the sensitizing effect of a given number of transfusions. Sensitization not only renders potential recipients untransplantable, but transplant outcome is also worsened in highly sensitized patients. Opelz et al. (1981c) have shown in a large retrospective and prospective multicentre study that the frequency of sensitization has been overestimated on the basis of previous retrospective studies (Russell, 1977; Solheim, 1979; Glass, 1980). In the study of Opelz et al. (1981c), it was prospectively shown that highly reactive antibodies (antibodies exhibiting $>$ 90% reactivity against a random test panel) did not occur in any of the male recipients or in females that had not previously been pregnant after up to 20 transfusions. Almost 90% of the male patients were practically unresponsive ($<$10% antibody reactivity against the panel). The situation was quite different for women with previous pregnancies; 10% of these developed highly reactive antibodies after 10 transfusions. Also T cell antibodies were more frequent in females. In the retrospective part of the study, the frequencies for all of these categories were higher. As opposed to what had previously been assumed, only one-third of the patients who became sensitized and received additional transfusions showed an increase in antibody reactivity, while one-third showed a decrease. The recipients showing an increase in antibody reactivity were also predominantly females who had had previous pregnancies. Further, recent evidence has been presented by the Los Angeles group that recipients showing a decrease in antibody levels after additional transfusions fare very well after transplantation (Opelz et al., 1981a). In several retrospective as well as prospective studies, the same group showed that an increasing number of transfusions is correlated with better graft survival (Opelz and Terasaki, 1980a, 1980b). Only 3% of the patients became highly sensitized (Opelz et al., 1981b) with consequent poor graft prognosis.

The desirable number of transfusions is one of the controversies alluded to. Some authors reported that after only one transfusion an extremely high one-year graft survival of approximately 80 percent was obtained (Persijn et al., 1977; Williams et al., 1979). Others found an optimal effect of about 5 transfusions, more transfusions exhibiting no additional benefit. The dose (i.e., number) effect as reported by Opelz and Terasaki was also confirmed by both Spees et al. (1980) in a large prospective study initiated by the Southeastern Organ Procurement Foundation and Fehrman et al. (1980).

Variable results have also been published regarding the blood products used. In the multicentre data analysed by Opelz and Terasaki, it was consistently found that frozen blood was less effective than whole blood, packed cells or washed packed cells

(Opelz and Terasaki, 1980a, 1980b). Two groups reported on a beneficial effect of frozen blood (Polesky et al., 1977; Fuller et al., 1977, 1982). Huggins et al. (1973) claim that frozen deglycerolized red blood cells cause less sensitization and are less frequently followed by serum hepatitis as compared with whole blood. They distinguish between erythrocytes deglycerolized by centrifugal washing and by agglomeration. The centrifugation method would leave more leukocytes viable and this would explain a higher sensitization rate, which was however not significant. Blood deglycerolized by centrifugation led to significantly better graft survival (Fuller et al., 1982). The importance of leukocytes for the induction of the transfusion effect was also demonstrated by the Leiden group (Persijn et al., 1979), which reported that leukocyte-free (cotton-wool-filtered) blood had virtually no beneficial effect on graft survival; washed erythrocytes showed about the same results as one blood transfusion of unidentified composition. It seems that leukocytes may be necessary for inducing a transfusion effect, but it should be realized that their administration also carries a considerable risk of recipient sensitization. It has been demonstrated that deliberate buffy-coat transfusions lead to excessive antibody formation (Takahashi H. et al., 1982). Nothing is known at present about the effect of plasma or platelet transfusions on graft survival. Recent evidence obtained in the rhesus monkey suggests that platelet-rich plasma is less immunogenic than fresh blood. In addition, blood stored for 3 weeks or more proved to be significantly less immunogenic than blood stored for 2 weeks or less (Oh et al., 1982). The latter finding was also confirmed in man following donor-specific transfusions using stored blood older than 4 days (Light et al., 1982).

Although it has been claimed by some authors that the time interval between the last transfusion and transplantation influences the outcome of transplantation, this was not demonstrated in other series (Persijn et al., 1979; Opelz et al., 1979a; Corry et al., 1980) and also the prospective data of the International Workshop Study (Opelz and Terasaki, 1980b) provided evidence that the transfusion-transplantation interval is irrelevant. The possible effect of such an interval could be ascribed to the fact that patients receiving a transfusion shortly before transplantation generally had previously received multiple transfusions.

Another controversial topic concerns the effectiveness of blood transfusions given during operation. Although some centres report a beneficial effect of peroperative transfusions (Stiller et al., 1978; Hunsicker et al., 1980; Williams et al., 1980), others contradict them. The International Workshop Study could not support a beneficial effect on graft survival.

Likewise, the latter study could not confirm that the time length of dialysis was correlated with graft survival, as was found by others (Guttmann, 1978; Fehrman et al., 1979). Dialysis time appeared to be irrelevant and graft survival proved to be primarily related tot the number of transfusions given before transplantation.

Festenstein et al. (1976) found blood transfusion to be maximally effective in well-matched (i.e., for 3 or 4 HLA-A or -B antigens) recipients. Opelz and Terasaki (1980a) reported a beneficial effect of transfusions independent of the number of antigens matched. The results of the International Workshop Study showed the

most prominent beneficial effect of histocompatibility matching in nontransfused recipients. This effect of HLA matching on graft survival decreased with an increasing number of transfusions, i.e., with a greater improvement of graft survival mediated by transfusions. This outcome was similar for both HLA-A, -B and -DR matching. Nevertheless, transfused patients with better matches showed a superior graft survival, although not significant. Apparently the administration of blood transfusions and matching are not really additive; transfusions probably improve graft survival to a level where additional HLA matching has little influence.

Matching not only of the kidney donor but also of the blood donor may influence graft survival. Persijn et al. (1979) observed that the HLA type of the blood transfusion donor had no bearing on graft survival in their study using a one transfusion protocol. Recently, interest has focused on the application of well-matched blood transfusions, in the hope that this might prevent sensitization while retaining the beneficial transfusion effect. Nubé et al. (1981) reported a favourable effect of two or three HLA-A and -B matched leukocyte-poor transfusions which was comparable with the effect of one random leukocyte-poor transfusion in the same centre. Sensitization seemed to be less than for one random transfusion.
On the other hand, Albert et al. (1981) administered one or three HLA-A, -B matched transfusions with an average mismatch of 0.86 and found no significant decrease in antibody production and no definite beneficial effect of transfusion.

Another new development in "transfusion therapy" is the administration of donor-specific tranfusions to one-haplotype related recipients (Salvatierra et al., 1980). It has been demonstrated that third-party blood transfusions also have a beneficial effect on graft survival in one-haplotype related transplantations (Opelz et al., 1981a). The protocol of donor-specific transfusions was started to prospectively select incompatible one-haplotype related donor recipient pairs and to alter the recipient immune response. It appeared that 30% of the potential recipients became sensitized against their blood donor but not against a random panel. Consequently, these sensitized patients did not recieve the respective related transplant, but were still suitable candidates for cadaver transplantation. A very favourable effect of these donor-specific transfusions was observed in nonsensitized patients transplanted with one-haplotype identical related kidneys, with one- and two-year survival rates of 98% and 95%, respectively (Salvatierra et al., 1981; Mendez et al., 1982). A peculiar finding when using this donor-specific transfusion protocol was that in many cases early, relatively mild, rejection crises which were quite amenable to therapy and seldom resulted in graft loss were seen (Salvatierra et al., 1982; Takahashi I. et al., 1982; Leivestad et al., 1982). In sharp contrast donor-specific transfusions from nonrelated individuals were followed by an extremely low graft survival (Ruzany et al., personal communication). Probably the implantation of a graft, following administration of blood containing the same histocompatibility antigens creates a donor-specific sensitization, which is not compensated for by the benefits of the related situation.

1.1.1. MECHANISM

Several hypotheses have been proposed to explain the working mechanism that underlies the beneficial effect of pretransplant blood transfusions.
In their initial publication, Opelz et al., (1973a) offered two possible explanations. In the first place, blood transfusions may act by a selection of the potential recipients into responders and nonresponders. Patients who have received many random blood transfusions and do not respond with lymphocytotoxic antibody formation are nonresponsive. This state of nonresponsiveness will also be reflected in a better acceptance of a subsequent kidney graft. On the other hand, responders easily produce antibodies after a few transfusions. These antibodies not only make it more difficult to find a suitable kidney donor but they are also a reflection of a more vigorous immune response towards a prospective graft which may eventually result in rejection. As a second hypothesis, it was suggested that blood transfusions induce a state of recipient unresponsiveness, probably by means of the formation of enhancing antibodies. The selection principle has been quoted for several years as the mechanism of action of blood transfusions. However, the demonstration of a transfusion effect in HLA-identical sibling transplantation makes this theory unlikely, at least as the sole mechanism (Opelz et al., 1981a). It cannot be excluded that selection plays a role in cadaver transplants. It definitely plays a role in some protocols applying donor specific blood transfusions in one-haplotype related kidney transplantation (Salvatierra et al., 1981).
The second hypothesis that blood transfusions would induce decreased immune reactivity was suggested by the finding that the plasma of dialysis patients and parous women contained a factor that blocked the mixed leukocyte reaction (Sengar et al., 1973). It was speculated that nonreactivity as a result of blood transfusions might be due to enhancing antibodies. Such antibodies have been demonstrated in skin transplantation in mice and kidney transplantation in rats and have been shown to exhibit a protective effect on graft survival (Jeekel and Westbroek, 1971; Stuart et al., 1968). Yet, enhancement is unlikely to play a role, since the transfusion effect in cadaver kidney recipients is not donor specific and because a single random transfusion is already effective. For the latter reasons, "classical" tolerance can also be ruled out as a likely causative phenomenon (van Rood and Balner, 1978). Also preformed cold B-cell antibodies have been implicated as being responsible for improved graft survival (Iwaki et al., 1979). However, an association between the administration of blood transfusions and the occurrence of pretransplant autoreactive B-cell antibodies could not be proved and this makes their role in the transfusion effect unlikely (Opelz et al., 1981c). Further, anti-idiotypic antibodies have been assigned a role in the transfusion mechanism (Bühlmann et al., 1978). In a recent study, it was found that anti-idiotypic antibodies could be shown only in recipients who had recieved pretransplant blood transfusions. In transfused recipients, these antibodies were demonstrable only in patients with functioning grafts and not in patients who had rejected the graft (Singal et al., 1982). These IgG antibodies caused inhibition in the MLR and were directed against responder T cells. The authors sug-

gest that anti-idiotypic antibodies could be induced by blood transfusions and these antibodies might be responsible for enhanced renal allograft survival. Other evidence for the existence of humoral factors that may play a role in evoking the beneficial transfusion effect has been presented by Proud et al. (1979), who showed that the plasma of multitransfused patients contained inhibiting activity that was related to the alpha-2-macroglobulin fraction. Keown and Descamps (1979) have proposed the hypothesis that blood transfusions would have a nonspecific immunosuppressive effect on the host immune system via a temporary functional modulation in mononuclear phagocytes. The transiently impaired function of mononuclear phagocytic cell function occurred after ingestion of altered red blood cells in vitro. Although it is an interesting hypothesis, the reported ineffectiveness of leukocyte-free blood transfusions (Persijn et al., 1979) makes it very improbable that the mechanism mentioned is the only factor responsible for the beneficial transfusion effect.

The argument has been advanced that the observed effect of pretransplant blood transfusions was merely a reflection of the duration of dialysis-dependent uremia, because a correlation was found between the number of transfusions and the time length of dialysis. However, experiments in animals suggest an immunosuppressive effect of pretransplant blood transfusions, as no uremia effect was observed in these healthy animals (Marquet et al., 1971; van Es et al., 1979; Obertop et al., 1978b).

Much recent work provides indirect evidence for a role of suppressor T cells in the blood transfusion effect. Fischer et al. (1980) found a marked suppression of cellular immunity after transfusion of third-party washed blood cells. A subsequent transfusion induced a more pronounced immunosuppressive effect. This phenomenon of immune suppression was not seen after transfusion of autologous blood in human volunteers. In a more recent communication, the same group reported that third-party cells were also inhibited in the suppressor MLR after transfusion. Further, Con A inducible suppressor activity was low during the first two weeks following transfusion and increased thereafter; the initially low suppressor activity coincided with a transient decrease in suppressor cells posttransfusion (Lenhard et al., 1982). The authors concluded that blood transfusions initially induce a nonspecific suppression of cellular immunity, probably mediated by monocytic cells, whereas increasing suppressor T-cell activity is present in a later phase; it was suggested that at least two cellular graft protective mechanisms are induced by blood transfusions. A suppressive effect of transfusion on cellular immunity, although limited to PHA-induced mitogenesis, was also found by Borleffs and Marquet (1981) in rhesus monkeys. Smith et al. (1981) showed a decrease in suppressor T-cell activity one week after transfusion of packed red cells in hemodialysis patients; this was followed by an increase in suppressor cell function twee weeks later. This increased suppressor T-cell function disappeared after five months. These data are in accord with the above-mentioned findings of Lenhard et al.

The finding that the blood transfusion effect is independent of the transfusion-transplantation interval (Opelz and Terasaki, 1980b) is not in agreement with the presumed suppressor T-cell activity as the only mechanism for explaining the beneficial transfusion effect if the above findings of Smith et al. are taken into account.

Finally, Marquet et al. (1982) demonstrated that suppressor cells can be the causative factor in prolonged graft survival following donor blood transfusion in a heterotopic heart allograft model using the BN/Ro and WAG/Ro rat strains as donors and recipients, respectively.
Reviewing all the data presented above, it is likely that more than a single immunologic phenomenon can be held responsible for the transfusion effect. Suppressor T cells rank high on the list of proposed factors.

1.1.2. SENSITIZATION

In the preceding pages, attention has been focused several times on the sensitizing effect of blood transfusions. However, antibodies against leukocytes may also develop as a result of pregnancy or graft rejection. About 30-60% of all patients waiting for a kidney transplant will have these antibodies (Ting, 1981). Antibodies are detected by screening the sera from dialysis patients against the lymphocytes of a random panel of volunteers. The presence of lymphocytotoxic antibodies to kidney donor cells, i.e., a positive crossmatch, was until recently a contra-indication for transplantation, since it had been demonstrated that a positive serological crossmatch could result in accelerated graft rejection (Kissmeyer - Nielsen et al., 1966; Patel and Terasaki 1969). At that time, it was still believed that these lymphocytotoxic antibodies were directed at HLA-A and -B antigens. Now it is known that antibodies are also directed at other antigen systems on the lymphocyte cell surface.
Antibodies to some of these antigens are not associated with graft loss and consequently a transplantation with a positive crossmatch can sometimes be carried out. By various screening procedures it is often possible to determine the specificity of these antibodies.
Lymphocytotoxic antibodies can be reactive with T or B cells. Reactivity with T and B cells may indicate the presence of HLA-A, -B or -C or autoreactive antibodies. Reactivity with B cells only may indicate the presence of HLA-DR, autoreactive, Lewis or weak HLA-A, -B, -C antibodies. Reactivity with T cells only may be due to T-cell alloantigens (Ting, 1981). It has been reported that kidneys may be transplanted in the presence of a positive B-cell crossmatch with a low risk of failure (Ting and Morris, 1977; Morris et al., 1977), although a hyperacute rejection has been reported (Dejelo and Williams, 1977). Certain B-cell antibodies appear to be associated with graft rejection and others with improved survival. B-cell antibodies can be detected at a temperature of either 5°C or 37°C. The cold B-cell antibodies (detected at 5°C) are autoreactive IgM immunoglobulins (Iwaki et al., 1979; Jeannet et al., 1980). The pretransplant cold B-cell antibodies are not induced by blood transfusions but rather by viral infections (Opelz et al., 1981c). Warm B-cell antibodies are considered to be directed against HLA-DR antigens and are IgG in nature. Anti-HLA-DR antibodies seem to be the equivalent of anti-Ia antibodies in rodents. Although Jeekel et al. (1976) could not demonstrate an enhancing effect of anti-LD antibodies in their rat kidney graft model, anti-Ia antibodies can have an enhancing effect on graft survival in rodents. Anti-HLA-DR antibodies in humans have rather

been shown to exert a deleterious effect in kidney transplantation (Sirchia et al., 1979, Ayoub et al., 1980). Both autoreactive and anti-HLA-DR antibodies have been reported to result in a minority of positive B-cell crossmatches (Ettenger et al., 1979; d'Apice and Tait, 1980).
Hyperacute rejection has been frequently observed with positive T-cell crossmatches (Opelz et al., 1979b), but it is relatively safe to perform a transplant with a positive B-cell crossmatch (Ettenger et al., 1976; Ting and Morris 1977; Reekers, 1982). However, it does not seem safe to transplant with a positive B-cell crossmatch (Ting and Morris, 1981) when non-autoreactive B-cell antibodies are present, when B-cell antibodies have developed after a failed graft and when anti-HLA-A, -B or -C antibodies are present.

1.2. IMMUNOSUPPRESSION

After initial attemps in France (Dubost et al., 1951; Küss et al., 1951) and in the USA (Hume et al., 1952) to transplant human kidney allografts without postoperative immunosuppression, it became clear by the middle of the 1950s that ways had to be found to suppress the immune response. As it was already known at that time that identical twins did not reject each other's tissues, the problem of rejection was circumvented in the first transplantation between monozygotic twins by Murray and associates in 1954. Meanwhile, it had been shown that total body irradiation could induce prolonged survival of kidney allografts in dogs. At the end of the 1950s, total body irradiation was introduced in clinical renal transplantation, but the results were not favourable.

1.2.1. AZATHIOPRINE AND PREDNISOLONE

Schwarz and Dameshek (1959) showed that the antimetabolite 6-mercaptopurine could induce a persisting immunosuppressive state in rabbits. Using this compound, prolonged renal allograft survival could be obtained in dogs (Calne, 1960). Azathioprine, an analogue of 6-mercaptopurine, was subsequently found to have a better immunosuppressive effect in the dog renal allograft model and to predispose to fewer infections (Calne, 1961). The clinical application of azathioprine as an immunosuppressive agent in human kidney transplantation soon followed. The experimental basis for the use of corticosteroids was laid by Billingham et al. (1951) and their usefulness in immunosuppressive therapy was soon confirmed by many others. Sinse the beginning of the 1960s, the combination of azathioprine and corticosteroids has been the "sheet anchor" of clinical immunosuppression. Although reasonably effective, the immunosuppressive potency of this regimen is far from optimal, as reflected in the average one-year survival rate of cadaver kidney transplants, which amounts to only slightly more than 50%.
The associated side-effects of azathioprine and corticosteroids also represent a major problem. Many of these side-effects such as the Cushingoid appearance, avascular necrosis of bone, diabetes and cataracts can be attributed to the steroids. Cardio-

vascular and cerebrovascular disease have currently replaced infection as a major cause of death after transplantation (Morris, 1981a); however, infections remain a problem. Increased incidences of malignant lymphomas and other tumors have also been reported (Penn, 1979, 1981). Further reported complications of chronic conventional immunosuppression are gastrointestinal ulcerations, pancreatitis, hyperparathyroidism, hypersplenism, liver malfunction and hyperlipidemia (Starzl et al., 1977). To diminish the side-effects related to steroid administration, several schedules for decreasing the total dose administered and consequently the inherent side-effects have been developed. Both alternate-day corticosteroid therapy (Fauci, 1978) and low dose steroid therapy (McGeown et al., 1980; Chan et al., 1980) have been shown to be as effective as high continuous doses in preventing graft rejection. Also recommendations have been made for a maximum total dose of 2 g of steroids in intravenous "pulse therapy" to reverse rejection crises (Kumar et al., 1978). Side-effects specific for azathioprine seem to be mainly related to its hepatotoxic effect (Schein and Winokur, 1975) and to the leucopenia resulting from its bone marrow toxicity.
Although side-effects which are mainly related to the corticosteroids used may be severe, conventional immunosuppression consisting of azathioprine and prednisolone (Aza/Pred) is still indispensible, because it is the best documented and therefore at present safest immunosuppressive therapy. While awaiting the final evaluation of the new promising agent Cyclosporin A (CyA), the low dose steroid therapy as propagated by McGeown et al. (1980) provides satisfactory immunosuppression with an acceptable number of unwanted side-effects.

1.2.2. CYCLOSPORIN A

Cyclosporin A (CyA) is a fungal metabolite which exhibits a very strong immunosuppressive effect in addition to weak antifungal activity. It was isolated at the Sandoz Laboratories from the soil fungi Trichoderma Polysporum Rifai and Cylindrocarpon Lucidum in a screening program for antibiotic agents. CyA is a nonpolar cyclic peptide consisting of 11 amino acids. One of these amino acids is unique and most are hydrophrobic, which explains its insolubility in water. The compound has a molecular weight of 1202.6 daltons and the molecular formula is $C_{62}H_{111}N_{11}O_{12}$. The antibiotic spectrum is very narrow; only few yeast species are sensitive to CyA and bacterial growth is not inhibited. Its immunosuppressive activity was first shown by Borel and co-workers (1976, 1977). The Cambridge group was the first to report marked prolongations of survival of vascularized organ allografts in animals (Kostakis et al., 1977; Calne and White, 1977); toxic effects were then still seen because of the lack of experience with the drug. Since 1977, many reports have appeared on the effect of CyA in various transplantation models using several species. A review of the various animal transplantation experiments is given in Tabel 1.2.2.A. Clinical transplantation studies have also been initiated in a number of centres which are concerned with kidney, pancreas, liver and heart transplants (Calne et al., 1981a; Starzl et al., 1981b; Oyer et al., personal communication).

In the following, some studies will be discussed in more detail.

Experimental transplantation

Rat. Most experiments using CyA have been performed in rats. Kidney and heart graft rejection can be suppressed by a short course (1 week) of CyA administered orally or intramuscularly (Homan et al., 1980e; Kawahara et al., 1980; Niessen et al., 1982a). This effect is dose-dependent and at an adequate dose indefinite graft survival is even achieved. If treatment with CyA is delayed until 4 days after transplantation, renal allograft rejection is no longer suppressed (Homan et al., 1980e), suggesting that the drug is effective mainly when administered at an early stage of the immune response. CyA is also far less effective in suppressing renal allograft rejection in recipients sensitized by a previous donor skin graft (Homan et al., 1980f).

Table 1.2.2.A. Transplantation experiments in various species using cyclosporin A for postoperative immunosuppression.

Species	transplant	reference
mouse	skin	Lems et al., 1980 Borel and Meszaros, 1980
	bone marrow	van Bekkum et al., 1980 Borel et al., 1976
rat	kidney	Homan et al., 1980 Simms et al., 1980
	heart	Kostakis et al., 1977 Kawahara et al., 1980 Niessen et al., 1982a
	pancreas	Rynasiewicz et al., 1980 Garvey et al., 1980
	skin	White et al., 1980
	bone marrow	Tutschka et al., 1979 Borel et al., 1976
	nerve	Zalewski and Gulati, 1981
rabbit	kidney	Dunn et al., 1978 Green and Allison, 1978
	skin	Gratwohl et al., 1981b
	cornea	Shepherd et al., 1980
dog	kidney	Calne and White, 1977 Homan et al., 1980a Niessen et al., 1981
	lung	Veith et al., 1981
	pancreas	McMaster et al., 1980
	skin	Deeg et al., 1980
	bone marrow	Deeg et al., 1981
monkey	kidney	Borleffs et al., 1981
	heart	Jamieson et al., 1979
pig	heart	Calne et al., 1978

These data indicate that CyA might be less effective in sensitized recipients and are also supported by in vitro findings demonstrating that the secondary MLR response is only poorly inhibited by CyA, while it has no effect on the generation of cytotoxic effector cells. In contrast to the above data, it was found in our WAG/Ro to BN/Ro heart allograft model in the rat that sensitization by donor blood could be overcome and that even permanent graft survival can be achieved at a dose of 15 mg/kg/day CyA given intramuscularly for 7 days (Niessen et al., 1982a). Preliminary results in the same model indicate that the source of the sensitizing donor antigen is important for the resulting strength of the rejection response, which must be overcome. For example, high dosis of CyA (up to 30 mg/kg/day) are necessary to obtain permanent heart graft survival in some recipients sensitized by a donor heart in the WAG/Ro to BN/Ro combination, whereas only moderately prolonged graft survival can be obtained following sensitization by donor skin at the same doses of CyA (unpublished observations). Although a short course of CyA can induce permanent heart or kidney allograft survival, this could not be achieved in vascularized segmental pancreatic allografts; grafts were rejected after discontinuation of therapy (Rynasiewicz et al., 1980). Prolongation of islet allograft survival could only be obtained across a minor histocompatibility barrier. Skin allografts were likewise rejected after termination of therapy (White et al., 1980); this was also observed in the mouse (Lems et al., 1980). The survival of cardiac xenografts from the Syrian hamster to the Lewis rat was found to be prolonged by high doses of CyA (35 mg/kg/day for 2 weeks) but only to a very limited extent (3 weeks) (Homan et al., 1981a). A similar experiment of Marquet et al. using the hamster to WAG/Ro rat model showed a prolongation of only a few days at equally high doses of CyA; this discrepancy can probably be explained by the stronger immunogenecity of the latter model.

In the rat, the suppression of the immune response by CyA seems to be both specific and nonspecific. During the administration of the drug, immune reactivity is nonspecifically suppressed. When the administration is discontinued, this stage is followed by an "unstable" period during which a secondary challenge with donor skin results in rejection of this skin graft as well as of the initial heterotopic heart graft; a third party secondary skin graft can in some cases also cause rejection of the initial heart graft in this period (Nagao et al., 1982). There is eventually a third phase during which only donor-specific skin grafts are tolerated. It was found that the lymphocytotoxic response to third-party allografts was suppressed in rats with these long-term surviving kidney allografts (Homan et al., 1979). This tolerant state proved to be similar to that found in enhanced organ graft recipients, irrespective of the way this state was induced (Morris, 1981b). Hutchinson et al. (1981a) found evidence for the existence of specific suppressor cells from 12 days after transplantation and following administration of CyA for 7 days after grafting.

Rabbit. Also in rabbits a short course of CyA proved to be sufficient for indefinite kidney allograft survival. The tolerance induced was found by some authors to be specific (Green et al., 1979) but nonspecific by another (Dunn, 1981). Although it was not investigated, the same pattern as in the rat might be found. Also comparable to the rat is the rejection of skin grafts after stoppage of CyA administration.

CyA does not prevent rejection of second-set skin grafts in recipients previously sensitized with donor skin (Gratwohl et al., 1981).

Dog. In the dog, a clear dose-response relationship in the prevention of renal allograft rejection was observed (Homan et al., 1980a; Niessen et al., 1981). CyA at a dose of 20-25 mg/kg/day is sufficient to maintain graft survival in all cases. In recipients treated with CyA as the only immunosuppressant, rejection is always seen after discontinuation of the drug. If recipients were transfused before operation and treated with the combination of CyA and Aza/Pred, prolonged kidney graft survival was obtained in 50% of the recipients when CyA was stopped and Aza/Pred continued (Niessen et al., 1982b); this prolonged survival could be attributed to the expression of the blood transfusion effect, which proved to be manifest only on treatment with Aza/Pred and not when CyA was used for immunosuppression (Niessen et al., 1981). In contrast to the findings in the rat, prolongation of kidney graft survival could nevertheless be achieved in some animals when CyA was administered starting on day 4 after transplantation; still, this indicates that also in outbred animals the value of the drug for the reversal of rejection crises is limited (Homan et al., 1980d). Beginning rejection of skin allografts could be prevented but rejection eventually occured after termination of CyA treatment (Deeg et al., 1980). Survival of vascularized segmental pancreatic allografts could be maintained only at high doses of CyA (up to 40 mg/kg/day) (Du Toit et al., 1982), whereas whole-organ pancreas allografts were rejected at a median survival time of 85 days at doses of 18 to 25 mg/kg/day (McMaster et al., 1980).

CyA was found to be relatively nontoxic in the dog. No signs of nephrotoxicity have been observed (Homan et al., 1981c; Niessen et al., 1981), not even in dogs receiving a kidney with poor function because of ischemic damage (Homan et al., 1980c). Liver function abnormalities resulting in jaundice have been reported, but high dosages of the drug (50 mg/kg/day) were used in that series (Calne et al., 1979b). In the same study, a considerable number of pulmonary infections was noted, although white cell counts did not fall significantly. Others found no significant increase in infectious complications. A side-effect which seems to be related to the use of high doses is the tendency to lose weight (Calne et al., 1979b; Du Toit et al., 1982). Gum hypertrophy, which also occurs in humans, was observed in dogs given high doses of CyA.

Monkey. Kidney graft rejection could be effectively suppressed by CyA in the rhesus monkey (Cosimi et al., 1978); doses of 10 mg/kg/day and 25 mg/kg/day were found to be about equally effective (Borleffs et al., 1981). In cynomolgus monkeys, heterotopic heart allograft survival was shown to be significantly prolonged using CyA; in combination with antithymocyte globulin (ATG), a high incidence of infection as well as lymphoma formation was seen (Pennock et al., 1981). In the latter study, the combination therapy was not more effective than CyA alone in terms of graft survival. Except for the reports of the Stanford group (Pennock et al.), no lymphoma formation has been noted by other investigators using CyA at therapeutic doses for a long period of time in the monkey. The same applies to the high frequency of infections (Borleffs et al., personal communication). Aside from the pro-

found immunosuppression caused by the combination of CyA and ATG (as reflected in the high number of infectious complications), contamination with a lymphoma inducing virus is probably also reponsible for the development of these tumors (Bieber, personal communication).

Clinical transplantation

CyA has been proved to be an extremely potent immunosuppressant also in man. By now, over 300 kidneys have been transplanted using CyA for immunosuppression, either alone or in combination with conventional immunosuppressants, in particular steroids. At the beginning, when CyA was used with other immunosuppressants, several patients developed severe infections (Calne et al., 1979a; Sweny et al., 1981). Calne et al. felt that the immunosuppression brought about by combination therapy was excessive and they consequently advised that CyA be used preferably as a single agent and to confine the use of corticosteroids to the intravenous treatment of rejection crises (Calne et al., 1981a). On the other hand, it has been proposed by Starzl et al. (1980) that not the nephrotoxic effect of CyA but early rejection may be the cause of renal malfunctioning; they recommended the combined use of CyA and prednisone to anticipate rejection. They noted that rejection in their liver patients was usually easy to control with small increases in prednisone when used concomitantly with CyA. Morris et al. (1981b) frequently found signs of cellular rejection in routine posttransplant biopsies while using CyA. Therefore, they favour the addition of steroids to prevent renal damage from chronic rejection.
The main problem in the use of CyA is its nephrotoxicity. This has been unequivocally established since the first reports of Calne et al. (1978b); it was observed not only in kidney transplant patients but also in bone marrow (Powles et al., 1980) and liver transplant recipients (Klintmalm et al., 1981). This awkward side effect, which also complicates the ready recognition of rejection, is fortunately reversible and can usually be managed with a reduction in the CyA dosage. However, kidney function is poorer in CyA patients than in Aza/Pred patients (Hamilton et al., 1981) and serum creatinine levels usually decrease after conversion from CyA to Aza/Pred (Calne et al., 1981b; Morris, 1981b). Another dose-dependent side effect of CyA is the hepatotoxicity which has been noted by several investigators (Starzl et al., 1981a; Calne et al., 1981a). This occurred less frequently than nephrotoxicity and could also be managed by reduction in dosage. Following the study of Calne et al. (1979a) reporting the occurrence of lymphomas in patients treated with CyA, the oncogenecity of the drug has remained a subject of continuous concern. The occurrence of lymphomas is probably related to excessive immunosuppression. Up to now, 5 of 300 renal allograft patients treated with CyA have developed a lymphoma and, except for one, they all had received additional immunosuppressants. This is one reason why Calne advises that CyA be preferably used not in combination with other immunosuppressants. Starzl, who uses prednisone concomitantly, had one patient of 123 transplant recipients with a lymphoma. Although one should remain alert considering the present data on CyA, there is no reason to preclude its further use in

humans. Other side effects in man are hirsutism, benign mammary fibroadenomas (Rolles and Calne, 1980), gum hypertrophy, tremor, neuresthesia and depressive psychoses (Calne et al., 1979a; Powles et al., 1980). Monitoring of CyA serum levels for clinical use has now become possible with a radioimmunoassay (Keown et al., 1981) or high pressure liquid chromatography (Gratwohl et al., 1981b). There is a good correlation between the serum level and the immunosuppressive activity, but the correlation between serum creatinine and CyA levels seems to be poor and it is not quite clear which level of the drug is nephrotoxic. CyA is variably absorbed and reaches a peak serum level 3-4 hours after administration; it is taken up by the liver and excreted via the bile (Taylor, 1980).
Of course, this potent drug has not been used only in kidney transplantation. In liver transplantation, lower doses of CyA (10 mg/kg/day) seem to be sufficient to suppress graft rejection. Hepatotoxicity appeared to be a minor problem (Calne et al., 1981a; Starzl et al., 1981b). Also in liver transplantation, Starzl added prednisone to cope with chronic rejection. Pancreas transplantation could be another indication to use CyA. Several pancreases have been transplanted, some in combination with kidney or liver transplants (Calne et al., 1981a). The results so far have been rather disappointing; graft failure was attributable to both chronic rejection and fibrosis of the exocrine pancreas caused by the occlusion of the pancreatic duct with Latex. This program has been abandoned for the time being. In bone marrow transplantation, CyA is very effective in preventing acute Graft Versus Host Disease (GVHD) (Powles et al., 1980) if administration is started shortly before transplantation. When given for treatment of an established GVHD, CyA resolved the skin manifestations during the course of treatment. Nephrotoxicity and hepatotoxicity have been problems in several of these patients (Hows et al., 1981; Gluckman et al., 1981).

Mechanism of action

Cyclosporin A acts selectively on T lymphocytes, as is evidenced by both its in vitro inhibition of the Con A and PHA induced proliferative response of rat lymphocytes (Burckhardt and Guggenheim, 1979) and human lymphocytes (Leapman et al., 1981) and its failure to suppress antibody synthesis to lipopolysaccharide antigens in nude mice (Borel et al., 1977). In general, B lymphocytes are thought not to be sensitive to CyA (Gordon and Singer, 1979). Further, a depletion of cellular elements has been seen in those areas of the reticuloendothelial system that are postulated to contain cytotoxic and helper T cells (Baldwin et al., 1981b).
The drug acts in the early phase of the immune response, although it is active only when present at the time of exposure of the recipient to antigen. Studying human lymphocytes in culture, it was observed that the drug is rapidly taken up by lymphocytes (within a few minutes) and that especially actively proliferating clones are involved; the most striking effect is the inhibition of uridine uptake soon after administration (Leoni et al., 1978). When given some time after transplantation, CyA is no longer effective in preventing kidney graft rejection in the rat (Homan et al.,

1980e) or GVHD in man.
The initially suggested clonal deletion which would explain the action of CyA has been refuted, considering the fact that in outbred species rejection nearly always occurs after discontinuation of the drug (Borleffs et al., 1981; Niessen et al., 1981). From experiments in rats, it could be deduced that CyA and enhancing serum operate on the same part of the immune response, because CyA acts synergistically with heterologous antilymphocyte serum but not with enhancing antisera (Homan et al., 1980b). Several studies support the concept that CyA inhibits the generation of cytotoxic T lymphocytes, but not the generation and expression of suppressor T cells (Gunn et al., 1980; Leapman et al., 1981). The immune suppression induced by CyA might be a reflection of its preventing the generation of cytotoxic T cells, concomitantly allowing the expression of suppressor cells (Hess et al., 1981). The "tolerance" induced by CyA has been discussed previously.

In conclusion, it can be stated that CyA is still a very promising immunosuppressant and with acceptable side effects. It can be steroid sparing and at least represents an alternative to the conventional immunosuppressants used. Its precise action remains unknown but it acts in the early phase of the immune response, selectively on proliferating helper or cytotoxic T cells. Its long-term effects will have to be awaited.

1.3. HISTOCOMPATIBILITY MATCHING

Many different histocompatibility antigens are expressed on the cells of each individual. The strong or major histocompatibility antigens on donor cells are more likely to evoke a rejection reaction in the recipient than weak or minor histocompatibility antigens. The genetic codes for the strong transplantation antigens that form the major histocompatibility system are clustered on a particular chromosomal region; genes of this specific chromosomal region make up what is called the Major Histocompatibility Complex (MHC) and such a complex has been defined in many species (Götze, 1977). In man, the MHC is located on the sixth chromosome. Its products can be detected on leukocytes by serological methods or by the mixed lymphocyte reaction (see Materials and Methods). The MHC in man has been designated as HLA (human leukocyte antigens) (Bach and van Rood, 1976) and that in the dog as DLA (dog leukocyte antigens) (Vriesendorp, 1980). Tissue typing makes possible the choice of donors that are more compatible with the recipient than are random individuals.
Ideally, a perfect match should allow for a significant increase in graft survival; however, in cadaver kidney grafting, this improvement in graft survival following HLA-A, -B matching is on the average not more than 10-15% at one year between the poorest and the best matched groups (Morris, 1981a). Only two centres have reported a greater benefit of matching (van Hooff et al., 1974; Festenstein et al., 1976). The relative value of HLA-A, -B matching in cadaveric kidney transplantation has also been confirmed by the multicentre prospective data presented at the Eighth Histocompatibility Workshop (Opelz and Terasaki, 1980b).

Better results can probably be obtained by matching for DR antigens (van Rood et al., 1979a, 1979b, 1981; Ting and Morris, 1981). DR antigens can be serologically determined; their name refers to the fact that these B-cell antigens — which are believed to be analogous to the murine Ia antigens — are closely associated with the D locus antigens. B-cell antigens are controlled by the chromosomal D region in man and could be defined only by the mixed lymphocyte reaction until four years ago. The possibility of using serological techniques instead of the relatively cumbersome cellular ones made prospective matching for D locus antigens clinically practical. It has not yet been fully clarified whether D and DR antigens are products of the same locus (Balner, 1979). Apart from the fact that DR matching seems to be more effective than matching for HLA-A, -B and -C, it is also more useful because of the restricted polymorphism of the DR locus, which exhibits only 7 allelic antigens, while the A series includes 17, the B series 27, the C series 6 and the MLR defined D series 11 antigens so far known. The effects of matching for HLA-DR can probably be increased by HLA-A and -B matching (van Rood et al., 1981); the results of matching for HLA-DR and for HLA-A, -B seem to be quite independent. However, the results of the Eighth International Workshop Study (Opelz and Terasaki, 1980b) still leave some doubts as to the influence of DR matching on graft outcome; the retrospective data of the Study in particular showed superior results. In the latter study, a remarkable correlation between HLA-DR matching and second transplant survival was found. Matching for HLA-A and -B loci was found to be not, or only marginally, significant in second cadaver transplants (Opelz, 1978; Opelz and Terasaki, 1978).

Within families, histocompatibility matching is of great importance and has been shown to be very effective from the beginning (Singal et al., 1969). Especially grafts between HLA-identical siblings show very favourable results: about 80 percent of grafts will still be functioning after 5 years (Calne, 1980b). Obviously, matching for loci other than those on the MHC circumvents the effect of responsiveness towards many other unknown incompatibilities.

Besides a prolonging effect on graft survival, HLA-A, -B matching probably also influences patient survival indirectly. It was found in a collaborative analysis of Eurotransplant and the European Dialysis and Transplant Association that HLA-A, -B matching had a significant influence on patient survival (van Rood et al., 1979b). This finding has been attributed to the lesser amount of immunosuppressants required to combat rejection crises when donor and recipient are well matched. This could be deduced from the correlation between the number of mismatches and the number of rejection crisis treatments given.

Although minor antigens also play a role in graft rejection, relatively little is known about the several minor histocompatibility systems. The ABO group antigens, for example, are minor antigens. Their effect is relatively well documented; they have been shown to shorten allograft survival time in cases of incompatibility (Dausset and Rapaport, 1966). At present, donor recipient matching always involves matching for the blood group antigens. Other minor systems that can influence graft survival are the Rhesus blood group (Rh) system, the Lewis blood group system (Fi-

scher et al., 1979) and a recently discovered system of endothelium/monocyte antigens (Baldwin et al., 1981a). The data of the International Workshop Study (Opelz and Terasaki, 1980b) surprisingly showed a cumulative effect on the simultaneous presence of several red cell mismatches, resulting in decreased graft survival time. This observation stresses the possible importance of these minor systems.
It will be necessary to study the minor systems more extensively to confirm the preliminary data obtained.

CHAPTER 2

MATERIALS AND METHODS

2.1. KIDNEY TRANSPLANTATION EXPERIMENTS IN DOGS

Animals

Beagle dogs were obtained from the Centraal Proefdierenbedrijf TNO, Austerlitz, The Netherlands. The beagles were 8 to 12 months of age and weighed 10 to 15 kg. Female beagle dogs had not previously been pregnant. Mongrels were obtained from local dog handlers via the Centraal Proefdierenbedrijf of the Erasmus University, Rotterdam. They were of approximately the same size and weight as the beagles. For female mongrels, no specific information as to previous pregnancies was available.

For the experiments on the effect of matched beagle blood, non-littermate beagles of the appropriate tissue type were used as blood donors. Nine unrelated tissue-typed mongrels were used as third party blood donors. They were selected for negativity for dog erythrocyte antigens DEA-1 and DEA-2 to avoid transfusion reactions which have been reported to occur when there were incompatibilities for these antigens (Swisher and Young, 1961).

Histocompatibility testing

Histocompatibility testing was performed by serological techniques as well as by the mixed lymphocyte reaction (MLR).
By serologic testing, the serologically defined (SD) dog leukocyte antigens of the major histocompatibility complex (MHC), called DLA in the dog, can be detected. For this testing, the one- and two- stage microlymphocytotoxicity tests were used to determine the cytotoxic activity of a panel of test sera towards the blood lymphocytes of a given individual. In the one-stage test, the donor lymphocytes are suspended in complement and are added to an equal amount of antiserum and incubated for 30 minutes at 37°C. In the two-stage test, equal volumes of cells and antiserum are incubated for 30 minutes at 37°C, followed by a second incubation period with complement (Smid-Mercx et al., 1975; Joint report of second international workshop on canine immunogenetics, 1976; Vriesendorp et al., 1977). The reproducibility of both tests has been between 90% and 95%. The selection of the test procedure depends mainly upon the way the antisera were raised. With the 120 alloantisera on hand in our laboratory, the three SD loci, DLA-A, -B and -C and their respective alleles (DLA-A: 1, 2, 3, 7, 8, 9, 10, blank; DLA-B: 4, 5, 6, 13, blank; DLA-C: 11, 12, R15, blank.) could be defined.

Lymphocyte defined (LD) antigens of the MHC can be detected by mixed lymphocyte reactions (MLR) (Bijnen et al., 1977; Bijnen, 1978). In the mixed lymphocyte culture (MLC), the lymphocytes of two individuals are mixed and under adequate culture conditions the responder cells can be stimulated by allogeneic (stimulator) lymphocytes to proliferate and thus incorporate radioactive thymidine into their DNA. The amount of incorporation was determined at the sixth day of culture. Many variables can influence the amount of DNA synthesized (Bijnen et al., 1979). Using homozygous typing cells as stimulator cells in unilateral MLC, the presence of DLA-D and -E loci and their alleles* can be detected. For each donor recipient combination, MLR's were performed as unilateral cultures in both directions in quadruplicate at one time. Dogs were only transplanted if they were positive in MLR with their donor, which implies mismatching for LD-antigens.
It is noteworthy that in the dog a high degree of linkage disequilibrium is present between the SD alleles of different loci, between SD and LD alleles and between LD alleles of different loci. This implies that certain combinations of alleles occur more frequently than could be expected on the basis of the population frequencies of the independent alleles of linked loci. The phenomenon of high linkage disequilibrium in the dog is attributable to the so-called "founder" effect, which means that a few ancestors contributed most of the genes to many next generations. For our beagle dogs the relationship between SD haplotypes and LD locus antigens is depicted in Table 2.1.A.

Blood transfusions

For each transfusion, 100 ml citrated blood was administered to the recipient immediately after collection.
In the experiment studying the effect of the number and timing of transfusions, blood was administered 2 weeks preoperatively or peroperatively, as mentioned in the respective sections.
In all other studies, blood transfusions were administered at 4, 3 and 2 weeks before transplantation.

Plasma transfusions

A volume of plasma equivalent to 100 ml blood and devoid of cells was also administered at 4, 3 and 2 weeks before transplantation. The blood from which the plasma was prepared was obtained from the same mongrel donors that were used for the fresh whole blood transfusions. The plasma was prepared by centrifugation of the blood at 1400 g for 20 minutes. It was stored for several days at a temperature of -70°C before administration.

** 50, 51, 52, 53, 54, 55, 56, 57, 58, R_1, R_2, R_3, R_4, blank; these alleles cannot yet be allocated to either locus.*

The plasma thus obtained is identical to the fresh frozen plasma in common clinical use; it contains less than 5% of the blood platelets present in the original amount of blood.

Kidney transplantation

Renal allografting was performed in a routine manner under ethrane anaesthesia as described elsewhere (Bijnen and Obertop, 1980). Kidneys were always placed in the iliac fossa of the recipient. The renal artery was anastomosed end-to-end to the external iliac artery and the renal vein was anastomosed end-to-side to the common iliac vein. In case of the presence of a second renal artery, the recipient tail artery was used for a second end-to-end anastomosis or the donor was sacrificed and an aortic (Carrel) patch was taken which was then sutured end-to-side to the external iliac artery. The ureter was implanted into the base of the urinary bladder with a submuscosal tunnel.
After removal of the transplant, it was perfused with cold (4°C) 0.9% saline to which heparin, 5000 IU/l, and procaine, 2 mg/l, were added. Ischemia times ranged from 15 to 35 minutes. Before the skin incision was made, all dogs received 375.000 IU procaine penicillin and 625 mg dihydrostreptomycin i.m.; this was repeated two days after transplantation. Bilateral nephrectomy of the recipient was always performed at the same operation. Parenteral fluid (4.5% saline and 2.5% glucose) was administered on the day of transplantation and on the first postoperative day.

Table 2.1.A. SD haplotypes and linked LD locus antigens.

SD haplotype	LD antigen
2, 4, 11	50
2, 5, 11	51
9, 6, 12	52
3, 11	53
10, 5	54
3, -	55
1, 13	56
7, 5	57
9, 4, 12	58
-, 13	57/59
7, 13	R1*
7, -	R2 (R2A, R2B)
2, 13	R3
3, 10	R4
3, 4	R5
5, 11, R20	R6
10, 4	R7

LD locus antigens are indicated by the rank number of the homozygous typing cells, with which they were determined.

* Rrefers to the fact that these antigens were discovered in Rotterdam and have not yet been approved at an official workshop

Fig. 2.1.I. Triplet model. C: mongrel; A and B: beagles

Postoperative immunosuppression

Azathioprine (Imuran^{R}, kindly provided by Wellcome Nederland BV) was used at a concentration of 10 mg/ml. It was dissolved in saline and titrated with NaOH to a PH of 10.
Prednisolone (Di-adreson-F-aquosum; Organon, Oss, The Netherlands) was used at the standard concentration of 25 mg/ml. It was dissolved in distilled water.
Both azathioprine and prednisolone (Aza/Pred) were administered by daily i.v. injection in doses of 2 mg/kg body weight and 1 mg/kg body weight respectively, starting on the day of operation. This medication was continued until day 65; thereafter, it was gradually tapered off during another 50 days, according to the following schedule:

Until day 65 : starting dose of both drugs
From day 65 to 72 : 2/3 × starting dose of both drugs
From day 72 to 93 : 1/3 × starting dose of both drugs
From day 93 to 105 : 1/6 × starting dose of both drugs

Routinely, azathioprine was temporarily withdrawn when the leukocyte count dropped to below 3×10^9/l.
Cyclosporin A (CyA, kindly provided by Sandoz Ltd., Basle, Switzerland) was supplied as a white powder. For intramuscular injection, it was dissolved in Migyol 812^{R} (Dynamit Nobel AG; Troisdorf-Oberlar, W-Germany) and absolute ethanol (40%^{V}/v) by stirring at a temperature of 50°C for 2 hours. For oral administration, it was dissolved in olive oil by stirring at a temperature of 60°C for 2 hours. CyA was given in daily doses of 2, 5, 10, 15 and 25 mg/kg body weight for 28 days, starting on the day of operation. During the first 4 days after operation, it was administered by intramuscular injection in the hind legs in volumes of 0.5 - 3.5 ml and by oral gavage thereafter.

Postoperative follow-up

Serum creatinine levels were determined daily during the first postoperative week and at least biweekly thereafter. If the levels increased to above 1000 μ mole/l, the dogs were killed. The postoperative day on which the animal died from rejection or on which the serum creatinine level exceeded 1000 μ mole/l was taken as the endpoint of graft survival.
Leukocyte counts were regularly performed; if these dropped to below 3×10^9/l, azathioprine administration was temporarily discontinued.
In the studies using CyA, serum levels of alkaline phosphatase and aspartate aminotransferase were also determined to detect possible hepatotoxicity.

Histopathology

A postmortem was always performed. Kidney specimens stained with hematoxylin and eosin were microscopically examined to confirm the diagnosis of rejection, to

score the histological severity of rejection and to check for concomitant disease. In the studies using CyA, liver specimens were also examined microscopically for signs of cholestasis. Nonimmunological graft failures were excluded from the study. If other organs were found to be diseased at autopsy, samples were taken.

Experimental design

The kidney transplantion experiments described in this thesis were always performed in a triplet model. The kidneys of a nonrelated mongrel donor were transplanted to two beagle recipients and a kidney from one of the beagles was transplanted to the mongrel (Fig. 2.1.I).
In the studies using CyA for postoperative immunosuppression, the beagle recipients were often DLA-identical sibs. In an outbred species like the dog, the existence of sibs, carrying the same transplantation antigens offers the most sensitive control group possible for any treatment in transplantation experiments. If the two beagles in a triplet are DLA-identical sibs, a difference in graft survival will most likely be the result of a difference in treatment. The treatment under study in our experiments using DLA-identical littermate pairs was the administration of blood transfusions.

Statistical evaluation

Unless stated otherwise, the Wilcoxon-rank sum test was used.

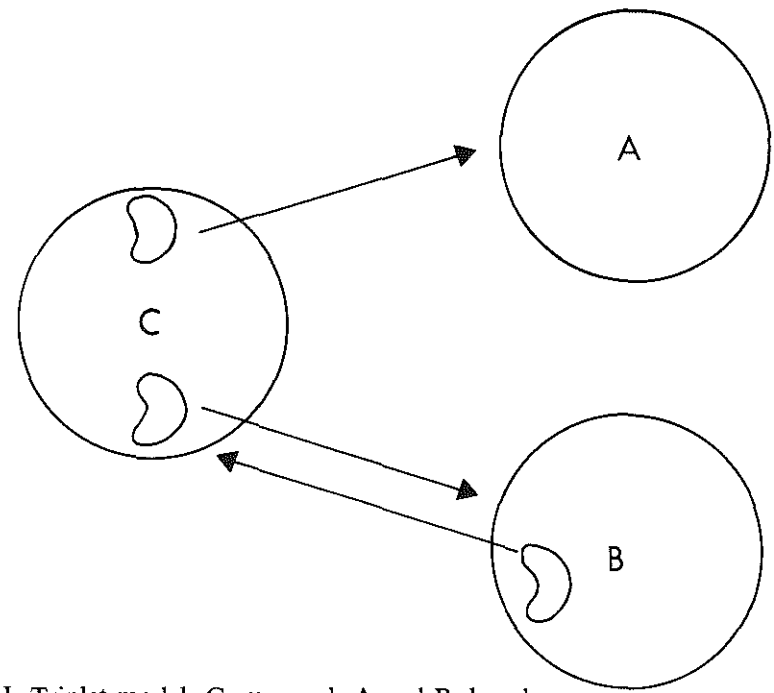

Fig. 2.1.I. Triplet model. C, mongrel; A and B, beagles

2.2. HEART TRANSPLANTATION EXPERIMENTS IN RATS

Rats

Adult male inbred rats of the WAG/Ro (RT 1^u) and BN/Ro (RT 1^n) strains and weighing 250-300 g, were used as both donors and recipients of pretransplant blood transfusions and cardiac allografts. The animals were obtained from the Centraal Proefdierenbedrijf of the Erasmus University, Rotterdam, The Netherlands.

Blood transfusions

Blood was obtained from the donor animal by cardiac puncture. Recipients received 1 ml of the citrated blood intravenously 7 days before heart transplantation.

Heart transplantation

Intra-abdominal auxiliary heart transplantation was performed according to the method described by Abbott and Lindsey (1964). Briefly, the ascending aorta of the donor heart was sutured end-to-side to the recipient's abdominal aorta, thus allowing a coronary perfusion of the graft; similarly, the pulmonary artery was sutured end-to-side to the inferior vena cava with 7-0 silk. Caval veins and pulmonary veins of the donor heart were ligated. The transplanted heart was finally covered with peritoneum. Rejection was defined as the cessation of palpable heart beats.

Postoperative immunosuppression

Only CyA was used for postoperative immunosuppression. It was dissolved in olive oil by continuous stirring at a temperature of 60°C for 2 hours. The solution was injected intramuscularly in the hind legs of the rats in volumes of 0.25-0.5 ml. Controls were injected with the same volume of olive oil.
The drug was administered for 7 days starting on the day of operation in doses of 5, 10 and 15 mg/kg body weight.

Histopathology

A postmortem was always performed. Histological preparations of the grafts stained with hematoxylin and eosin were microscopically examined to confirm the diagnosis of rejection. Nonimmunological graft failures were excluded from the study. Animals with concomitant disease as revealed at autopsy were also excluded from the study.

CHAPTER 3

THE EFFECT OF VARIOUS PRETRANSPLANT TRANSFUSION PROTOCOLS ON CANINE KIDNEY GRAFT SURVIVAL

Though it is generally accepted that pretransplant blood transfusions have a beneficial effect on renal allograft survival the following unsolved questions still remain.

The effect of blood transfusions per se, without the usual postoperative immunosuppressive treatment consisting of azathioprine and prednisolone has not yet been evaluated. Of course it would not be ethical to test this in the clinical situation, but it should be studied in a well defined preclinical model.

There have been conflicting reports regarding the optimal number and the timing of blood transfusions; these items also await further evaluation.

Except for improved graft survival blood transfusions can also cause sensitization of a future kidney transplant recipient. Therefore it seemed logical to gain the maximal benefit from a deliberate transfusion policy by administering blood from well matched donors, thus maximally avoiding the risk of sensitization.

Further the effect of the different blood components remains controversial with regard to the questions of both sensitization and the effectiveness in prolonging graft survival; some blood components have not yet been evaluated in this context, e.g., fresh frozen plasma.

Before a deliberate blood transfusion policy can be recommended, consensus has to be reached on the effectiveness and inherent risks of a particular blood transfusion protocol. The present studies in dogs are an attempt to provide answers to these questions.

3.1. THE EFFECT OF THIRD-PARTY BLOOD TRANSFUSIONS WITHOUT POSTOPERATIVE IMMUNOSUPPRESSION

Introduction

Blood transfusions represent the most important variable improving graft outcome in human cadaveric kidney transplantation (Opelz and Terasaki, 1980a). It has been demonstrated that third-party blood transfusions also have a significant effect on graft survival in dogs receiving Aza/Pred for postoperative immunosuppression (Obertop et al., 1978b, Fabre et al., 1978). The use of third-party transfusions best mimics the clinical situation in cadaveric kidney transplantation, where recipients are transfused with blood from different third-party individuals. This transfusion effect has been proved to be very reproducible in our dog model using beagles as recipients and mongrels as kidney donors (Obertop et al., 1979; Bijnen et al., 1982).

In a previous communication from this laboratory, it was reported that repeated administration of pooled third-party blood did not result in prolonged renal allograft survival in nonimmunosuppressed beagle recipients (Bull et al., 1978).
It remained to be established, however, whether random third-party mongrel blood transfusions would have an effect on renal allograft survival in nonimmunosuppressed recipients. As it would be unethical to test this in humans, our dog model seemed very suitable for further evaluation of the blood transfusion effect in view of the reproducible effects obtained.
In the present study the same protocol was used as described previously (Obertop et al., 1978b) (see Materials and Methods), without administration of postoperative immunosuppression; this makes the current results comparable to Obertop's study. Recipients were transplanted irrespective of a positive crossmatch test. Nontransfused nonimmunosuppressed dogs, which received a mismatched kidney were used as controls.

Results

The survival times of the transfused and nontransfused dogs are graphically shown in Fig. 3.1.I. The individual survival times and the crossmatch data are given separately in Table 3.1.A. It is evident that third-party blood transfusions have no effect on graft survival in nonimmunosuppressed recipients. The number of transfused dogs in this series is too small to allow an evaluation of a possible relation between a positive crossmatch test and graft survival.

Discussion

From the presented data, it can be concluded that pretransplant third-party blood transfusions have no effect on graft survival when compared with nontransfused controls. Obviously, the effect of blood transfusions alone on the immune response is not sufficient to cause prolonged kidney allograft survival.
With regard to the immune suppressive effect of third-party blood pretreatment, the findings of this study bear a close resemblance to previous active enhancement studies in the dog. Attempting to obtain prolonged renal allograft survival, Zimmerman et al. (1968) pretreated mongrel dogs with repeated injections of subcellular donor antigen without immunosuppression; no significant prolongation of graft survival was found, although a tendency for longer survival was noted. Modifying the dosage or duration of antigen administration merely resulted in worsening of the results but not in prolonged survival. Wilson et al. (1969) did a similar experiment with postoperative administration of Aza/Pred; they found a very significant prolongation of graft survival. Also Jeekel et al. (1975) could not demonstrate a significant improvement of kidney graft survival studying both passive and active enhancement in the dog without postoperative immunosuppression. Our data for third-party blood transfusions are in accord with these findings with regard to the necessity of immunosuppression to obtain prolongation of graft survival. When postopera-

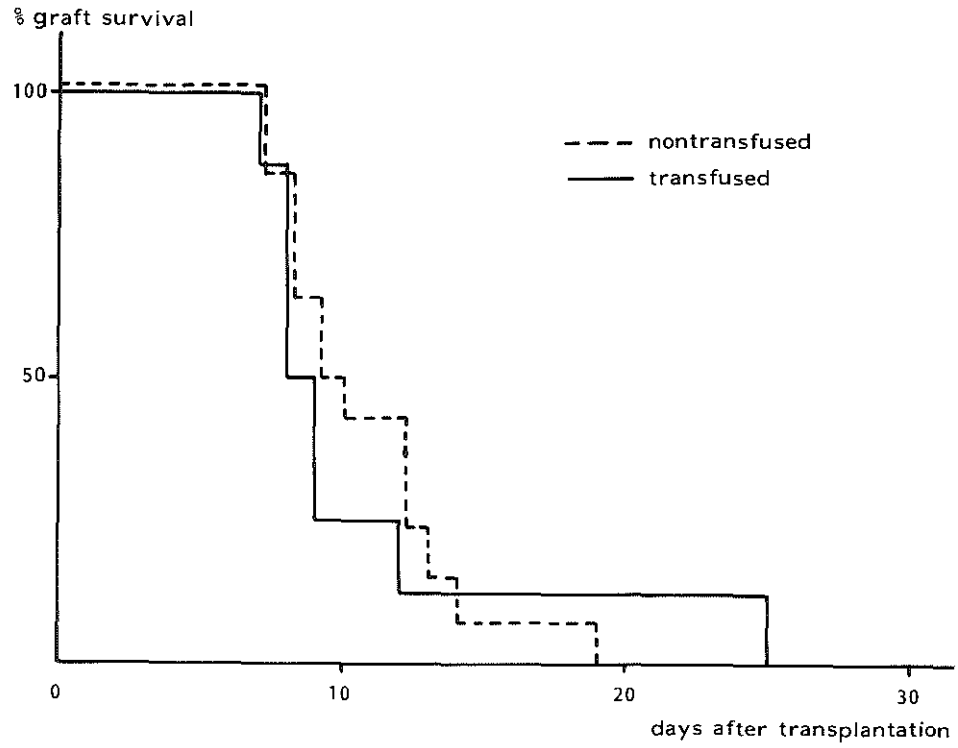

Fig. 3.1.I. Kidney graft survival in transfused and nontransfused dogs without postoperative immunosuppressive treatment. Transfusions consisted of random third-party mongrel blood.

Table 3.1.A. Survival times (days) of dogs depicted in Fig. 3.1.I.

Transfusions	7^{ND}	8^{+}	8^{ND}	8
	9^{+}	9^{+}	12	25
No transfusions	7	7	8	8
	8	9	9	10
	12	12	12	13
	14	19		

+ : positive crossmatch test (one- or two-stage microlymphocytotoxicity test)
ND: crossmatch tests not done

tive immunosuppression is given it does not seem to matter whether repeated pooled blood transfusions or random third-party transfusions are administered. For, as mentioned above, repeated pooled blood transfusions without postoperative immunosuppression did not result in significantly prolonged graft survival (Bull et al., 1978); however, it was found in another study that addition of posttransplant immunosuppressive treatment resulted in the beneficial blood transfusion effect becoming manifest (Abouna et al., 1977).

It seems that, in outbred animals such as dogs, postoperative immunosuppression is required for the expression of a beneficial effect induced by third-party or donor-specific antigen pretreatment. However, not all authors have unequivocally found similar results: Calne et al. (1966) failed to demonstrate any prolongation of canine renal allograft survival using third-party blood and different forms of donor antigen, whether immunosuppressants were or were not used.

Many protocols designed to obtain prolonged allograft survival in outbred animals were adapted from studies in rats to serve as a preclinical model. In inbred rat strains, immunosuppression is not required to obtain prolongation of graft survival in different active enhancement protocols (Stuart et al., 1968; Nelson, 1961; Marquet, 1978; Fabre, 1980). The same applies to the effect of blood transfusions. In the rat, only donor-specific transfusions are effective (Marquet et al., 1971, 1973; Fabre and Morris, 1972) and immunosuppression is not necessary to obtain prolonged graft survival. Third-party transfusions in dogs exert an effect only when followed by posttransplant immunosuppression. Donor blood transfusions in these animals have not been proved to be effective without postoperative immunosuppression (Obertop et al., 1975; Sutherland et al., 1979); however, studies on the effect of pretransplant donor blood transfusions in immunosuppressed dog recipients have not been reported. The study of Halasz et al. (1964) should not be taken into account because two separate studies using tissue-typed dogs could not confirm their finding that subcutaneous administration of donor blood gave prolonged kidney allograft survival in nonimmunosuppressed mongrel recipients (Obertop et al., 1978a; Sutherland et al., 1979). It is also known that the method of administration may be critical in enhancement: subcutaneous antigen administration often results in accelerated rejection.

Although there is a close resemblance between the findings from clinical studies and preclinical dog models regarding the effect of blood transfusions, the question arises as to whether pretransplant blood transfusions in themselves might influence graft survival in the clinical situation. As opposed to the dog recipients in this study, patients who are eligible for transplantation are nearly always more or less uremic and on maintenance dialysis treatment. The diminished immune reponsiveness accompanying the uremic state might make the effect of pretransplant transfusions more pronounced. An indication for the influence of the pretransplant immunosuppressed state on the expression of the transfusion effect can probably be found in the clinical and experimental survival data after a single transfusion. The effectiveness of one pretransplant transfusion seems variable in different centres but can be determined in multicentre data (Opelz and Terasaki, 1980a); very high one-year survival ra-

tes amounting to 80 percent have been reported only from some transplantation centres (Persijn et al., 1977, 1979; Williams et al., 1979) and may be the result of a better posttransplant follow-up (Opelz and Persijn, 1981). In the dog model, a single transfusion did not appear to be effecttive (Obertop et al., 1981) unless pretransplant immunosuppression was concomitantly given (van der Linden et al., 1982). It may be that the effect of only one transfusion is rather weak; however, it can be made more pronounced by pretransplant immunosuppression. The above reasoning concerning the importance of a pretransplant immunosuppressed state, remains a matter of speculation but is of interest with respect to the mechanisms involved.

In conclusion, it does not seem justified to treat patients with only pretransplant blood transfusions and not with postoperative immunosuppressants.

3.2. THE EFFECT OF NUMBER AND TIMING OF THIRD-PARTY BLOOD TRANSFUSIONS

Introduction

Blood transfusion has been shown to be the most consistent and most dominant variable influencing renal allograft survival (Opelz and Terasaki, 1980a; Spees et al., 1980). It seems unethical nowadays to withold blood transfusions from prospective transplant recipients and consequently many transplantation centres have changed their formerly rather restrictive transfusion policies. The inherent risks of transfusions consist of the sensitization of future recipients and the transmission of infections such as hepatitis B. These risks increase with the number of transfusions (Opelz et al., 1981c; Fehrman et al., 1980). Although the benefit of transfusions may largely outweigh the risk of inducing broadly reactive lymphocytotoxic antibodies, a transfusion policy that achieves an optimal protective effect without incurring the above-mentioned risks should be developed. Therefore, it seems logical to administer the minimal number of transfusions evoking a maximal effect.

No consensus has yet been reached as to the optimal number of transfusions. In both retrospective and prospective studies, Opelz and Terasaki (1978, 1979a, 1980a, 1980b) consistently found a dose-response relationship, i.e., the beneficial effect of blood transfusions increases with the number administered; this has been confirmed by others (Fehrman et al., 1980; Spees et al., 1980). In their multicentre analysis, Opelz and Terasaki (1980a) also found an effect of a single pretransplant transfusion, but only if packed cells were administered. Several authors, mainly reporting retrospective single centre analyses, found that about five transfusions was the optimal number to be administered to obtain improvement of graft survival; more transfusions often gave no additional benefit (Werner-Favre et al., 1979; Feduska et al., 1979; Hourmant et al., 1979; Corry et al., 1980). Only two communications have reported an extremely high one-year graft survival ($\pm 80\%$) after only one pretransplant transfusion (Persijn et al., 1977; Williams et al., 1979). Because of the wi-

de variation in findings and opinions, it is of interest to evaluate the effect of a single pretransplant transfusion in an established preclinical model. Both the graft protective effect and the frequency of sensitization can be considered.

An even more certain way to prevent recipient sensitization would be the administration of blood during transplantation, provided, of course, that peroperative transfusions also improve allograft survival. Stiller et al. (1978) initially reported a beneficial effect of peroperative transfusions in a small retrospective study. Many reports soon followed from other centres, both confirming (Hunsicker et al., 1980; Williams et al., 1980; Rashid and Sengar, 1978) and refuting (Brynger et al., 1978; Salaman, 1978; Persijn and van Rood, 1978; Opelz and Terasaki, 1981) that report. Recently, Stiller et al. could no longer confirm their initial findings in a subsequent prospective trial (Opelz and Persijn, 1980). In summary, it can be said that the effect of peroperative transfusions in man is, at the least, controversial. The beneficial effects of both a single pretransplant transfusion and a peroperative one have been demonstrated in the rhesus monkey (van Es et al., 1978) and in the dog (van der Linden et al., 1982).

The contradictory findings hitherto prompted us to investigate both questions in our dog model using beagles and mongrels. The beagles received one pretransplant blood transfusion four weeks or two weeks before operation. Mongrels received one blood transfusion during operation. Kidneys were exchanged between mongrels and beagles. A standard regimen of Aza/Pred was given postoperatively. Nontransfused immunosuppessed dogs which received a MLC mismatched kidney were considered suitable as controls. Recipient sera were not screened for lymphocytotoxic antibo-

Table 3.2.A. Survival times (days) of immunosuppressed (Aza/Pred) dogs which received one pretransplant, one peroperative or no blood transfusion

1 Preoperative transfusion (-2 weeks)	9	14^{+}	14	16^{+}
	16^{+}	42^{+}	55	150^{+}
(-4 weeks)	19^{+}	19^{ND}	54^{ND}	
1 Peroperative transfusion	12	12	16	16
No transfusion (controls)	9	10	11	12
	12	12	12	13
	15	15	16	16
	19	19	19	20
	21	23	26	30
	35	41	48	110
	>463			

+ : positive crossmatch test (one- or two-stage microlymphocytotoxicity test)
ND: crossmatch tests not done

dies but one- and two-stage crossmatch tests were performed to check for possible sensitization. In earlier experiments, a good correlation was found between positive crossmatch tests and the presence of lymphocytotoxic antibodies in recipient serum (Obertop et al., 1979). Transfused dogs exhibiting a positive crossmatch test nearly always had broadly reactive antibodies (A.M. Dekkers-Bijma, personal communication). Kidneys were transplanted irrespective of the outcome of the crossmatch tests.

Results

The survival times of the experimental groups and the crossmatch data are given in Table 3.2.A.
The survival times of the transfused groups were not significantly different from the controls. Crossmatch tests were done for 9 of 11 preoperatively transfused recipients; 6 of these 9 dogs showed a positive test. Positive crossmatches were not correlated with altered graft survival.

Discussion

In this study neither one pretransplant transfusion nor one peroperative transfusion exerted an improving effect on graft survival. Also in the dog model, van der Linden et al. (1982) found an effect of one peroperative whole blood transfusion, whereas one pretransplant transfusion was shown to be only marginally effective with concurrent pretransplant immunosuppression.
Considering the absence of a peroperative transfusion effect in our study, it should be taken into account that an unexpected variable may have been introduced by transplantation of kidneys from transfused donors. It has been reported that transfusions to the donor shortly before transplantation reduced renal allograft survival in the dog (Jeekel et al., 1980). It remains to be determined to what extent earlier transfusions to the donor influence renal allograft survival.
The fact that one pretransplant or peroperative transfusion had no improvement effect is also at variance with the monkey data (van Es et al., 1978). In mismatched rhesus monkeys, one pretransplant blood transfusion caused both prolonged and accelerated rejection. The accelerated rejection could not be foreseen because conventional serological tests did not reveal sensitization. Recipients transfused during or shortly before transplantation almost uniformly exhibited prolonged survival of approximately the same duration. This improvement was less than after five transfusions. In rhesus monkeys, the beneficial effect obviously increases with the number of transfusions, as has also been described by Opelz and Terasaki (1980a) for the clinical situation. This dose-effect probably also exists in dogs, but the threshold may lie at a different level. This could be deduced from the earlier observations (Obertop et al., 1978b) that three transfusions caused markedly prolonged survival (>>60 days) in 6 of 10 recipients; however, there is no information on the effect of more transfusions in immunosuppressed dogs.

The reasoning that one transfusion should be safer from the viewpoint of sensitization does not appear to be true. After one pretransplant transfusion, two-thirds of recipients exhibited a positive crossmatch, which is a good measure for broad sensitization. In a compilation of dogs from recent foregoing studies, approximately 40% showed a positive crossmatch following three transfusions (unpublished observations). The finding of a greater number of sensitized recipients after one transfusion may be explained by the fact that a loss of antibody reactivity can occur in spite of repeated additional transfusions. This phenomenon has been reported by Opelz et al. (1973b) and has been subsequently confirmed in a prospective study with human volunteers by Ferrara et al. (1974), but in a donor-specific situation. Patients who showed such a loss of antibody reactivity had a better graft survival than non-sensitized ones, which seems indicative for a special state of unresponsiveness (Opelz et al., 1981a). This phenomenon could also be observed in our dog model (Obertop et al., 1978b), where a decrease in immune (antibody) reactivity in some animals receiving three intermittent pretransplant transfusions was shown. Also in that study loss of antibody reactivity was correlated with markedly prolonged graft survival in all cases.

Because one preoperative transfusion led to no significant improvement in dogs, the possible effect of the time interval between the last transfusion and transplantation cannot be properly evaluated. When three transfusions were given four, three and two weeks before transplantation, a strongly positive effect was seen, while one transfusion at four or two weeks pretransplant gave no prolonged survival. Opelz and Terasaki (1980b) could not report an effect of the time interval between transfusion and transplantation from the prospective data of the International Workshop Study; this interval was found to be inversely related to the number of transfusions. Thus, a beneficial effect of transfusions shortly before operation merely reflects the effect of an increasing number.

Theoretically, this interval might be important when proposed mechanisms for the transfusion effect are considered. Several studies in man (Fischer et al., 1980; Smith et al., 1981) and experimental animals (Marquet et al., 1982) suggest that suppressor cells play a role. It has been found that suppressor cell activity in man is optimally effective three weeks after transfusion and remains present for about five months (Smith et al., 1981). This may have implications if only one transfusion is administered within three weeks before transplantation. Yet, the data from the few dogs transfused four weeks pretransplant do not support this hypothesis. Also the effect of one transfusion administered more than one year before transplantation (Persijn et al., 1979) cannot be fully explained by the above-mentioned findings regarding T suppressor cells.

In summary, the findings presented show that one pretransplant transfusion does not significantly improve kidney graft survival and do not prove that one transfusion would give less sensitization. On the contrary, when these data are considered in connection with Obertop's study, the results are suggestive for a decrease in immune reactivity with an increasing number of transfusions. The effect of peroperative transfusions is still open to question.

3.3. THE EFFECT OF MATCHED BEAGLE BLOOD TRANSFUSIONS WITH POSTOPERATIVE ADMINISTRATION OF AZATHIOPRINE AND PREDNISOLONE

Introduction

The ideal of a clinical transfusion policy remains the achievement of a protective effect against graft rejection without the risk of sensitization. Sensitization may lead to accelerated graft rejection or render patients practically unsuitable as transplantation candidates. Theoretically, sensitization can be prevented as much as possible by administering matched blood, i.e., blood exhibiting the same, defined, transplantation antigens as are present on recipient tissue. On the other hand, it is probably necessary to administer a certain load of different antigens to induce a state of unresponsiveness (Fehrman et al., 1980) that is sufficient to have an effect on graft survival. Little is known about the effect of matched transfusions. In a preliminary report on 10 patients, Nubé et al. (1981) showed that two or three HLA-A, -B matched transfusions had a beneficial effect on graft outcome. However, Albert et al. (1981) found that the administration of one or three matched transfusions resulted in relatively poor graft survival. In the rhesus monkey, one SD-matched blood transfusion resulted in prolonged graft survival in five of seven SD-identical immunosuppressed recipients (van Es et al., 1978).

In the present study, an attempt was made to evaluate the effect of matched blood transfusions on renal allograft survival in our dog model.

Experimental Design

Beagle dogs were used as recipients and mongrels as kidney donors. As in all other studies all combinations of kidney donors and recipients were positive in the MLR, indicating LD incompatibility (see Materials and Methods). Tissue typed beagle dogs were chosen as blood donors and kidney recipients, because many of their histocompatibility antigens are defined and their pedigrees are known.

In the first experiment three mutually DLA- (SD- and LD-) identical blood transfusions from unrelated blood donors were administered to unrelated mutually DLA-identical kidney recipients (□0) and to unrelated, mutually DLA-identical, kidney recipients, which were DLA-mismatched with the blood donors (□2). Because of the unexpected results of this first experiment a second one was done, involving the administration of three mutually mismatched beagle blood transfusions to three unrelated mutually mismatched recipients (□2D) (see Table 3.3.A.). In view of the restricted variation in MHC antigens and the limited number of dogs (!), it was impossible to always administer fully mismatched transfusions to the recipients of group □2D. In these dogs certain SD antigen haplotypes nearly always occur in combination with the same LD antigens as a result of high linkage disequilibrium (see Materials and Methods table 2.1.A.).

Table 3.3.A. Serological tissue-typing data of beagle recipients and beagle blood donors

Experimental group	Dog number	Recipient DLA-antigens	Blood donor DLA-antigens	Survival time (days)
Group □0	800657	7,-/9,6,12	7,-/9,6,12	9
			7,-/9,6,12	
			7,-/9,6,12	
	791814	9,6,12/10,4	9,6,12/10,4	12
			9,6,12/10,4	
			9,6,12/10,4	
	801606	9,6,12/1,13	9,6,12/1,13	12
			9,6,12/1,13	
			9,6,12/1,13	
	801539	9,4,12/3,10	9,4,12/3,10	13
			9,4,12/3,10	
			9,4,12/3,10	
	791827	9,6,12/1,13	9,6,12/1,13	13
			9,6,12/1,13	
			9,6,12/1,13	
	801496	7,-/9,6,12	7,-/9,6,12	16
			7,-/9,6,12	
			7,-/9,6,12	
	791718	7,-/9,6,12	7,-/9,6,12	19
			7,-/9,6,12	
			7,-/9,6,12	
	8253	9,6,12/1,13	9,6,12/1,13	26
			9,6,12/1,13	
			9,6,12/1,13	
Group □2	800474	9,4,12/3,10	9,6,12/1,13	10
			9,6,12/1,13	
			9,6,12/1,13	
	791728	R20,5,11/2,5,11	7,-/9,6,12	10
			7,-/9,6,12	
			7,-/9,6,12	
	791719	7,-/9,6,12	9,6,12/1,13	12
			9,6,12/1,13	
			9,6,12/1,13	
	800713	7,-/3,10	9,6,12/4,10	13
			9,6,12/4,10	
			9,6,12/4,10	
	800716	3,10/10,4	7,-/9,6,12	13
			7,-/9,6,12	
			7,-/9,6,12	

	790771	2,4,11/9,6,12	9,4,12/3,10	16
			9,4,12/3,10	
			9,4,12/3,10	
	791727	R10,5,11/9,4,12	9,6,12/1,13	48
			9,6,12/1,13	
			9,6,12/1,13	
	801538	9,4,12/3,10	7,-/9,6,12	54
			7,-/9,6,12	
			7,-/9,6,12	
Group □2D	800556	5,11,R20/6,10	5,11,R20/6,10	10
			6,-/9,4,12	
			4,10/7,-	
	800577	5,11,R20/6,10	2,4,11/3,10	12
			9,4,12/1,13	
			9,6,12/2,5,11	
	791721	7,-/9,6,12	7,-/9,4,12	12
			9,6,12/2,5,11	
			1,13/7,5	
	790893	9,6,12/1,13	5,11,R20/6,10	13
			6,-/9,4,12	
			4,10/7,-	
	800789	2,4,11/7,5	9,6,12/2,5,11	14
			3,10/7,5	
			1,13/9,6,12	
	801497	9,6,12/7,5,R15	9,6,12/3,10	30
			7,-/9,4,12	
			1,13/9,6,12	
	801440	6,-/9,4,12	9,6,12/3,10	36
			7,-/9,4,12	
			1,13/9,6,12	
	801707	7,-/9,6,12	9,4,12/3,10	61
			9,4,12/1,13	
			9,6,12/2,5,11	
	791712	7,-/9,6,12	9,6,12/2,5,11	>200
			3,10/7,5	
			1,13/9,6,12	

□o : three DLA-identical blood transfusions
□2 : three DLA-mismatched blood transfusions
□2D : three different DLA-mismatched blood transfusions
NT : no transfusion

The sera of the transfused recipients were not screened for the presence of cytotoxic antibodies; instead one- and two-stage crossmatch tests were performed (see section 3.2.). Kidneys were transplanted regardless of the outcome of the crossmatch tests. All dogs received the standard immunosuppressive treatment of Aza/Pred.

Results

The survival times of the three experimental groups are graphically represented in Fig. 3.3.I. For comparison, a control group was included, consisting of nontransfused dogs which were grafted with mismatched kidneys and subsequently treated with Aza/Pred. The individual survival times and the crossmatch data are given in Table 3.3.B.
No significant difference in graft survival between the three experimental groups and the control group was demonstrable. In the groups receiving DLA-mismatched blood (□2 and □2D), there seemed to be a slight tendency for longer survival when compared with the group receiving DLA-identical blood (□0). Yet no significant correlation could be found between the number of mismatched antigens administered and the survival times or between the number of mismatched antigens and the occurrence of positive crossmatch tests (see Table 3.3.A.).
Positive crossmatches occurred in only 1 of 8 dogs receiving DLA-identical transfusions (□0), in 4 of 8 receiving mutually identical DLA-mismatched transfusions (□2) and in 3 of 9 receiving different DLA-mismatched transfusions (□2D).

Discussion

In the experiment described in the preceding section (3.2) the administration of only one pretransplant transfusion or of a peroperative one has been discussed as a possible alternative to obtain a blood transfusion effect without the risk of sensitization. Another way to prevent sensitization to a great extent is to avoid a challenge with incompatible transplantation antigens by administration of blood from SD- and LD-identical individuals, i.e., well-matched blood transfusions. Although the underlying mechanism is not fully understood, several findings point to the fact that the transfusion effect might be dependent upon the presentation of a sufficient amount of antigen. This could be derived from the findings that leukocyte-free blood (Persijn et al., 1979) and also frozen blood in large multicentre studies (Opelz and Terasaki, 1978, 1980a) do not seem to induce a prolongation of kidney graft survival, or do so to a lesser extent; it has been further demonstrated in several studies that a certain minimal number of transfusions, e.g., five or more, is optimally effective in obtaining a beneficial effect on graft survival. The latter reasoning would imply that the administration of matched blood with as few antigenic incompatibilities as possible might not result in prolongation of graft survival. Thus far, little research has been done on this relatively new topic. A preliminary report from the Leiden group (Nubé et al., 1981), dealing with relatively few patients and using historical controls, suggests that HLA-A and -B matched blood can have a prolonging effect on graft

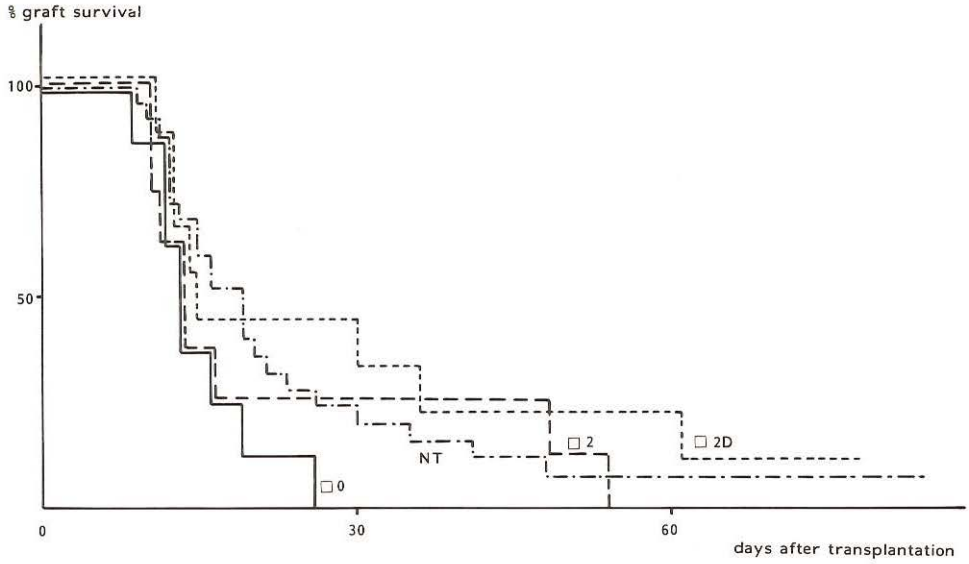

Fig. 3.3.I. Kidney graft survival in immunosuppressed (Aza/Pred) beagles which were transfused with beagle blood.

□0 : three DLA-identical blood transfusions
□2 : three DLA-mismatched blood transfusions
□2D : three different DLA-mismatched blood transfusions
NT : No transfusion

Table 3.3.B Survival times of dogs (days) depicted in Fig. 3.3.I.

□0 : DLA-identical transfusions	9	12	12	13
	13	16	19+	26
□2 : DLA-mismatched transfusions	10	10+	12+	13+
	13	16	48	54+
□2D : Different DLA-mismatched transfusions	10	12+	12	13
	14+	30+	36	61
	> 100			
NT : No transfusions	9	10	11	12
	12	12	12	13
	15	15	16	16
	19	19	19	20
	21	23	26	30
	35	41	48	110
	>>463			

\+ : positive crossmatch test (one- or two-stage microlymphocytotoxicity test)

outcome which is comparable with the excellent results of one random pretransplant transfusion in this centre. The Munich group (Albert et al., 1981), on the other hand, has already discontinued this matched-transfusion policy, because in comparison with mismatched transfusions poor allograft survival was observed. On the basis of the results obtained with leukocyte-free blood and matched transfusions, they concluded that HLA antigens present on leukocytes play a role in the transfusion effect.

In both studies, the frequency of cytotoxic antibody production seemed to be lower than average. In the Leiden study, only two patients exhibited weak cytotoxic antibody activity, which was probably directed against HLA-DR antigens, and a third one showed a temporary strong anti-HLA-A and -B activity. However, no contemporary controls who received mismatched transfusions were available for comparison. In the Munich study, antibody production tended to be less but was not significantly decreased as compared with the group receiving mismatched transfusions.

A single SD-matched transfusion caused prolongation of graft survival in the rhesus monkey (van Es et al., 1978); however in that study, kidney donors and recipients were also optimally matched for A and B (SD) locus antigens. It was further found that five of seven recipients developed cytotoxic antibodies which were not related with graft survival. It is therefore difficult to state anything conclusive about the effect of matched blood tranfusions in considering the above-mentioned data. But the impression remains that the decrease in cytotoxic antibody induction mediated by this transfusion policy is not a dramatic one.

Our study in tissue-typed beagles which were transfused with matched beagle blood showed quite unexpected results. It was shown in the first experiment that three mutually identical transfusions had no significantly different effect in both DLA-identical and DLA-mismatched recipients. Because mongrel blood transfusions had a prolonging effect under the same circumstances, the present findings indicated that the antigenic composition of three identical beagle blood transfusions was not suitable for the induction of a transfusion effect. An explanation might be that three identical beagle blood transfusions might not offer a sufficiently varied amount of antigens and that they were in this sense comparable with only one transfusion, which did not prolong graft survival in our model using standard conventional immunosuppression postoperatively. However, various differently mismatched transfusions also produced no significantly prolonged graft survival. Therefore it is a more likely hypothesis that beagle blood lacks the decisive antigens to induce a beneficial transfusion effect in beagle recipients. Obviously, the MHC antigenic differences for which we can type at present do not play a major role in the induction of a blood transfusion effect in this model. Probably undefined, minor histocompability antigens or as yet unknown MHC antigens or the combination of several types of antigenic differences are required to induce an improved graft survival. The results obtained may be best explained by the existence of an in-breeding effect in these beagle dogs, which originated also a smaller pool of undefined antigenic differences. The antigenic substrate seems to be confined to the cellular blood elements (see section 3.4).

In the present study, DLA-identical transfusions indeed caused little sensitization, as can be deduced from the number of positive crossmatches. As earlier explained (section 3.2), the number of positive crossmatch tests can give an impression of the frequency of broadly reactive antibodies. In a compilation of 83 dogs from recent previous studies an average percentage of 40% positive crossmatch tests was found after three third-party blood transfusions. DLA-mismatched beagle blood transfusions resulted in as many positive crossmatches as did mismatched mongrel blood, indicating that the MHC antigenic differences in beagle blood equally cause sensitization.

Briefly, this study supports the idea that (known) MHC antigenic differences are not very important for the induction of the transfusion effect. Furthermore optimally matched beagle blood transfusions indeed seem to induce much less sentitization in beagle dogs.

3.4. THE EFFECT OF THIRD-PARTY PLASMA WITH POSTOPERATIVE ADMINISTRATION OF AZATHIOPRINE AND PREDNISOLONE.

Introduction

Blood transfusions have a beneficial effect on renal allograft survival. Several blood products have already been analysed for their prolonging effect. Leukocyte-free blood seems to be ineffective in humans (Persijn et al., 1979). There have been conflicting reports concerning the effect of frozen blood; some publications from single centres have claimed a beneficial effect of frozen blood (Polesky, 1977; Fuller, 1977), whereas Opelz and Terasaki (1974, 1978, 1980a) in their multicentre analysis data found that frozen blood is definitely less effective; this was confirmed by the prospective data from the International Workshop Study (Opelz and Terasaki, 1980b). Whole blood, packed cells and washed packed cells all have about the same effect (Spees et al., 1980; Opelz and Terasaki, 1980a).
In preclinical models, both leukocytes and erythrocytes have been shown to result in prolonged graft survival (Obertop et al., 1979; van Es et al., 1978).
Nothing is known from clinical studies about the effect of plasma transfusions before transplantation; the same applies to platelets. Plasma and platelets contain HLA antigens in soluble and particulate form, respectively (Pellegrino et al., 1974). Also from experimental studies in outbred animals, little is known about the effect of pretreatment with third-party plasma on renal allograft survival. Harder et al. (1979) investigated the combined effect of different blood products and donor pretreatment on renal allograft survival in dogs. They found that donor blood transfusions, donor plasma transfusions and third-party plasma transfusions were equally effective in prolonging kidney graft survival, when combined with preoperative nonspecific immunosuppression, but without postoperative immunosuppression.

In the present study, third-party plasma from three mongrel donors was investigated for its effect on kidney graft survival in our dog model, employing the same pro-

tocol that had been used to demonstrate the effect of whole blood, erythrocyte and leukocyte transfusions. Plasma was prepared according to the method employed for the clinical preparation of fresh frozen plasma (see Materials and Methods). It was administered to the beagle recipients at 4, 3, and 2 weeks before transplantation. Aza/Pred were used for postoperative immunosuppression at standard dosages.

Results

The survival times of the dogs transfused with plasma are graphically shown in Fig. 3.4.I. For comparison, we included a control group consisting of nontransfused dogs, which were also grafted with mismatched kidneys and subsequently treated with conventional immunosuppression; their survival times have been published previously (Bijnen et al., 1980). The individual survival times are given in Table 3.4.A. The dogs transfused with plasma tend to survive for shorter periods than the nontransfused controls; however, the difference is not statistically significant. All crossmatch tests between recipient sera and donor lymphocytes were negative in the experimental group.

Discussion

From the results presented, it can be concluded that third-party plasma has no significant effect on kidney allograft survival in dogs when conventional immunosuppressants are administrered postoperatively. The fresh-frozen plasma used in this study contained HLA antigens in both soluble form and rep.esented on platelet fragments; only five percent of the platelets present in blood are retained in the plasma solution after centrifugation. Obviously, this method of presenting antigens does not induce a similar effect as the equivalent amount of whole blood in combination with postoperative immunosuppression.
It has been shown by van Rood et al. (1970) that plasma is effective only in a donor-specific setting and that the effect was dependent upon the composition of the antigen mixture present in the plasma. It was found that donor plasma caused accelerated rejection of a donor skin graft, but not of third-party skin. On the other hand, plasma free of platelets and particulate matter caused prolongation of skin allograft survival in the donor specific situation. This finding supports the concept that particulate antigens tend to be immunogenic and soluble ones tend to be tolerogenic (Fabre, 1980; Marquet, 1978). Harder et al. (1979) showed that third-party plasma can influence renal allograft survival in a beneficial way when preoperative immunosuppressive treatment employing procarbazine and antithymocyte serum is given after the plasma transfusions. In their dog model, third-party plasma, donor plasma and donor blood were equally effective and this suggests that the underlying mechanism is not donor specific. Several experiments using donor-specific plasma have been performed. Tinbergen (1971) and Jenkins et al. (1971) administered donor strain plasma in rat models, which showed no prolongation of allograft survival as opposed to whole blood. Fagiolo et al. (1979) transfused donor plasma in outbred rab-

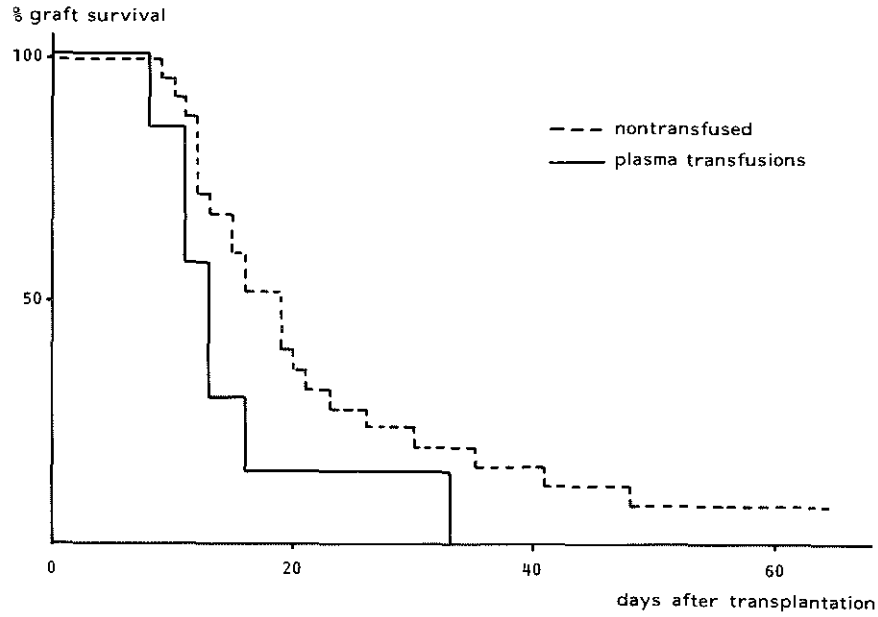

Fig. 3.4.I. Kidney allograft survival in immunosuppressed (Aza/Pred) dogs which received pretransplant plasma transfusions.

Table 3.4.A. Survival times (days) of dogs depicted in Fig. 3.4.I.

Plasma transfusions*	8	11	11	13
	13	16	33	
No transfusion	9	10	11	12
	12	12	12	13
	15	15	16	16
	19	19	19	20
	21	23	26	30
	35	41	48	110
	>463			

* : all crossmatch tests were negative

bits; they found a significantly prolonged skin allograft survival, which was even more pronounced than the effect of whole blood. Calne et al. (1970) found that pigs transfused with donor blood or donor plasma exhibited prolonged kidney graft survival; they ascribe their finding to the presence of soluble tolerogenic transplantation antigens. The latter experiments were done without postoperative immunosuppression. They cannot be compared to our study in dogs using third-party plasma and postoperative immunosuppression. Those experiments are more comparable with other active enhancement studies that employ donor antigen pretreatment to achieve prolonged allograft survival in a donor specific way (Calne et al., 1970; Wilson et al., 1969; Marquet, 1978). In view of the findings of Harder et al., (1979), it remains to be elucidated whether a nonspecific component is involved in the abovementioned experiments.

In our study, there were no positive crossmatch tests following repeated preoperative plasma transfusions. On the average, 40% of dogs, which received three blood transfusions, have a positive crossmatch against donor lymphocytes (unpublished observations). Therefore it does not seem likely that many broadly reactive lymphocytotoxins were produced; however, the sera of the transfused dogs have not been tested against a panel of lymphocytes. It has been hypothesized that the use of certain blood derivatives such as the plasma protein fraction might shorten renal allograft survival because of sensitization to HLA antigens (Pattison et al., 1974). No evidence supporting this hypothesis has been reported up to now.

Pretreatment with third-party plasma in combination with postoperative conventional immunosuppression did not result in prolongation of renal allograft survival. To obtain a beneficial effect on graft survival, it might be necessary to add pretransplant immunosuppression to the present protocol on the basis of the data of Harder et al. (1979) and the results obtained with one pretransplant transfusion (see section 3.2). It was found that one pretransplant transfusion was effective only when concomitant pretransplant immunosuppression was given (van der Linden et al., 1982). Extrapolating these findings to the clinical situation, pretransplant plasma transfusions might have an improving effect on renal allograft survival when administered to uremic patients who are known to have a depressed immune responsiveness.

At present, the administration of whole blood remains the most certain and effective means to obtain prolonged kidney graft survival.

CHAPTER 4

THE EFFECT OF POSTTRANSPLANT ADMINISTRATION OF CYCLOSPORIN A ON RAT HEART ALLOGRAFT SURVIVAL IN TRANSFUSED AND NONTRANSFUSED RECIPIENTS

Introduction

The novel immunosuppressant cylosporinA (CyA) has been shown to be very effective in suppressing graft rejection, especially in nonsensitized recipients (Brent, 1980). There is still much debate about its action on the secondary immune response (Borel et al., 1977; Brent, 1980; Deeg et al., 1980), which is an important consideration with respect to the possibilities of treatment of sensitized patients.
In the clinical situation pretransplant third-party (Opelz and Terasaki, 1980a) and donor-specific (Salvatierra et al., 1980) blood transfusions given to prospective (cadaver) kidney graft recipients may have a beneficial effect on allograft survival. This beneficial blood transfusion effect has also been shown in outbred animals such as dogs (Obertop et al., 1978b) and monkeys (van Es et al., 1977) and in inbred rat strains (Marquet et al., 1971). However, blood transfusions carry the risk of sensitization (Patel and Terasaki, 1969) with subsequent poor graft prognosis. In the inbred WAG/Ro (RT 1^u) and BN/Ro (RT 1^n) rat strains a beneficial or deleterious effect of pretransplant donor blood transfusions on graft survival can be seen depending upon the donor recipient combination used (Marquet et al., 1971, 1973). After conditioning with 1 donor blood transfusion one week before transplantation indefinite survival of a BN/Ro heart allograft is seen in WAG/Ro recipients, whereas accelerated rejection (graft survival 5-6 days) of a WAG/Ro heart occurs in BN/Ro recipients. Normally, when nontransfused recipients are involved, each strain rejects a heart allograft from the other in 8-9 days. Both pretransplant blood transfusions and CyA are important tools to improve allograft survival.
It was investigated in this rat model whether postoperative administration of CyA would interfere with the effect of pretransplant blood transfusions in either donor-recipient combination.
CyA was administered in doses of 5, 10 and 15 mg/kg during 7 day, starting on the day of operation.

Results

The survival times of the different experimental groups are shown in Table 4.A.

In the BN/Ro to WAG/Ro combination, a low dose of 5 mg/kg CyA administered during 7 days was sufficient to give indefinite graft survival in nonsensitized recipients (group 2A). As earlier shown, one blood transfusion led to indefinite graft survival in this donor-recipient combination (group 4A). This beneficial transfusion

Table 4.A. Rat heart allograft survival in transfused and nontransfused recipients treated with cyclosporin A.

		A(BN/Ro to WAG/Ro)		B(WAG/Ro to BN/Ro)	
Group	*Treatment*	*Survival (days)*	*n*	*Survival (days)*	*n*
1	None	8-9	25	8-9	25
2	CyA, 5 mg/kg/day	P	8	16, 16, 18, P, P	5
3	CyA, 15 mg/kg/day			13, 28, P, P, P	5
4	PBT	P	25	5-6	25
5	PBT + olive oil	P	5	5-6	6
6	PBT + CyA, 5 mg/kg/day	P	8	2, 3, 4, 4, 6, 7, 9	7
7	PBT + CyA, 10 mg/kg/day			3, 5, 11, 12, 14, 25, 35, 35, 37	9
8	PBT + CyA, 15 mg/kg/day	P	7	P	7

PBT: pretransplant blood transfusion; P: permanent; CyA: cyclosporin A.

effect was not affected by any dose of CyA or the solvent (groups 5A, 6A, 8A).

In the WAG/Ro to BN/Ro combination, the low and the high doses of CyA appeared to be almost equally effective in prolonging heart allograft survival in nonsensitized recipients (groups 2B, 3B). In BN/Ro recipients, transfusion of WAG/Ro blood led to sensitization which resulted in accelerated rejection (5-6 days) of WAG/Ro hearts (group 4B). In the sensitized BN recipients, a clear dose-effect relationship for CyA was shown. A dose of 5 mg/kg CyA led to rejection within 4 days in about half of the cases (group 6B), whereas the 10 mg/kg dose prolonged allograft survival considerably in most recipients (group 7B). The 15 mg/kg dose of CyA abolished the sensitizing effect of a pretransplant blood transfusion (group 8B) and resulted in permanent graft survival in all instances.

From the observations in the WAG/Ro to BN/Ro donor-recipient combination, it is clear that the dose of CyA needed for prolongation of allograft survival is higher in sensitized recipients than in nonsensitized ones.

Discussion

In earlier studies, is was shown that, when administered for a limited period of time, CyA can effectively result in long-term renal allograft survival in nonsensitized rodents (Dunn et al., 1980a; Homan et al., 1980e). It has also been reported that CyA can prolong cardiac allograft survival in rats, although mostly under continuous treatment (Jamieson et al., 1979; Kawahara et al., 1980).

This study shows that postoperative administration of CyA for 7 days can lead to indefinite or prolonged cardiac allograft survival in nonsensitized recipients, depending upon the donor-recipient combination used. In the weak BN/Ro to WAG/Ro combination, a low dose of 5 mg/kg CyA was sufficient to obtain permanent graft survival; in the reverse situation a higher dose was needed. This CyA induced phenomenon of permanent organ graft acceptance has been observed only in rodents up to now; other mammals reject their transplants after termination of drug administration (Calne, 1980a; Niessen et al., 1981). On considering the survival time elapsing after discontinuance of CyA treatment in species other than rodents, an al-

most normal (control) graft survival is observed in many cases (Homan et al., 1981c; Niessen et al., 1981). It seems that these animals react like naive recipients from that time on and that CyA only delays the onset of an otherwise normal immune response. The induction of long or permanent graft survival in rats is not confined to CyA. It has also been observed after administration of other immunosuppressants, such as antilymphocyte serum, cyclophosphamide and the combination of azathioprine and prednisolone (van Bekkum et al., 1969).

The data obtained in this study also lend support to the opinion that CyA can act as an effective immunosuppressant in sensitized recipients, provided that a sufficiently high dosage is administered. This is in contradiction with an earlier statement that CyA might never be effective in sensitized patients (Lindsey et al., 1980). The support is especially strengthened by the fact that, in our model, the sensitizing effect of pretransplant blood transfusions was definitely abolished by an acceptable dose of CyA, while the beneficial effect of such pretreatment was unaltered. This latter finding might be in accord with the presumed suppressor T cell sparing by CyA (Leapman et al., 1980). In the BN/Ro to WAG/Ro donor-recipient combination, we have evidence that long-term allograft acceptance after the administration of a pretransplant blood transfusion is mediated by suppressor T cells (Marquet et al., 1982). Because the beneficial effect of a pretransplant blood transfusion is not affected by CyA, it is tentative to conclude that CyA does not interfere with the action of these suppressor cells. On the other hand, it cannot be excluded that a 5 mg/kg dose of CyA even in blood conditioned WAG/Ro recipients is sufficient to cause the prolongation of graft survival merely by its immunosuppressive effect, as was observed in nontransfused recipients in this donor-recipient combination.

The markedly accelerated rejection observed at the lower dose of CyA in the WAG/Ro to BN/Ro donor-recipient combination is difficult to explain. A possible explanation may be that it was caused by IgM antibodies. There is suggestive evidence that CyA acts on T helper cells (Homan et al., 1980e). T helper cells enable the shift from IgM to IgG antibody production, especially in a secondary immune response. Logically, CyA will suppress this shift and this may lead to a selective production of IgM; the latter antibodies have been extensively shown to cause accelerated graft rejection (Mullen et al., 1977).

In summary, CyA treatment can result in indefinite cardiac allograft survival in both sensitized and nonsensitized rats; in the sensitized WAG/Ro to BN/Ro model, this effect is dose dependent. This finding is encouraging, since it does not preclude the use of CyA in the treatment of sensitized graft recipients.

CHAPTER 5

THE EFFECT OF POSTTRANSPLANT ADMINISTRATION OF CYCLOSPORIN A ON CANINE KIDNEY GRAFT SURVIVAL IN TRANSFUSED AND NONTRANSFUSED RECIPIENTS

Cyclosporin A (CyA) is probably the most potent single immunosuppressive agent known at present (Brent, 1980; Morris, 1981b). Its immunosuppressive action has been demonstrated for many different kinds of allografts in a number of species and for a variety of antigens. It also effectively suppresses renal allograft rejection in man (Calne, 1980a) and in the dog (Calne et al., 1977; Homan et al., 1980a). Several side effects have been reported in man, in contrast to experimental animals (Calne, 1980a). Serious, often lethal, infections were observed when CyA was combined with other immunosuppressants postoperatively. Some of these side effects are clearly dose-dependent, e.g. the nephrotoxic effect. It is still too early to form an impression of the long-term effects of CyA. To avoid the occurrence of serious side effects, it is desirable to use the minimal dosage of CyA that is required to obtain an adequate immunosuppressive effect. Pretransplant blood transfusions have a beneficial effect on renal allograft survival in man (Opelz et al., 1980a); this effect was also found in dogs when Aza/Pred were administered postoperatively (Obertop et al., 1978b, 1979).

It is not known whether an additive or potentiating effect could be achieved by combining the administration of CyA and pretransplant blood transfusions. Yet, in the clinical situation CyA is used both in transfused and nontransfused recipients (Starzl, 1981a). Therefore it is important to investigate the effect of blood transfusions in relation to the different immunosuppressants used, CyA in particular.

In the present studies the effect of CyA was evaluated both as a single agent and in combination with conventional immunosuppressants in transfused and nontransfused dogs. Further dose-response studies were done using CyA in the canine renal allograft model; concomitantly the effect of conversion from CyA to Aza/Pred was investigated.

5.1. THE EFFECT OF CYCLOSPORIN A AS THE ONLY IMMUNOSUPPRESSANT IN TRANSFUSED AND NONTRANSFUSED RECIPIENTS

Introduction

It is an established fact that pretransplant blood transfusions prolong renal allograft survival. However, this has been demonstrated only for transfused recipients who received azathioprine and prednisolone postoperatively. Up to now, no communications have appeared concerning the use of CyA and pretransplant blood transfusi-

ons in renal allograft patients. It is important to consider transfusion data when evaluating new immunosuppressive drugs, because blood transfusion is the most dominant variable influencing cadaver kidney graft survival (Opelz and Terasaki, 1980a; Opelz et al., 1981a).

The purpose of the present study was to determine whether a beneficial effect of pretransplant third-party blood transfusions would be evident when CyA was administered as the only immunosuppressant for 28 days. Since daily doses of 10 to 25 mg/kg CyA have already been shown to give very effective immunosuppression in dogs (Calne et al., 1977; Homan et al., 1980a), lower doses of CyA were used to allow an evaluation of the possible additive beneficial effect of pretransplant blood transfusions.

At operation, two beagles each received a kidney from the same mongrel. Some of the beagle pairs (Fig. 5.1.II) consisted of DLA-identical littermates of the same sex. One dog of each pair was transfused. Mongrels were not transfused and received a kidney from the nontransfused beagle at the same operation. Kidneys were transplanted irrespective of a positive crossmatch test. CyA was administered in doses of 2, 5 and 10 mg/kg/day for 28 days. Control dogs, transfused or nontransfused, also received a DLA-mismatched kidney and were not treated postoperatively. Serum levels of creatinine, alkaline phosphatase and aspartate aminotransferase were determined postoperatively in the CyA treated groups. For the evaluation of liver and kidney function under CyA treatment, only dogs surviving longer than three weeks were considered; values were included until rejection was imminent to rule out the effect of severe uremia on liver function or the effect of rejection on kidney function. Leukocyte counts were routinely done. The experimental groups are given in Table 5.1.A.

Results

The survival times of the dogs are depicted in Fig. 5.1.I; the individual survival times and the crossmatch data are given in Table 5.1.A. When the survival times of the transfused and the nontransfused recipients are compared, no significant difference is demonstrable in any dose group. Comparison of the transfused and nontransfused sibs of the DLA-identical littermate pairs illustrates in an even better controlled way that there is no difference in graft survival (Fig. 5.1.II).

Dogs treated with CyA at 5 mg/kg/day survived significantly longer in both the transfused and the nontransfused group. In nontransfused dogs, the 10 mg/kg/day dose showed a further improvement in graft survival over the 5 mg dose, which was significant ($p < 0.025$). In the transfused recipients, this difference was not statistically significant. Only 5 of 20 recipients in the 5 mg group survived for the duration of CyA treatment (28 days), as opposed to 10 of 13 in the 10 mg group. After termination of CyA treatment, graft rejection always followed. Graft survival after discontinuation of CyA therapy was of about the same duration as for untreated controls.

No side effects were seen in the CyA treated dogs.

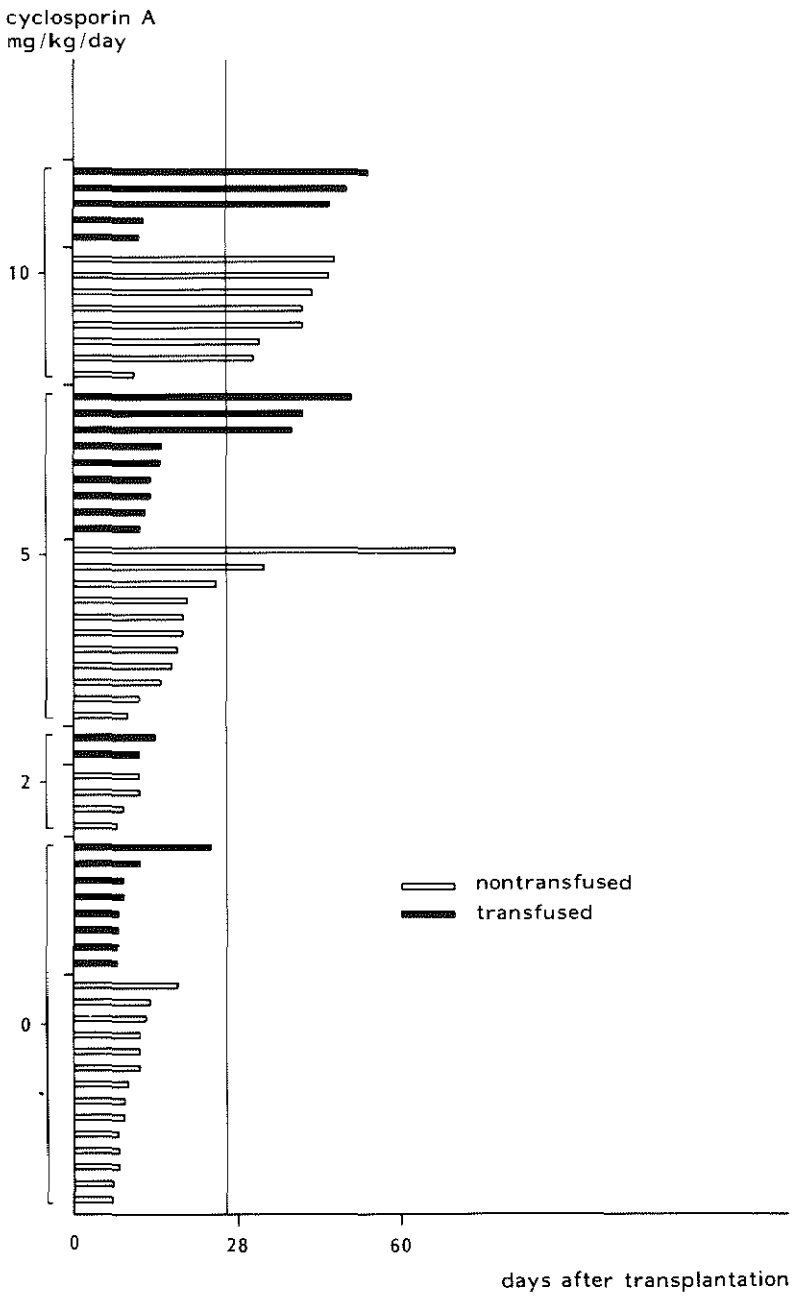

Fig. 5.1.I. Kidney graft survival in transfused and nontransfused dogs treated with cyclosporin A in doses of 0 (controls), 2, 5 and 10 mg/kg/day. Cyclosporin A was administered for 28 days.

Table 5.1.A. Survival times (days) and crossmatch data of dogs depicted in Fig. 5.1.I.

	No Immuno-Suppression				CyclosporinA 2 mg/kg				CyclosporinA 5 mg/kg				CyclosporinA 10 mg/kg			
No Transfusions	7	7	8	8	8	9	12	12	10	12	16	18	11	33	34	42
	8	9	9	10					19	20	20	21	42^{+}	44	47^{+}	48
	12	12	12	13					26	35	60					
	14	19														
Transfusions	7^{ND}	8	8^{ND}	8	12	15^{+}			12^{+}	13	14	14^{+}	12^{+}	13^{+}	37	40
	9^{+}	9^{+}	12	25					16	16^{+}	40^{+}	42	44			
									51							

$^{+}$: positive crossmatch test (one- or two- stage microlymphocytotoxicity test)
ND : crossmatch tests not done

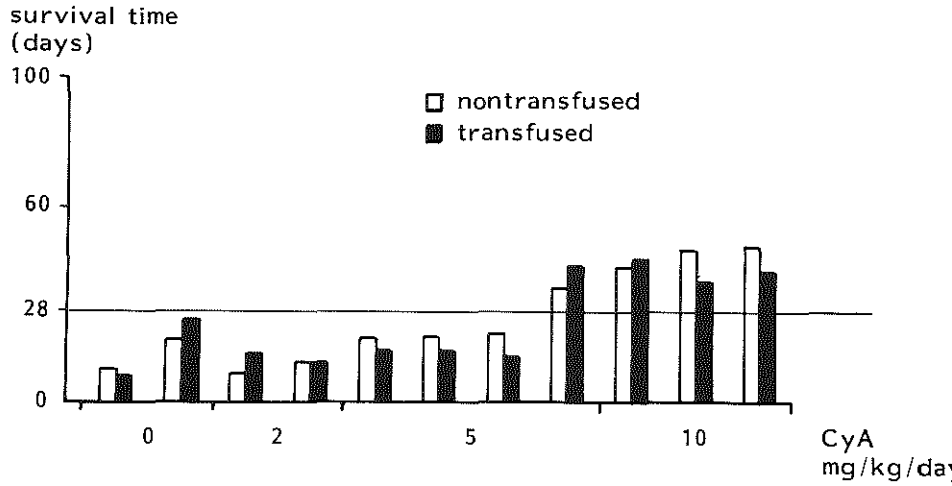

Fig. 5.1.II. Paired comparison of kidney graft survival in transfused and nontransfused DLA-identical littermates treated with cyclosporin A postoperatively. Cyclosporin A was administered for 28 days.

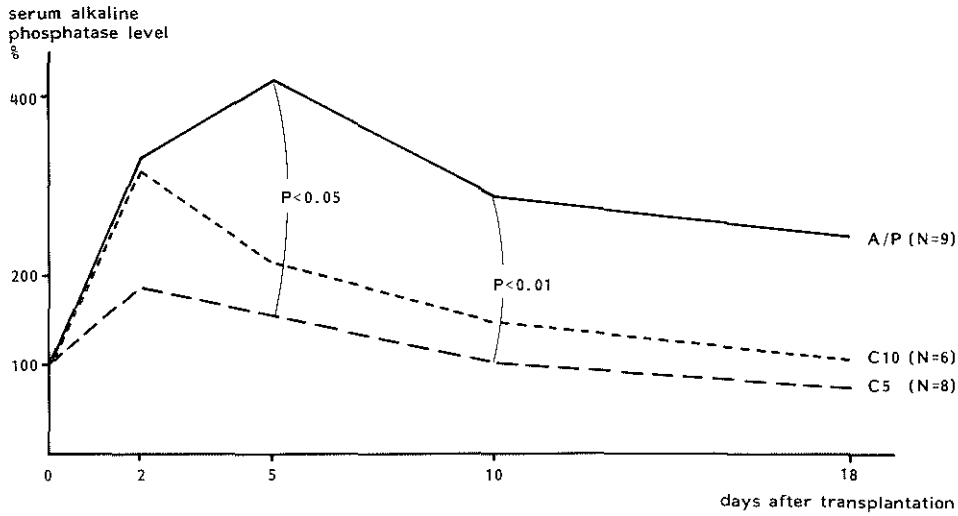

Fig. 5.1.III. The effect of different posttransplant immunosuppressive regimens on serum alkaline phosphatase levels. Values given are mean values expressed as percentages of preoperative control levels. The numbers of dogs considered are given in parentheses. A/P: azathioprine and prednisolone; C5: cyclosporin A 5 mg/kg/day; C10: cyclosporin A 10 mg/kg/day. Significant differences are indicated (Student's t-test).

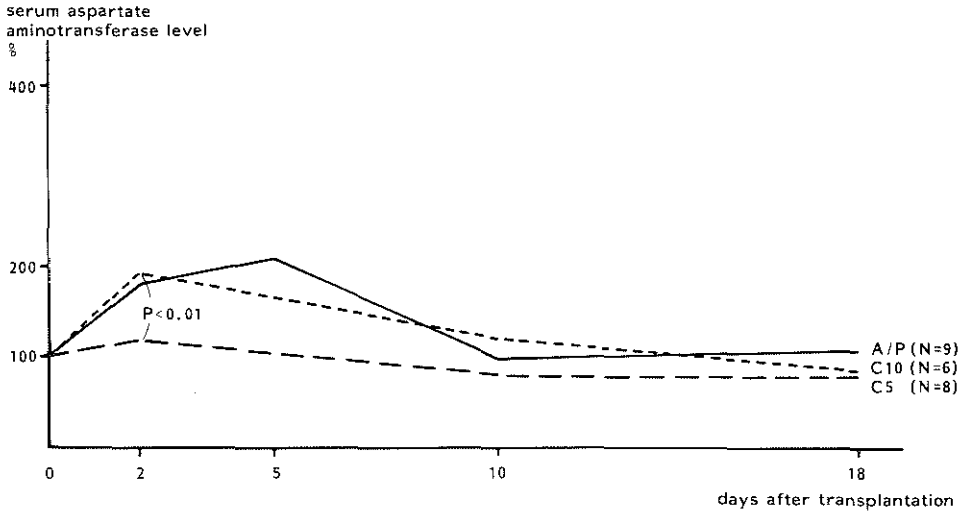

Fig. 5.1.IV. The effect of different posttransplant immunosuppressive regimens on serum aspartate transaminase levels. Values given are mean values expressed as percentages of preoperative control levels. The numbers of dogs considered are given in parentheses. A/P: azathioprine and prednisolone; C_5: cyclosporin A 5 mg/kg/day; C_{10}: cyclosporin A 10 mg/kg/day. Significant differences are indicated (Student's t-test).

In the group of transfused dogs, no significant difference in survival was noticeable between those showing a positive crossmatch and those which did not.
On day seven, serum creatinine levels were significantly elevated ($p < 0.05$) only in the 5 mg group, in comparison with control dogs from a previous study which received Aza/Pred (Fig. 5.3.II). The effect of CyA on kidney function will be more extensively evaluated and discussed in section 5.3. Serum levels of alkaline phosphatase and aspartate aminotransferase are graphically shown in Figs. 5.1.III and 5.1.IV; values expressed are percentages of the preoperative control values. Alkaline phosphatase levels are significantly lower on days five ($p < 0.05$) and ten ($P < 0.10$) in the group treated with 5 mg CyA as compared with control dogs receiving Aza/Pred. Serum levels in dogs treated with 10 mg CyA were lower than in the Aza/Pred group, though not at a significant level. On day 2, serum aspartate aminotransferase levels were significantly lower ($p < 0.01$) in dogs treated with 5 mg CyA than in those treated with 10 mg CyA. Normally in nonimmunosuppressed recipients, serum levels of liver enzymes are elevated for about ten days after transplantation, with a maximum at two to five days posttransplant (Westbroek, unpublished results).
No decrease in the leukocyte counts to below the "critical" number of $3.10^9/1$ were seen in dogs treated with CyA only; count varied from 4.4 to 52 x $10^9/1$.
At autopsy, no abnormalities were found except for rejected kidneys, in particular no tumours. Histology of the kidney specimens showed that signs of rejection were slightly less severe, though mononuclear infiltration was sometimes more pronounced in dogs receiving CyA only than in those on Aza/Pred or without immunosuppression. Liver specimens showed no signs of cholestasis or other specific damage.

Discussion

It is apparent from the data presented that CyA does not allow the expression of the blood transfusion effect when it is used postoperatively as the only immunosuppressant. This is in contrast with the results obtained when Aza/Pred were used for postoperative immunosuppression (Obertop et al., 1978b). It may be argued that an administration period of 28 days is a relatively short time for the transfusion effect to become manifest. However, this period is long enough to allow a clear dissociation between the survival times of transfused and nontransfused recipients on Aza/Pred (80% versus 24% respectively). The immunosuppressive dominance of CyA does not seem responsible for the absence of the blood transfusion effect. At a dose of 5 mg/kg/day, which was even too low for maintenance of the majority of the grafts during the course of therapy, no difference in survival time between transfused and nontransfused recipients was observed. Obviously, a transfusion effect is manifest only when conventional immunosuppressants are used postoperatively. It is more likely that the absence of the transfusion effect can be explained by a different interaction between the immunosuppressants used and the immunological mechanism that underlies the expression of the transfusion effect.
At present, indirect evidence suggests that nonspecific suppressor cells may play a role in the beneficial effect of blood transfusion on graft survival (Lenhard et al.,

1982; Marquet et al., 1982; Opelz and Persijn, 1981). Some authors have presented evidence that CyA selectively spares the expression of T suppressor cells (Leapman et al., 1980; Hess et al., 1981) and also does not affect their generation (Gunn et al., 1981). Considering these data, it is unlikely that the absence of the transfusion effect can be explained by the interference of CyA with suppressor cell action. On the other hand, preliminary evidence has been obtained by Marquet et al. (manuscript in preparation) that the action of suppressor cells was effectively abrogated by CyA in an adoptive cell transfer assay. This finding would readily explain the phenomenon observed in our study. Other T-cell-subset functions that are of crucial importance for the expression of a transfusion effect are possibly affected by CyA. It should be realized that attemps to elucidate the findings presented are speculative because the exact working mechanisms of both pretransplant blood transfusions and CyA are still poorly understood.

In the present study, a dose-response relationship for CyA and kidney graft survival was found, thus confirming reports from other workers (Homan et al., 1980a; Calne et al., 1979b). Yet, a dose of 10 mg CyA was suboptimal, 10 of 13 recipients surviving the administration course. At dosages of 5 to 10 mg, continuous administration of the drug seems necessary to maintain graft survival; after termination of CyA treatment, rejection inevitably occurred. Most dogs surviving for 28 days reject their graft one to three weeks after stoppage of CyA treatment, which is in the range of survival of nontreated controls. It seems therefore as if CyA reversibly blocks the immune response towards the graft. The findings in dogs are in agreement with those in rhesus monkeys (Borleffs et al., 1981), but are at variance with those in rodents. In rats and rabbits, a short course of CyA can induce permanent organ graft survival (Homan et al., 1980e; Niessen et al., 1982a; Dunn et al 1980a).

Serum creatinine levels on day seven were significantly elevated with 5 mg but not with 10 mg CyA, as compared with conventional immunosuppression; this finding indicates that the observed disturbance in kidney function is not primarily caused by a nephrotoxic effect of CyA. Further evidence supporting this speculation was obtained in subsequent studies (see section 5.3). However, it is evident from clinical and experimental studies that CyA can definitely exert a nephrotoxic effect which is reversible after discontinuation or reduction of the dosage (Gluckman et al., 1981; Klintmalm et al., 1981; Hamilton et al., 1981; Thomson et al., 1981). A hepatotoxic effect of CyA which occurs at higher doses of CyA and is likewise reversible has also been reported from clinical studies (Calne et al., 1979a; Powles et al., 1980; Starzl et al., 1981a). In the present study, the 5 mg dose caused a significantly lower increase in alkaline phosphatase level than did Aza/Pred, whereas the 10 mg dose did not. This phenomenon was observed on days five and ten. The difference between the 5 mg/kg/day dose and the 10 mg/kg/day dose was not significant on any day. As was known from earlier studies in nonimmunosuppressed dogs (Westbroek et al., unpublished results), transient increases in serum liver enzyme levels are evident until ten days following transplantation. Those values could not be compared with the present findings because determinations and baseline levels were different. Although the mentioned posttransplant increase makes the interpretation of the current data

somewhat difficult, it is likely that the higher - though not significant - alkaline phosphatase levels at 10 mg CyA are caused by a superimposed hepatotoxic effect of the drug during a period when the liver is more vulnerable. As a matter of fact, it appears from these data that conventional immunosuppression, i.e., azathioprine, may be significantly more hepatotoxic than 5 mg/kg/day CyA during this early posttransplant period. In contradiction is the report by Starzl et al. (1981a) that one patient had to be switched from CyA to azathioprine because of the hepatotoxicity of CyA more than a year postoperatively. It seems that hepatotoxicity is no longer a serious problem now that more information is available on the optimal dosages and serum levels of CyA can be determined (Calne et al., 1981b).
No indication of a myelotoxic effect could be shown in the present study. This contrasts sharply with the decrease in leukocyte counts seen on Aza/Pred in about 70% of the recipients surviving for longer than one month.

In summary, the beneficial blood transfusion effect was not demonstrable when CyA was used as the only postoperative immunosuppressant. A dose-effect relationship was found. No nephrotoxic effect or lower leukocyte counts were found at the dosages of the drug used. Slight hepatotoxicity seemed to occur only during the early posttransplant phase. Kidney transplant recipients treated with CyA only may receive no additional benefit from deliberate pretransplant blood transfusions.

5.2. THE EFFECT OF CYCLOSPORIN A IN COMBINATION WITH AZATHIOPRINE AND PREDNISOLONE IN TRANSFUSED AND NONTRANSFUSED RECIPIENTS

Introduction

As has been shown in the preceding section a blood transfusion effect cannot be demonstrated when CyA is used as the only immunosuppressant. Administration of Aza/Pred seems to be necessary for the expression of the transfusion effect in dogs. It would be worthwhile to combine a suboptimal dosage of CyA (10 mg/kg/day) with the conventional immunosuppressants azathioprine and prednisolone to study whether a blood transfusion effect was demonstrable and whether immunosuppression would be more optimal.
The combination of CyA with Aza/Pred has been considered harmful or at least a therapy that should be avoided to decrease the incidence of serious, often lethal, infections in clinical practice (Calne et al., 1979a; Calne, 1980a; Sweny et al., 1981). Also in a rabbit kidney graft model, the combination of CyA with corticosteroids proved to be deleterious (Dunn et al., 1980b). Early death and sepsis occurred significantly more often in recipients treated with CyA and steroids than in rabbits receiving CyA only. In contrast, a clinical study by Starzl et al. (1980) stresses that the addition of corticosteroids might increase the value of CyA. The side effects reported by these authors were not serious. Studying several drug regimens using CyA in dogs Homan et al. (1981c) found that the combination of CyA with low-dose prednisolone did not result in increased morbidity.

The present study was undertaken to investigate whether:

1) a blood transfusion effect would be demonstrable when Aza/Pred were used for background immunosuppression and CyA was administered for a limited period of time at a suboptimal dosage;
2) the combined immunosuppressive therapy would result in better graft survival than either CyA alone for 28 days or the combination of Aza/Pred alone;
3) a combined drug regimen would result in more side effects than either of the two constituents separately.

At operation, two beagle dogs which were mostly DLA-identical littermates of the same sex received a kidney from the same mongrel donor. CyA was administered at a dose of 10 mg/kg/day for 28 days. Aza/Pred were administered at standard dosages, viz., 2 mg/kg/day and 1 mg/kg/day for 65 days; thereafter, the drugs were tapered off for another 50 days.

Results

The survival times of the experimental groups are graphically shown in Fig. 5.2.I. For comparison, the survival times of dogs treated with Aza/Pred only are also depicted. The individual survival times and the crossmatch data are given in Table 5.2.A.; the survival data of recipients treated with CyA 10 mg/kg/day only are also included (see section 5.1.).

In the group treated with combined immunosuppression, a dichotomy of the survival times of transfused and nontransfused recipients can be seen; 50% (4) transfused recipients survived for sixty days versus 13% (1) of nontransfused recipients. For the purpose of comparison with the Aza/Pred group, a cut-off point of sixty days was chosen: in reality, the transfused dogs are still alive (>1 year) and the remaining nontransfused one survived for 139 days. The tendency of prolonged survival of transfused recipients over nontransfused recipients is again demonstrable when the DLA-identical littermates are compared pairwise (Fig. 5.2.II). The difference between transfused and nontransfused recipients is however not significant. A similar dichotomy effect as was observed on combined immunosuppression can be seen to occur earlier and more pronounced in the group treated with Aza/Pred; 60% of transfused recipients survived for sixty days versus 11% of nontransfused recipients ($p < 0.01$).

Nontransfused dogs treated with combined immunosuppression and subsequently with conventional drugs did not survive longer than nontransfused recipients treated with CyA only. These nontransfused dogs rejected their transplants after stoppage of CyA, irrespective of concomitant Aza/Pred administration, at a time interval which is approximately normal for untreated controls. The same observation applies to the transfused dogs which rejected their grafts.

Two transfused dogs which were treated with combined immunosuppression developed infections. One had to be killed because of a serious eye infection, with a stable kidney function, at 47 days. Another long surviving dog was striken with pneumonia four days after termination of CyA treatment; this could be succesfully treated

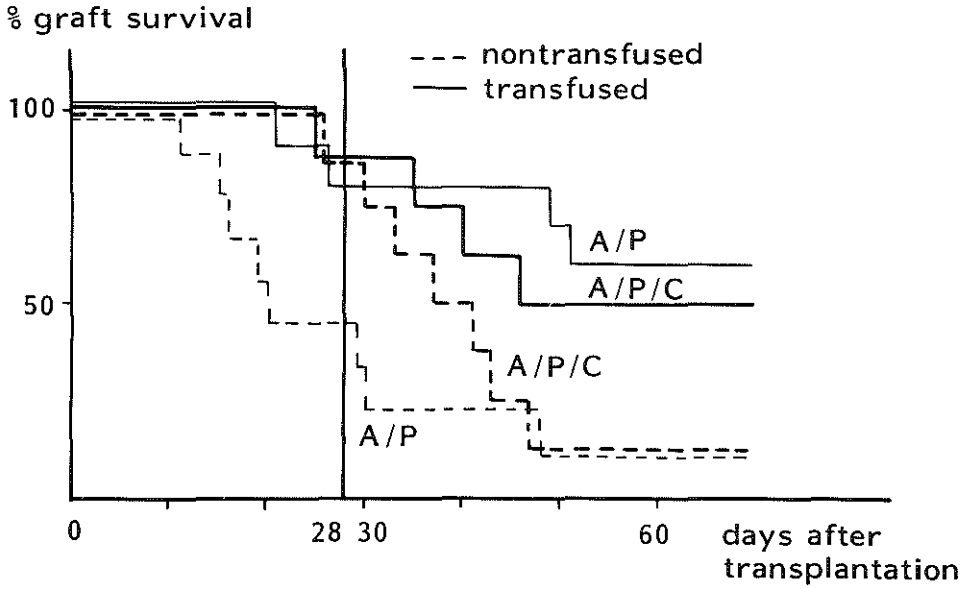

Fig. 5.2.I. Kidney graft survival in transfused and nontransfused dogs treated with the combination of azathioprine, prednisolone and cyclosporin A at 10 mg/kg/day (A/P/C) or with azathioprine and prednisolone only (A/P). Cyclosporin A was administered for 28 days.

Table 5.2.A. Survival times of transfused and nontransfused dogs treated with cyclosporin A only, with cyclosporin A in combination with azathioprine and prednisolone and with azathioprine and prednisolone only.

	Cyclosporin A 10 mg/kg				CyclosporinA 10 mg/kg Azathioprine 2 mg/kg Prednisolone 1 mg/kg				Azathioprine 2 mg/kg Prednisolone 1 mg/kg			
No transfusion	11	33	34	42	26	30	33	37	11	15	16	19
	42	44	47	48	41	43	47	>60	20	29	30	48
									>60			
Transfusions	12+	13+	37	40	25	35	40+	46	21	26	49	51
	44				>47*	>60	>60	>60	>60	>60	>60	>60
									>60	>60		

\+ : positive crossmatch test (one- or two- stage microlymphocytotoxicity test)
* : killed because of eye infection, with stable kidney function (creatinine 127 μmole/l)

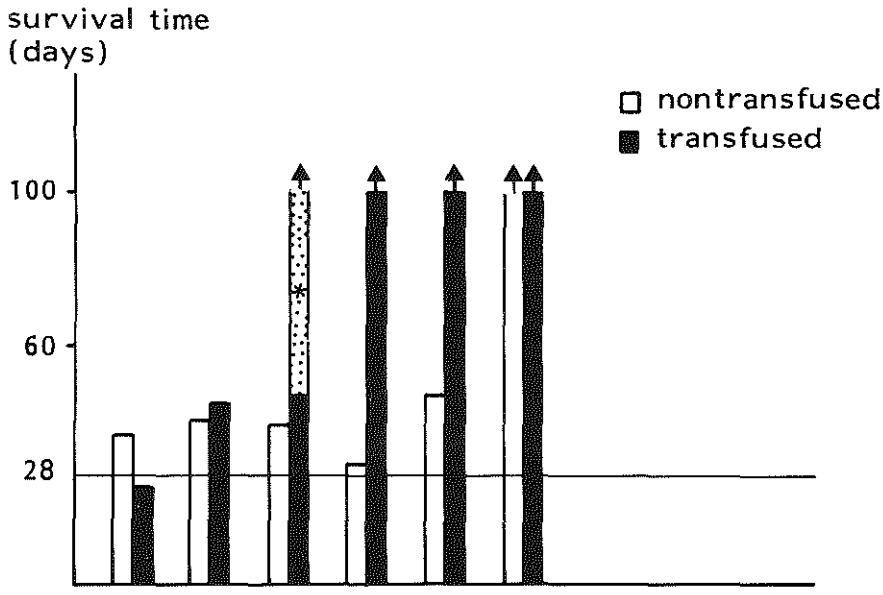

Fig. 5.2.II. Paired comparison of kidney graft survival in transfused and nontransfused DLA-identical littermates treated postoperatively with cyclosporin A, azathioprine and prednisolone. Cyclosporin A was administered for 28 days. * : Killed because of eye infection, with stable kidney function.

with a five-day course of lincomycin at 300 mg/day and kanamycin at 250 mg/day. In both animals infections occurred during a period in which leukocyte counts dropped below $3 \times 10^9/1$. These decreases in the leukocyte counts on combined immunosuppression were seen somewhat less frequently than on Aza/Pred (55% versus 67%) and were of equal duration (2-21 days; median 14 days). The serum creatinine level at day seven was significantly lower in dogs on combined immunosuppression as compared with those on Aza/Pred ($p < 0.05$); kidney function on different immunosuppressive regimens will be more extensively evaluated and discussed in section 5.3. Except for diseased kidneys and the eye infection mentioned no abnormalities were found at autopsy. Kidney specimens were not different from those seen on cenventional immunosuppression.

Discussion

In the present study, graft survival in transfused dogs which received CyA and Aza/Pred was prolonged (> 60 days) in 3 (4) of 8 recipients, as opposed to 1 of 8 nontransfused recipients; the only nontransfused dog ultimately died at 139 days, whereas the long-term surviving transfused dogs are still alive (> 1 year). A dissociation of survival times between transfused and nontransfused recipients can be seen only after stoppage of CyA (Fig. 5.2.I). When CyA was used as the only postopera-

tive immunosuppressant for a limited period of time, all grafts were rejected (section 5.1.) in both transfused and nontransfused recipients. When Aza/Pred were used for postoperative immunosuppression, a significant transfusion effect could be observed, 6 of 10 transfused dogs showing prolonged survival versus 1 of 9 nontransfused dogs (Obertop et al., 1978b). Thus, the results from the present study show an improvement in survival that is intermediate between those of the two regimens separately. Although the difference in survival between transfused and nontransfused recipients is not significant, it seems logical to attribute the greater prolongation in transfused dogs to the pretransplant transfusions in view of the results from the preceding experiments.

Considering the results of these experiments in relation to each other, it is likely that the presence or absence of the transfusion effect can be explained by a differential action of Aza/Pred or CyA on the immunological mechanism that is responsible for the transfusion effect. The different mechanism of CyA may have resulted in a partial interference with the action of Aza/Pred, which probably gave rise to the diminished expression of the transfusion effect in the present study. It remains to be determined whether a better transfusion effect can be obtained when recipients are initially treated with CyA alone and subsequently converted to Aza/Pred or vice versa.

When CyA was discontinued in the present study, all nontransfused dogs rejected their transplants after a period of time that was normal for nontreated controls. The same phenomenon was observed after discontinuation of CyA as the only immunosuppressant (section 5.1.). Obviously, the addition and continuation of Aza/Pred was not additive to the immunosuppressive regimen used in terms of graft survival. It is known that the combination of Aza/Pred in itself has a moderate immunosuppressive effect in nontranfused dogs (Obertop et al., 1978b, Fabre et al., 1978; Homan et al., 1981c). The lack of additional effect in this study might be related to the fact that a dose of 10 mg CyA is suboptimally effective. This could be deduced from the finding that converting from an optimal dose of CyA to Aza/Pred resulted in prolonged survival which could not be explained by the immunosuppressive activity of Aza/Pred alone (section 5.3.; Homan et al., 1981c).

Several authors have warned of the danger of combining CyA with conventional immunosuppressants, especially with regard to the occurrence of serious infections (Calne et al., 1979a; Sweny et al., 1981). In the current study, two dogs developed a serious infection; normally, the occurrence of an infection is rare in our dogs on Aza/Pred. Both infections occurred while the leukocyte counts were below $3.10^9/1$. At that time, the animals were already off CyA and azathioprine administration had been stopped according to protocol (see Materials and Methods). The frequency and duration of the depressed leukocyte counts give no indication for a more myelotoxic effect of the combined regimen (CyA/Aza/Pred) than of Aza/Pred. It is not realistic to conclude from these two observations that combined immunosuppression predisposes to severe infections, but they serve as a warning to be alert.

Though not measurable in graft survival, the combined regimen seems to exert a more profound immunosuppression in terms of kidney function. The combination of CyA and Aza/Pred results in a significantly better ($p < 0.05$) kidney function

than either of the two regimens on the seventh posttransplant day. According to the other results obtained, this better kidney function is a reflection of better immunosuppression (see section 5.3.).

Briefly summarizing, for the expression of the beneficial blood transfusion effect, Aza/Pred seems to be necessary, either as initial therapy or after discontinuation of CyA. Addition of Aza/Pred to a suboptimal dose of 10 mg CyA does not lead to an additional prolongation of graft survival. No dramatic increase in infection frequency was observed.

5.3. DOSE-RESPONSE STUDIES OF CYCLOSPORIN A AND THE EFFECT OF CONVERTING TO CONVENTIONAL IMMUNOSUPPRESSANTS IN NONTRANSFUSED DOGS.

Introduction

Especially in the early days of the clinical use of cyclosporin A (CyA) precise knowledge concerning an optimal dose-effect relationship was lacking (Calne et al., 1979a, 1979b). This knowledge is particularly important with respect to its dose-dependent side-effects such as the described nephrotoxicity and hepatotoxicity (Calne et al., 1978b; Powles et al., 1980). Several transplantation studies in experimental animals have yielded conflicting results for different species. In rodents, a short course of CyA proved to be sufficient to result in indefinite organ graft survival (Green et al., 1978; Marquet et al., 1981), although skin grafts were rejected after termination of CyA administration (Borel et al., 1976). In outbred animals, rejection of both organ and skin grafts inevitably followed stoppage of CyA administration (Homan et al., 1980a, Niessen et al., 1981; Deeg et al., 1980). Also the minimal dose that is required to prevent rejection proved to be different in various studies; among others, this is possibly dependent upon the method of administration (Kostakis et al., 1977a, Niessen et al., 1982a; Homan et al., 1980e).
It appears from clinical studies that changing to and from CyA endangers seldom the function of the kidney (Calne et al., 1981b). Homan et al., (1981c) showed that a change to conventional immunosuppressants (Aza/Pred) after a long-term course of CyA was followed by prolonged renal allograft survival which could not be explained by the immunosuppressive effect of Aza/Pred only. This observation suggests that prior CyA administration can influence graft survival after changing to conventional immunosuppressants.

In our model, we attempted to define a dose-response relationship for CyA, seeking for the minimal dose that would prevent rejection of kidney allografts during the period of CyA therapy. Also the effect of converting to Aza/Pred after a 28-day course of CyA administration was studied to see whether effective suppression of graft rejection could be safely continued by changing to conventional immunosuppressants at that time. In evaluating the dose-effect relationship for CyA in canine renal allografting, this study is an extension of the nontransfused group described in

section 5.1. Apart from the beagles in the previous group, the other recipients presented here were all mongrel dogs that received a kidney from an incompatible beagle donor. CyA was administered at different doses for 28 days. Seven of ten dogs that received CyA at 15 mg/kg/day and all of those that received the drug at 25 mg/kg/day were converted to Aza/Pred after discontinuation of CyA treatment. Administered from 65 days after transplantation, Aza/Pred were tapered off for another 50 days.

Results

The survival times of the dogs, including the recipients that were converted to Aza/Pred, are plotted in Fig. 5.3.I.: for completion, the pertinent data from section 5.1. were added. The individual survival times and other information pertaining to the mongrel recipients are given in Table 5.3.A. The survival times of control dogs treated with Aza/Pred postoperatively are given in Chapter 3. Administration of CyA at a dose of 2 mg/kg/day did not prolong renal allograft survival. CyA 5 mg/kg/day led to significant prolongation of renal allograft survival ($p < 0.005$) and doubling the dose of the drug to 10 mg/kg/day further improved allograft survival significantly over that of the 5 mg/kg/day dose ($p < 0.025$). Seven of 8 dogs treated with CyA at 10 mg/kg/day survived for the duration of therapy; 5 of these 7 dogs rejected their kidneys at an untreated control survival time if calculated from the day of stoppage of CyA. Unexpectedly short were the survival times of the dogs on 15 mg/kg/day; only 3 of 10 animals survived CyA treatment and were subsequently treated with Aza/Pred. One of these dogs survived for 170 days and the other two rejected their kidneys at untreated control survival times if calculated from the day of converting. The 7 dogs that rejected their kidneys under CyA therapy (15 mg/kg/day) did so at the same survival time as did untreated controls. All four dogs treated with CyA at 25 mg/kg survived for 28 days. One of these animals is still alive for more than one year; a second survived for 123 days and the other two rejected their kidneys at a control time for dogs on Aza/Pred if the day of converting was taken as the day of transplantation.
The serum creatinine levels for all experimental groups mentioned in this chapter are graphically shown in Fig. 5.3.II. Only dogs surviving for longer than four weeks were considered and values were included until rejection was imminent. A tendency of improving kidney function with increasingly potent immunosuppression seems evident. Yet, only some differences are significant at day seven (Student's t-test). Serum creatinine levels are significantly higher during treatment with CyA at 5 mg/kg/day when compared with Aza/Pred ($p < 0.05$) or Aza/Pred/CyA ($p < 0.05$). Levels were also significantly higher on CyA 10 mg/kg/day as compared with Aza/Pred/CyA (< 0.05). Further, creatinine levels are significantly higher on Aza/Pred than on Aza/Pred/CyA ($p < 0.05$). Autopsies and histology resulted in no peculiar findings.

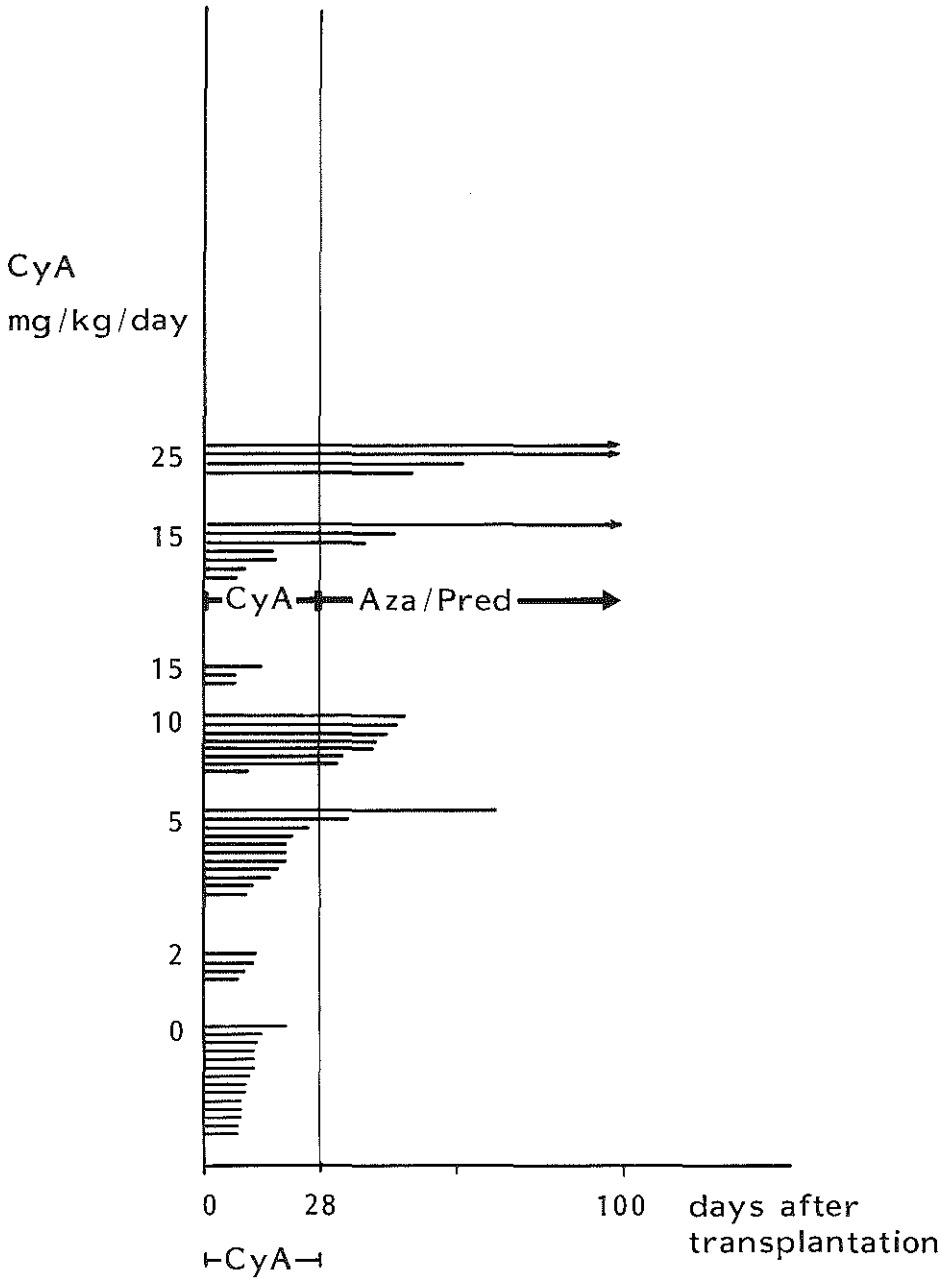

Fig. 5.3.I. Kidney graft survival in dogs treated postoperatively with different doses of cyclosporin A. Cyclosporin A (CyA) was stopped at 28 days after transplantation. Seven dogs on CyA at 15 mg/kg/day and all of those on CyA at 25 mg/kg/day were converted to azathioprine and prednisolone after cessation of CyA treatment.

Table 5.3.A. Survival times (days) of dogs depicted in Fig. 5.3.1.

CyA	0 mg/kg/day	7 (F)	7 (M)	8 (M)	8 (F)
		8 (F)	9 (F)	9 (M)	10
		12 (M)	12 (M)	12 (M)	13 (F)
		14 (M)	19		
CyA	2 mg/kg/day	8 (F)	9	12 (M)	12
CyA	5 mg/kg/day	10 (F)	12 (M)	16 (M)	18
		19	20 (M)	20	21
		26 (F)	35	70	
CyA	10 mg/kg/day	11 (F)	33	34 (M)	42 (M)
		42	44 (M)	47	48
CyA	15 mg/kg/day	7 (F)	8 (F)	8 (F)	9 (F)
		14 (F)	17 (F)	18 (F)	39 (F)
		46 (M)	170 (M)		
CyA	25 mg/kg/day	49 (M)	62 (M)	123 (M)	> 200 (M)

CyA: cyclosporin A. For mongrel recipients a sex indication is added. M: male, F: female.

Discussion

It is evident from the data presented that CyA is a very potent immunosuppressant. A dose-effect relationship for CyA in canine kidney transplantation had already been shown (Homan et al., 1980a) and could be confirmed in our dog model, with the exception of the 15 mg dose (see also section 5.1.).

The survival data in the 15 mg group were rather unexpected because 5 of 10 recipients rejected their grafts at a time appropriate for nontreated controls, while only 1 of 8 recipients did so on 10 mg CyA. Therefore, we attempted to analyse the different recipient-related factors that might influence graft survival. The major factors influencing graft survival have been found to be recipient-related (Jonasson and Moses, 1981). All recipients in the 15 mg and 25 mg groups were mongrels. As mentioned under "Materials and Methods" these mongrels were obtained indirectly from local dog handlers and hardly anything was known about their history. It was known that some females had been pregnant but the ages could only be estimated. Considering the 15 mg group, it is striking that only 2 of 10 recipients were male; they survived for 46 to 170 days. Three of 8 bitches were known to have borne puppies; these survived for 7, 8 and 8 days, respectively. Two of the remaining 5 bitches appeared morphologically old and were found to have a multiparous uterus at operation; they survived for 7 and 14 days. The other three bitches seemed young and proved to have a virgin uterus at operation; they died at 17, 18 and 39 days. From the above-mentioned data, it can be speculated that old age and pregnancies pre-

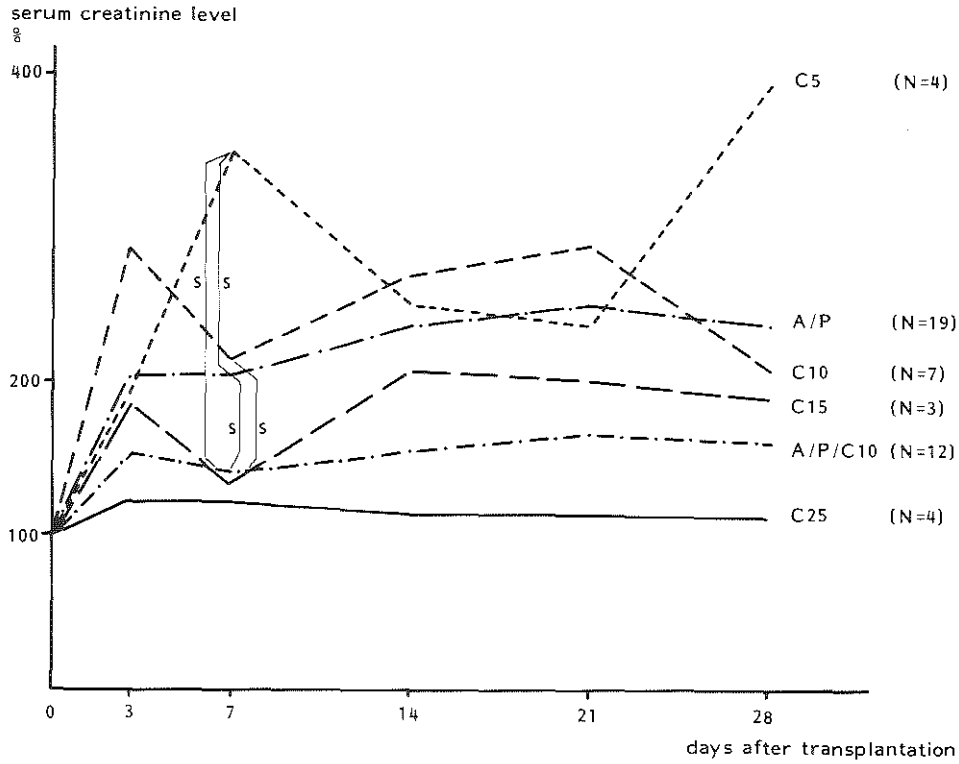

Fig. 5.3.II. Posttransplant serum creatinine levels, before beginning of rejection, on different immunosuppressive regimens. Values given are mean values expressed as percentages of preoperative control levels. The number of dogs are given in parentheses. C_5, C_{10}, C_{15}, C_{25}: cyclosporin A at 5, 10, 15 or 25 mg/kg/day. A/P: azathioprine and prednisolone (conventional immunosuppression); A/P/C_{10}: azathioprine and prednisolone in combination with cyclosporin A at 10 mg/kg/day (combined immunosuppression); S: significant at a level of $p < 0.05$ (Student's t-test).

dispose to shorter graft survival. Dogs which have been pregnant several times are more likely to be old. It has been found in clinical studies that cytotoxic antibodies are more frequently present in parous females (Opelz et al., 1981c).
Still, some authors report a beneficial effect of previous pregnancies comparable with that of blood transfusions (Editorial, Lancet 1978; Hourmant et al., 1979; Fauchet et al., 1979), a finding that could not be confirmed in large multicentre studies (Opelz and Terasaki, 1978, 1980b; Spees et al., 1980; Jonasson and Moses, 1981). The latter studies do indicate a relation between old age and shorter graft survival. It is possible than some old breeding bitches were included in this 15 mg group and that graft survival was consequently influenced by advanced age. Although not very likely in view of the concomitant prolonged survivals, something may have been wrong with the CyA solution. The present system for obtaining mongrel dogs makes a reliable evaluation of factors that may influence graft survival impossible. In fact, no difference in survival was seen between beagle recipients and mongrel recipients receiving a mismatched kidney.

A dosage of 25 mg CyA seems to be optimal, as all dogs fully survived the administration period. No side effects were seen on either this fairly high dosage or the lower ones used in this study.

Dogs that survived 28 days in the 15 mg or 25 mg group and were subsequently converted to Aza/Pred showed a dual pattern of survival. Either they rejected their graft at a time which would coincide with Aza/Pred treated recipients if calculated from the day of conversion or they showed markedly prolonged survival times (> 100 days). This marked prolongation cannot be explained by the effect of Aza/Pred alone but probably reflects a special state of diminished immune responsiveness induced by the previous CyA administration. A similar phenomenon has been observed by Homan et al., (1981c) when CyA at a dose of 20 mg/kg/day was administered for 100 days and then substituted or not by Aza/Pred. Dogs that were converted to Aza/Pred also showed a markedly prolonged survival, whereas those that were not converted rejected their grafts at control survival times. These authors did not investigate whether short-term administration of 20 mg CyA would give the same results. In view of our data on doses of 15 mg and 25 mg, it could be expected that prolonged graft survival could be noted after converting.

When we consider the creatinine levels of the recipients that survived for more than 28 days on CyA or on combined immunosuppression (Aza/Pred/CyA), a tendency for better kidney function with improving immunosuppression is suggested (Fig. 5.3.II). Some differences were significant ($p < 0.05$) on day seven: creatinine levels on 5 mg CyA were higher than on Aza/Pred or on Aza/Pred/CyA; levels on 10 mg CyA were also higher than on Aza/Pred/CyA. These data merely underline the observed tendency that better immunosuppression coincides with lower creatinine levels, which is identical to better kidney function. In this respect, our data would support the view of Starzl et al. (1980) that deterioration of kidney function often merely reflects the presence of chronic rejection rather than the nephrotoxic effect of the instituted therapy.

No nephrotoxic effects by CyA were observed in the present study, since increasing doses of the drug resulted not in decreases in kidney function but rather in better kidney function. This is in accord with experimental data from studies in dogs and monkeys, also showing no nephrotoxic effect of doses of up to 20 mg/kg/day or 25 mg/kg/day CyA, respectively (Homan et al., 1980a; Borleffs et al., 1981). The data in outbred experimental animals are in contrast with those from clinical studies: a nephrotoxic effect has already been observed by most clinicians at therapeutic doses (Calne et al., 1979a; Powles et al., 1980; Starzl et al., 1981a; Gluckman et al., 1981).

To summarize, a dose-response relationship could be confirmed for CyA in canine kidney transplantation up to a dose of 25 mg/kg/day. Several short-term survivals on 15 mg/kg/day could not be readily explained.

Short-term (28 days) treatment with 25 mg/kg/day CyA proved to have a beneficial influence on graft survival when a subsequent change to Aza/Pred was made. CyA was not nephrotoxic in this dog model.

CHAPTER 6

GENERAL DISCUSSION AND CONCLUSIONS

An attempt has been made in this study to provide answers to the several unsolved questions concerning the effects of blood transfusion and immunosuppression on graft survival.

6.1. BLOOD TRANSFUSION

Although a beneficial blood transfusion effect has been generally recognized (Opelz and Terasaki, 1980a), several questions remain to be answered before a deliberate clinical blood transfusion policy can be adopted.
Patients in whom the blood transfusion effect has been studied were mostly treated with the conventional immunosuppressants azathioprine and prednisolone. Consequently, nothing was known with respect to the immune suppressive, or more precisely, the survival promoting effect of pretransplant blood transfusion per se. Some studies had been performed on the effect of blood transfusions without the use of immunosuppressants in preclinical dog models. No significant beneficial effect on graft survival could be demonstrated using either donor-specific (Currier and Pierce, 1974; Sutherland et al., 1979) or pooled third-party blood (Bull et al., 1978). Only in the previously mentioned study by Halasz et al. (1964) was prolonged graft survival obtained following subcutaneous administration of donor blood. The data from the latter study must be considered an artifact, as the results were not reproducible in two independent studies following the same protocol (Obertop et al., 1975; Sutherland et al., 1979).
The experiment on the transfusion effect per se reported here clearly demonstrated that no beneficial effect on graft survival was obtained without conventional immunosuppression. Yet, this finding cannot be readily extrapolated to the clinical situation, because patients on dialysis are uremic. It is known that uremia causes a depressed immune reactivity (Fehrman et al., 1981) which might have some bearing on the expression of the transfusion effect. It seems not justified, however, to treat patients with pretransplant transfusions only on the basis of our findings.

Although pretransplant blood transfusions alone may have no manifest effect on graft survival without postoperative immunosuppression, another indication of the influence of blood transfusion on the immune response in man was observed in immunosuppressed recipients receiving a kidney from donors who were transfused shortly before death. It was found in two retrospective studies reporting on three transplantation centers that grafts from transfused cadaveric donors showed significantly prolonged survival times, especially when the recipients had also been transfused before transplantation (Jeekel et al., 1981; Frisk et al., 1981). The retrospective finding that transfusions to the donor can influence graft survival is supported by

the data from experiments in rats (Jeekel et al., 1982). However, in rats, the effect of transfusions to the kidney donor is mainly detrimental for the graft. It should be realized that the effect of donor transfusion, if it should be proved to be real, may have obscured transfusion data from previous studies.

If a deliberate blood transfusion policy should be adopted, a minimum number of transfusions would be preferable from both a practical point of view and in view of the fear of sensitization. If a similar beneficial effect could be obtained by peroperative transfusions only, that would be the ideal way of treatment, since sensitization would be prevented and the transfusion would constitute no extra burden to the patient. An optimal or near-optimal beneficial effect (80% one-year graft survival) of 1 pretransplant blood transfusion was reported from only two centres (Persijn et al., 1979; Williams et al., 1979). However, a single transfusion in the dog model proved to be only marginally effective, with concomitant pretransplant immunosuppression (van der Linden et al., 1982). In our dog model using Aza/Pred for immunosuppression, no significant beneficial effect of a single pretransplant blood transfusion could be shown. This could be explained by a threshold effect in the dog; possibly, a certain number of transfusions is necessary to exert an effect, as three transfusions cause a significant prolongation of graft survival in the same model (Obertop et al., 1978b). In the dog, there seems to be a dose-response relationship, just as was shown in the monkey (van Es et al., 1977, 1978) and in man (Opelz and Terasaki, 1980a).

There is even more controversy over the effect of peroperative transfusions in man. After the initial communication of Stiller et al. (1978) reporting a positive effect of transfusion during operation, the majority of the following reports did not support their finding (Brynger et al., 1978; Salaman, 1978; Persijn and van Rood, 1978); also in multicentre data no improvement effect could be shown (Opelz and Terasaki, 1980b, 1981). Although van der Linden et al. (1982) could demonstrate a positive effect of peroperative transfusions in the dog, we could not confirm this in a small series of recipients. A conclusive answer to the question concerning the effectiveness of peroperative transfusion cannot be given.

In an attempt to avoid sensitization as much as possible, studies were designed to obtain a beneficial transfusion effect while avoiding concomitant sensitization by means of matched blood transfusions. The results of two clinical pilot studies were controversial both with respect to the degree of sensitization and to the effect on graft survival (Nubé et al., 1981; Albert et al., 1981). We tried to evaluate both aspects in our mongrel to beagle donor recipient combination. Because tissue typing in our beagles has been well established, as opposed to mongrels, beagle blood transfusions of different match grades were administered to beagle recipients. It was found that beagle blood had no beneficial effect on subsequent graft survival, irrespective of the number of different MHC antigens administered. This means that beagle blood lacks the factor(s) contained in mongrel blood which are necessary to induce the transfusion effect. It may be that beagle blood contains fewer non-MHC antigenic differences that are probably important. This might be the result of the limited polymorphism of minor histocompatibility systems as a consequence of selec-

ted breeding in the beagle. In any event, this model using beagle blood in beagle recipients is not suitable for studying the effect of matched transfusions.
The relatively limited typing possibilities in mongrels preclude further study in the same donor-recipient combinations. On the other hand, it has become apparent from this study that MHC differences per se are not sufficient for the induction of a transfusion effect. It was also demonstrated that beagle blood can cause sensitization in beagles as well as can mongrel blood, but matched blood seems to lead to less sensitization than mismatched blood.

From clinical studies, it became evident that the type of blood product administered is important with regard to the transfusion effect. Frozen red blood cells appear less effective in multicentre studies than whole blood, packed cells or washed erythrocytes (Spees et al., 1980; Opelz and Terasaki, 1980a), although this was not universally confirmed (Fuller et al., 1977). This lesser or no effectiveness of frozen blood is probably attributable to the very low content of leukocytes. The absence of a transfusion effect after leukocyte-free blood transfusions supports this explanation (Persijn et al., 1979). However, in monkeys (van Es et al., 1978) and dogs (Obertop et al., 1979), both erythrocytes and leukocytes gave prolonged kidney graft survival. Little information existed about the effect of plasma and platelets on graft survival. In dogs, Harder et al. (1979) found an equivalent prolongation of graft survival following pretransplant administration of donor blood, donor plasma or third party blood in combination with preoperative immunosuppression but without postoperative immunosuppression. In our study, we found no effect of pretransplant plasma transfusions with postoperative Aza/Pred on canine kidney graft survival. So, pretransplant immunosuppression might be critical for the expression of a transfusion effect in the dog model following pretransplant administration of plasma or a single blood transfusion (van der Linden et al., 1982). Another outstanding finding in the study of Harder et al. was the equal effect of donor blood and third party blood.
Others have found that donor specific transfusion in dogs carries a high risk of donor specific sensitization with an inherent poorer prognosis for the transplant (Currier and Pierce, 1974; Obertop et al., 1975; Sutherland et al., 1979). However, in these experiments no postoperative immunosuppression was given, which makes the graft survival data difficult to interpret. A high sensitization rate following donor specific transfusion was also confirmed for the human situation in one-haplotype related recipients. However, the nonsensitized patients did extremely well (Salvatierra et al., 1981), suggesting that in addition to the nonspecific effect a donor specific enhancement effect may also play a role in the transfusion induced improvement in graft survival. Undoubtedly, the fact that donor and recipient were one-haplotype related makes a major contribution to the favourable results in this series, which can be derived from the low graft survival following donor specific transfusions from nonrelated individuals (Ruzany, personal communication).

The possible mechanisms that underly the blood transfusion effect were discussed in the Introduction: there is ample evidence for a role of suppressor cells. In our rat model, direct evidence was found that suppressor T cells present after donor specific

transfusion could induce prolongation of heart allograft survival after adoptive transfer to syngeneic recipients (Marquet et al., 1982). In the same communication, it was shown that donor specific transfusion produces both a specific and a nonspecific immunosuppression, which are both transferable by cells. However, in the rat model, the nonspecific immunosuppression induced by donor specific transfusions proved not to be sufficient to give rise to prolonged survival of third party heart allografts.
It cannot be denied that these findings bear some resemblance to the human situation. Also in humans indirect evidence has been presented for the role of cell mediated immune mechanisms, especially suppressor T cells, in the transfusion effect (Lenhard et al., 1982).
Summarizing, it can be said that present experimental and clinical evidence points to an important role of specific and nonspecific suppressor cells in the beneficial blood transfusion effect.

6.2. IMMUNOSUPPRESSION

In man, transplantation became feasible only after the introduction of the immunosuppressants azathioprine and prednisolone.
Since their introduction, these immunosuppressants have remained the mainstay of immunosuppressive therapy in transplant patients. The effect of pretransplant blood transfusions on graft survival has consequently been studied in recipients who were almost universally treated with Aza/Pred. It is possible therefore that the combination of Aza/Pred is a prerequisite for the manifestation of the transfusion effect. In dogs, immunosuppression with Aza/Pred had a significant but not dramatic effect on graft survival ($p < 0.01$) (See sections 3.1 and 3.2). This conventional immunosuppressive regimen alone seldom gave rise to prolonged survival (> 60 days). When pretransplant blood transfusions were given, a pronounced improvement ($p < 0.01$) in graft survival was seen when Aza/Pred were used postoperatively (Obertop et al., 1978b). These drugs rarely caused serious side effects in our dogs. Although a leukopenia was seen in 70% of recipients that survived for longer than one month, infections were seldom observed. No clinical signs of altered liver function were seen, despite the elevated serum alkaline phosphatase levels on Aza/Pred. In rats, Aza/Pred had a marginal effect on heart allograft survival (van Bekkum et al., 1969). This might be attributable to the fact that in rodents azathioprine is rapidly metabolized via an alternative pathway, thus diminishing its immunosuppressive effect (Elion, 1977); on the other hand, corticosteroids are more "lympholytic" in rats than in monkeys or man, which are relatively corticosteroid resistant.

The new immunosuppressant CyA has proved to be very potent in several experimental transplantation models (Homan et al., 1980a, 1980e; Borel and Meszaros, 1980; Dunn et al., 1980a; Pennock et al., 1981). The drug exhibited remarkably few side effects in experimental animals, as opposed to man (Calne, 1980). Our experiments in rats indicated that CyA can overcome sensitization by donor-specific blood transfusion in the WAG/Ro to BN/Ro rat model. This is in contradiction with the

opinion generally held that CyA does not act on the secondary immune response and consequently would not be of value for the treatment of sensitized patients (Morris, 1981b). In the reverse BN/Ro to WAG/Ro model, which exhibits a beneficial blood transfusion effect after donor-specific transfusion, CyA did not interfere with the permanent survival effectuated by transfusion. To explain this permanent survival after CyA treatment, two hypotheses that suppose different actions of CyA can be advanced. If it is assumed that suppressor T cells are responsible for the transfusion effect, the present findings of permanent graft survival in CyA treated transfused recipients might be in accord with the claimed suppressor cell sparing action of CyA (Leapman et al., 1980; Hess et al., 1981). The second hypothesis is that CyA prolongs graft survival merely by its immunosuppressive action. An indication for this may be found in the low dose of 5 mg/kg CyA which is sufficient to obtain permanent graft survival in the weak BN/Ro to WAG/Ro model. Thus, it is not impossible that CyA interferes with the expression of the transfusion effect by inhibition of suppressor cells, because it suppresses allograft rejection at the same time. The evidence presented by Hutchinson et al., (1981b) that CyA allows the emergence of specific suppressor cells is not totally convincing. They claim the emergency of specific suppressor T cells. Adoptive spleen or thymus cell transfer was performed on days 7, 11, 21, 50 and 100 after transplantation. CyA was administered for seven days posttransplant. Although the presence of suppressor cells on day 11 is in favour of their theory, the absence of a suppressor effect casts doubt on it. It would have been more convincing if CyA had been administered for a longer period of time and led to the same results. If CyA were to allow free proliferation of suppressor T cells, these cells might have been present on day 7 as well. Their presence at four days after termination of CyA treatment might mean that these cells only appear after discontinuation of CyA administration. Their findings could equally mean that CyA acts by vigorous nonspecific T-cell suppression, with the effect of CyA prevailling on more numerous cytotoxic T cells. It is known that rats with long-standing heart or kidney allografts, induced by any method, exhibit donor-specific immunity which can be transferred by adoptive cell transfer of spleen suppressor cells (Morris, 1981b; Marquet, 1978). The demonstration of a CyA induced phase of nonspecific immunosuppression followed by a later specific phase (Nagao et al., 1982) is not in contradiction with the latter phenomenon.

The concept of CyA allowing the expression of suppressor T cells by suppression of cytotoxic T cells is attractive, but the experimental evidence is not conclusive, as already mentioned by Morris (1981b).

Our experiments in dogs showed a clear dose-effect relationship for CyA in renal transplantation, thus confirming previous experiments in dogs (Homan et al., 1980a). Only the survival rates on 15 mg/kg CyA were unexpectedly low; the possible factors underlying this finding have been extensively discussed in section 5.3. It was found that rejection always occurred after cessation of CyA therapy unless other immunosuppressive treatment was given. In most animals, rejection occurred at 8-25 days after cessation of CyA, in both transfused and nontransfused recipients. The average amount of time elapsed after discontinuation of therapy is simi-

lar to the average survival time of nonimmunosuppressed recipients. From these data, it seems that CyA temporarily delays the onset of the immune response towards foreign antigens. This would also explain the reappearance of the blood transfusion effect on Aza/Pred following discontinuation of CyA therapy.

In species other than rodents, rejection of organ allografts was almost always seen after discontinuation of CyA (Homan et al., 1980a; Borleffs et al., 1981; Deeg et al., 1980; Pennock et al., 1981). A remarkable exception to this was observed in the pig (Calne et al., 1978a). CyA proved to also be an extremely potent immunosuppressant in the dog model. On 10 mg, approximately 80% of the recipients survived the duration of CyA therapy (28 days). Graft survival was significantly prolonged as compared with standard doses of Aza/Pred.

When a combined therapy using both CyA and Aza/Pred was given, the addition of Aza/Pred did not contribute effectively to the immunosuppressive potency of the instituted regimen in terms of graft survival. However, kidney function was better on combined immunosuppression than on either CyA (10 mg/kg) or Aza/Pred alone.

A dose of 25 mg/kg CyA was optimal or above optimal; all recipients fully survived the administration time. The subsequent survival of two of four recipients after conversion to Aza/Pred cannot be explained on the basis of the immunosuppressive effect of Aza/Pred only. The extraordinarily long survival times may also be attributable to the lasting decreased immune responsiveness induced by a relatively high dose of CyA administered for a limited period of time. The same observation of a markedly prolonged survival on Aza/Pred was made by Homan et al. (1981c) after 100 days of treatment at a lower dose of CyA (10 or 20 mg/kg).

From the above-mentioned data, it can be deduced that conversion from CyA to Aza/Pred does not seem to create serious hazards but may rather be advantageous. Probably, a switch from Aza/Pred to CyA would not do any harm either. The easy convertibility from and to either regimen seems to hold true for the clinical situation as well (Calne et al., 1981b). Although not many, some side effects were noticed in our animals. In some rats a general wasting, which was reminiscent of a picture described for Graft Versus Host Disease and which eventually led to death, was observed on doses of 15 mg/kg CyA given for seven days (unpublished observations). This situation persisted or developed after cessation of CyA therapy. No further untoward side effects were seen after the employed short course of CyA. In dogs, the elevated serum creatinine levels observed proved to be a reflection of suboptimal immunosuppression. With increasingly better immunosuppressive treatment in terms of graft survival, serum creatinine levels were lower. This finding supports the view of Starzl et al., (1980) that elevated serum creatinine levels are not necessarily a sign of nephrotoxicity but can also be a reflection of persistent chronic rejection.

A light subclinical form of hepatotoxicity as evidenced by elevated serum alkaline phosphatase levels was also observed in our dogs. No significantly greater number of severe infections was seen in our dogs on combined immunosuppression consisting of CyA and Aza/Pred, as has been described for the clinical situation (Calne et al., 1980a). In particular, no lymphomas were seen at autopsy; this is not surpri-

sing in consideration of the short administration time. It can be concluded that CyA has a very high therapeutic index, certainly in experimental animals. It is a very potent immunosuppressant with only few side effects, especially when used at therapeutic dosages. Contrary to the prevailing opinion, it has been shown that CyA can also be used with success in sensitized rat recipients. It would be worthwhile to test the drug in more numerous outbred animal models using sensitized recipients. Conversion to conventional immunosuppression and probably also vice versa does not seem to constitute a serious problem.

6.3. THE RELATIONSHIP BETWEEN BLOOD TRANSFUSION AND IMMUNOSUPPRESSION

Pretransplant blood transfusions prolong kidney graft survival in man (Opelz and Terasaki, 1980a). However, it should be realized that this has been proved only for patients who received azathioprine and prednisolone for postoperative immunosuppression.

It was shown in our dog model that pretransplant blood transfusions exert a beneficial effect on graft survival only in combination with postoperative conventional immunosuppression. Obviously, the effect of blood transfusions per se on the immune response is not sufficient to cause a significant prolongation of graft survival.

When CyA was used as the only postoperative immunosuppressant for 28 days, no transfusion effect was observable. Even at a low dose of 5 mg/kg CyA, which is not sufficient to maintain graft survival during the period of administration, no difference in survival between transfused and nontransfused recipients could be shown. The latter observation precludes the overriding immunosuppressive effect of CyA as the causative factor in explaining the absence of the transfusion effect. On conventional immunosuppression, a clear divergence of graft survival time between transfused and nontransfused dogs was noticeable at 28 days: 80 vs. 24 percent, respectively (Obertop et al., 1978b). When CyA and Aza/Pred were administered concomitantly, a divergence in graft survival between transfused and nontransfused recipients became apparent only after discontinuation of CyA. Although not statistically significant, this difference can only be attributed to the pretransplant transfusions, since a considerable number of recipients (3 (4) of 8) are now long-term survivors (> 1 year) and are off immunosuppression, a phenomenon which never occurs without previous transfusions.

The differential expression of the transfusion effect using different immunosuppressants must be due to the interaction of these agents with the various components of the immune response that are involved in the immune reaction evoked by the administration of third-party blood and/or other tissue antigens. Sufficient evidence is now available to assume that blood transfusions induce both specific and nonspecific T suppressor cells (Marquet et al., 1982, Lenhard et al., 1982). In nonimmunosuppressed rats, these transfusion induced suppressor cells have been shown to cause prolonged graft survival in an adoptive cell transfer model, dependent upon the number of cells transferred (Marquet et al., 1982). Assuming that these cell media-

ted suppressor mechanisms are responsible for the prolonged graft survival following transfusion, it can be speculated that the combination of Aza/Pred probably affects not the suppressor mechanism but rather the cytotoxic component of the immune response.
CyA obviously acts in a different manner. Although its working mechanism is not fully elucidated, CyA appeared to inhibit especially T helper cells in a reversible way (Homan et al., 1980e); suppressor cell induction and expression were found to be unaffected on the other hand (Leapman et al., 1981; Hess et al., 1981; Morris, 1981b). It seems that CyA temporarily stops the immunologic clock set to work by the introduction of antigens; when CyA was no longer administered, rejection occurred at a control survival time if calculated from that very time. It may be that CyA blocks the immune response in vivo in a rather nonselective manner. Preliminary experiments in the rat model showed that it may affect the prolonged heart graft survival seen after adoptive cell transfer from blood conditioned syngeneic rats (Marquet and Niessen, unpublished results). In the latter experiment, it was shown that rats receiving 100 x 10^6 mononuclear cells from blood conditioned syngeneic rats showed no permanent graft survival if they were treated with CyA at transfer, as opposed to animals that were not treated with CyA. This may suggest that CyA does indeed also inhibit suppressor cells, but it may also be that CyA blocks the action of certain immunocompetent cells or perhaps T helper cells that are essential for the expression of the transfusion effect.
A comparable explanation would apply to the absence of the transfusion effect in dogs.

It was demonstrated in the rat model that postoperative administration of CyA in the BN/Ro to WAG/Ro donor recipient combination did not alter the permanent graft survival that is seen following donor specific blood transfusion. This observation would be in accord with the supposed suppressor cell sparing effect of CyA. Still, it cannot be excluded that merely the immunosuppressive effect of CyA is manifested in this relatively weak donor recipient combination, because CyA could also induce permanent graft survival in nonconditioned recipients at similar dosages.
Using the reverse donor recipient combination, viz. BN/Ro to WAG/Ro, it was found that CyA at a dose of 15 mg/kg/day could effectively overcome the deleterious effect of sensitization by donor specific blood transfusion, which normally results in accelerated heart graft rejection. This finding is at variance with other studies reporting that CyA does not prevent graft rejection in sensitized animals (Morris, 1981b). The above findings may be promising with respect to the treatment of sensitized transplant patients. However, preliminary findings in the same rat model indicate that sensitization by donor heart or skin grafts is more difficult to overcome with high doses of CyA. Following sensitization by a donor strain heart, permanent survival of a second heart graft can eventually be obtained in 40% of recipients using CyA at doses of 15-30 mg/kg/day for seven days. Sensitization by donor skin grafts, which is normally followed by accelerated rejection of a subsequent heart graft, can only be modified to prolonged but not to indefinite graft survival using

these high doses of CyA.

Transfused recipients who are treated with CyA as the only immunosuppressant following transplantation do not benefit from the transfusions administered, unless they are converted from CyA to Aza/Pred. On the other hand, the immunosuppressive potency of CyA alone may prove to be sufficient to maintain graft survival. Further clinical studies using CyA will have to establish its place in the treatment of kidney graft recipients. For the time being, most patients are still being treated with the conventional immunosuppressants azathioprine and prednisolone and many will still have the benefit of prolonged graft survival when the combination of pretransplant blood transfusions and postoperative immunosuppression is used.

SUMMARY

Kidney transplantation is presently the therapy of choice for end-stage renal failure. The major threat for a graft remains rejection, despite all efforts and recent advances in the area of transplantation biology. Although graft survival has been improved only moderately, patient mortality has decreased considerably during the last decade. The three mainstays in the treatment of transplant patients are pretransplant blood transfusions, chemical nonspecific immunosuppression and histocompatibility matching. It was only recently established that pretransplant blood transfusions can prolong kidney graft survival. However, several parameters regarding the type and number of transfusions remained to be determined to obtain the optimal effect from a deliberate blood transfusion "therapy". It had not previously been investigated to what extent immunosuppression influences the expression of the transfusion effect. In particular, little was known about the effect of the new immunosuppressant cyclosporin A in blood conditioned recipients. As a matter of fact, the presence of a transfusion effect had been confirmed only for postoperative immunosuppression by azathioprine and prednisolone.
The effects of blood transfusion and immunosuppression on the survival of canine renal allografts and rat cardiac allografts are described in this thesis.

In Chapter 1, a review is given of the present state of transplantation, mainly with regard to the three modes of treatment mentioned above. Ample attention is paid to the properties of cyclosporin A.

The various materials and methods used are described in Chapter 2.

Chapter 3 deals with the questions that still remained open and awaiting a definite answer before considering a deliberate blood transfusion "therapy". Various blood transfusion protocols were studied in the canine renal allograft model.
It was found that blood transfusions per se, i.e., without azathioprine and prednisolone for postoperative immunosuppression, do not prolong kidney graft survival. Neither one pretransplant third-party blood transfusion nor three third-party plasma transfusions were effective when using conventional postoperative immunosuppression. It was concluded that such transfusions dit not provide sufficient amounts or types of antigens to induce prolongation of graft survival. However, these regimens might have been successful if the animal had been given pretransplant immunosuppression. Although only few animals were involved (which precludes a decisive answer), peroperative transfusions did not seem to prolong graft survival in dogs treated postoperatively with azathioprine and prednisolone. In both man an the dog, this topic of the peroperative transfusion effect remains controversial. Beagle blood transfusions did not induce a beneficial transfusion effect in beagle dogs receiving mongrel kidney grafts. The absence of the transfusion effect was independent of the fact of whether these transfusions were DLA-matched or DLA-mismatched. This phenomenon can be attributed to the high degree of inbreeding in the beagle. It is suggested that known MHC antigenic differences are not very important for the induction of the transfusion effect. Matched transfusions seem to induce less sensitization than do mismatched transfusions.

Chapter 4 describes the effect of cyclosporin A on heart allograft survival in blood conditioned recipients using two rat models. After conditioning with one donor-specific pretransplant blood transfusion, accelerated rejection was seen in the WAG/Ro to BN/Ro donor recipient combination, whereas permanent survival occurred in the reverse combination. Postoperative administration of cyclosporin A for one week did not affect permanent graft survival in blood conditioned WAG/Ro recipients, whereas sensitization was overcome in transfused BN/Ro recipients; at a dose of 15 mg/kg CyA, even permanent graft survival was achieved in all BN/Ro recipients. The finding that CyA can be used to prevent graft rejection in sensitized recipients was in contrast to the prevailing opinion in the literature.

Experiments studying the effect of cyclosporin A on kidney graft survival in transfused and nontransfused dogs are described in Chapter 5. The influence of the combined use of cyclosporin A and the combination of azathioprine and prednisolone as well as the effect of conversion from cyclosporin A to conventional immunosuppression were also studied. A beneficial blood transfusion effect could not be observed during the use of cyclosporin A for postoperative immunosuppression, either as a single agent or in combination with azathioprine and prednisolone. When the administration of cyclosporin A was stopped and the conventional immunosuppressants continued, a beneficial transfusion effect was again observable. Obviously, azathioprine and prednisolone are necessary for the expression of the blood transfusion effect. Using cyclosporin A as the only immunosuppressant, a clear dose response relationship was seen for kidney graft survival. At a dose of 25 mg/kg/day all recipients survived the duration of the cyclosporin A administration. After conversion from a high (e.g., 25 mg/kg/day) dose of CyA to conventional immunosuppressants, a markedly prolonged graft survival which could not be explained solely by the immunosuppressive effect of azathioprine and prednisolone was observed. In the present dog model, altered kidney function seemed to be a reflection of suboptimal immunosuppression rather than a reflection of the nephrotoxic effect of cyclosporin A, which has been described in clinical studies. Shortly after transplantation, a slight hepatotoxic effect of cyclosporin A was observed.

In Chapter 6, the data of the foregoing experimental paragraphs are discussed in a wider context. The possible importance of blood transfusions administered to the kidney donor is mentioned. Further, reference is made to the recent clinical studies employing donor-specific transfusions in one-haplotype related individuals. These transfusions have a 30 percent risk of sensitization, but in the nonsensitized recipients a very high early graft survival (98% at 1 year) is observed. Considering the mechanism of blood transfusions, it is suggested that T suppressor cells may play an important role in the transfusion effect; this could be derived from in vitro studies in man and adoptive transfer experiments in the BN/Ro to WAG/Ro rat model.

At present, most transplant recipients are still treated with azathioprine and prednisolone, which allow for the expression of the transfusion effect. However, in the near future, with increased experience in the use of cyclosporin A, the benefits and immunosuppressive potency of this new drug might prove to outweigh those of the transfusion effect seen when conventional immunosuppression is used.

SAMENVATTING

Niertransplantatie is tegenwoordig de voorkeursbehandeling van patienten met een terminale nierinsufficiëntie. Ondanks alle moderne ontwikkelingen vormt afstoting de grootste bedreiging voor een getransplanteerde nier. Wereldwijd gezien is de niertransplantaat overleving slechts in bescheiden mate verbeterd gedurende de laatste tien jaren. De mortaliteit na transplantatie daalde daarentegen aanzienlijk. De huidige behandeling van transplantatie patienten rust in hoofdzaak op drie pijlers: postoperatieve farmacologische inmunosuppressie, weefseltypering en de toediening van bloedtransfusies aan dialyse patienten vóór transplantatie. Het feit, dat bloedtransfusies de transplantaat overleving in gunstige zin beïnvloeden, is pas sinds kort algemeen aanvaard. Om deze ''transfusie therapie'' optimaal te kunnen toepassen moeten echter nog een aantal vragen opgelost worden. Het is bijvoorbeeld niet precies bekend wat de juiste samenstelling van het transfusaat moet zijn en wat het optimale aantal transfusies is om het beste resultaat te verkrijgen. Voorts is nog nooit onderzocht in hoeverre de toegediende immunosuppressiva van belang zijn voor het tot uitdrukking komen van de beschermende werking van preoperatieve bloedtransfusies. Met name is er nauwelijks iets bekend over het effect van preoperatieve bloedtransfusies bij patienten die na transplantatie worden behandeld met het nieuwe immunosuppressivum cyclosporine A. Bijna alle voorgaande studies naar het effect van bloedtranfusies werden immers verricht bij patienten die postoperatief azathioprine en prednisolon kregen toegediend.
In dit proefschrift worden de effecten van bloedtransfusies en immunosuppressie op de transplantaat overleving nader bestudeerd.

In Hoofdstuk 1 wordt een actueel overzicht gegeven van de factoren die van belang zijn voor de overleving van niertransplantaten; dit zijn vooral de drie bovengenoemde parameters. Ook wordt ruim aandacht besteed aan de eigenschappen van cyclosporine A.

In Hoofdstuk 2 worden de gebruikte materialen en methoden beschreven.

De experimenten, die in Hoofdstuk 3 besproken worden, hebben betrekking op de optimale toepassing van ''bloedtransfusie therapie'' bij niertransplantatie kandidaten. Daartoe werden verscheidene bloedtransfusie protocollen uitgetest in het hondenmodel.
Het bleek dat preoperatieve bloedtransfusies per se, zonder postoperatieve toediening van azathioprine en prednisolon, geen verlenging van transplantaatoverleving gaven. Eén preoperatieve bloedtransfusie had geen beschermend effect, evenmin als drie plasma-transfusies. Wellicht werden met deze transfusies niet die hoeveelheden of soorten van antigenen toegediend, welke noodzakelijk zijn om een langere overleving te bewerkstelligen. Mogelijkerwijs zouden deze protocollen wel effect gesorteerd hebben indien, naast postoperatieve, ook preoperatieve immunosuppressie zou zijn toegediend. Ofschoon slechts een kleine groep honden bloedtransfusies kreeg toegediend tijdens operatie, leek deze wijze van toediening niet effectief. Het effect van peroperatieve transfusies is dus zowel bij de mens als bij de hond niet eenduidig. Indien beagle ontvangers preoperatief getransfundeerd werden met beagle

bloed, bleek er geen sprake te zijn van een beschermend effect van transfusies. Dit uitblijven van het transfusie effect na de toediening van beagle bloed was onafhankelijk van de mate van overeenkomst van MHC antigenen tussen bloeddonor en nierontvanger en was waarschijnlijk te wijten aan de hoge mate van inteelt in de beagle. Bloedtransfusies met een maximale hoeveelheid overeenkomende weefsel antigenen gaven wel in mindere mate aanleiding tot sensibilisatie, zoals viel af te lezen aan het aantal positieve kruisproeven.

In Hoofdstuk 4 wordt de invloed van cyclosporine A op de harttransplantaat overleving in ratten bestudeerd. Daarvoor werden twee donor-ontvanger combinaties gebruikt, waarin de ontvangers tegengesteld reageerden op donor-specifieke bloedtransfusies. In de BN/Ro naar WAG/Ro combinatie geeft een preoperatieve donor bloedtransfusie permanente overleving van een heterotoop harttransplantaat, terwijl in de WAG/Ro naar BN/Ro combinatie na toediening van donor bloed juist versnelde afstoting optreedt. Postoperatieve toediening van cyclosporine A aan getransfundeerde WAG/Ro ontvangers bleek eveneens gepaard te gaan met permanente transplantaat overleving. Ook in de WAG/Ro naar BN/Ro donor-ontvanger combinatie werd verlengde of zelfs permanente transplantaat overleving verkregen indien getransfundeerde BN/Ro ontvangers behandeld werden met cyclosporine A. Bij een dosering van 15 mg/kg/dag werd in alle gevallen permanente transplantaat overleving gezien. Kennelijk kan cyclosporine A ook afstoting voorkomen in gesensibiliseerde ontvangers, hetgeen op grond van voorgaande publicaties onwaarschijnlijk leek.

In Hoofdstuk 5 wordt het effect van cyclosporine A op de niertransplantaat overleving in honden besproken; vooral de expressie van het bloedtransfusie effect bij postoperatieve toediening van cyclosporine A en/of conventionele immunosuppressie staat daarbij centraal. Daarnaast komt ook de invloed van gelijktijdige toediening van azathioprine en prednisolon, alsook het effect van omschakeling van cyclosporine A naar deze conventionele immunosuppressiva, ter sprake.
Preoperatieve bloedtransfusies bleken geen additionele verlenging van de transplantaat overleving te bewerkstelligen, indien postoperatief cyclosporine A werd toegediend. Ook indien dit middel werd toegepast in combinatie met conventionele immunosuppressie werd geen transfusie effect waargenomen. Slechts indien de cyclosporine A toediening werd gestaakt en de conventionele immunosuppressiva werden gecontinueerd, trad er een verlenging van overleving op in getransfundeerde ontvangers in vergelijking met de niet getransfundeerde controle groep. Azathioprine en prednisolon bleken dus onontbeerlijk voor de expressie van het transfusie effect. Bij gebruik van cyclosporine A werd een dosis-afhankelijke verlenging van de overleving gezien. Als een dosis van 25 mg/kg/dag werd toegediend, overleefden alle ontvangers de tijdsduur van toediening, waaruit blijkt dat de immunosuppressieve werking van cyclosporine A alleen voldoende is om afstoting te voorkomen, mits toegediend in een adequate dosering. De gestoorde nierfunctie, die bij doseringen van 5 of 10 mg/kg meer uitgesproken was, leek in het onderhavige hondenmodel niet te berusten op een nefrotoxisch effect van cyclosporine A, maar op de aanwezigheid van een chronische afstotingsreactie ten gevolge van onvoldoende immuno-

suppressie. De hepatotoxiciteit van cyclosporine A leek gering en in ieder geval significant minder dan bij conventionele immunosuppressie.

De gegevens van de voorgaande hoofdstukken worden in Hoofdstuk 6 in een ruimere context besproken. Ook wordt melding gemaakt van recente ontwikkelingen zoals het mogelijkerwijze belangrijke effect van bloedtransfusies die aan de nierdonor worden toegediend en het gebruik van donor-specifieke bloedtransfusies bij verwante niertransplantaties, waarbij nierdonor en -ontvanger een haplotype van elkaar verschillen. Donor-specifieke transfusies veroorzaken bij deze laatste groep patienten in 30% van de gevallen sensibilisatie; in niet gesensibiliseerde ontvangers daarentegen is de niertransplantaat overleving na 1 jaar zeer hoog (tot 98%). Op basis van de bekende gegevens lijkt het aannemelijk dat suppressor T cellen een belangrijke rol spelen bij het tot uitdrukking komen van het beschermende effect van bloedtransfusies. Vooral in vitro studies met humane cellen en "adoptive transfer" experimenten in het BN/Ro naar WAG/Ro rattenmodel leveren daartoe belangrijke aanwijzingen.

Thans wordt de meerderheid van de transplantatie patienten nog steeds behandeld met azathioprine en prednisolon, waarbij het beschermend effect van preoperatieve bloedtransfusies ondubbelzinnig werd aangetoond. In de nabije toekomst zal blijken in hoeverre de resultaten bij gebruik van cyclosporine A beter zullen zijn dan bij gebruik van azathioprine en prednisolon in combinatie met preoperatieve bloedtransfusies.

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