MODIFICATION OF GRAFT SURVIVAL BY TRANSFUSION OF THE DONOR

A study in rats

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A study in rats

HET EFFECT VAN DONOR-TRANSFUSIE OP DE OVERLEVING VAN ORGAANTRANSPLANTATEN

Een onderzoek bij ratten

PROEFSCHRIFT

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Simone, Heike, Jikke
kām sakyo
aba bidā!

Abbreviations used

Ag	: Antigen
ALG	: Anti lymphocyte globulin
APC	: Antigen presenting cell
ATG	: Anti thymocyte globulin
B cell	: Bone marrow-derived lymphocyte
BUN level	: Blood urea nitrogen level
CsA	: Cyclosporin A
DST	: Donor specific transfusion
FTLI	: Fractionated total lymphoid irradiation
GvH	: Graft-versus-Host
HBSS	: Hanks' balanced salt solution
HLA	: Human leucocyte antigen
Ia antigen	: Serologically detectable I-region coded antigen
IL	: Interleukin
i.v.	: intravenous
MHC	: Major histocompatibility complex
ml	: milliliter
PLN assay	: Popliteal lymph node assay
T cell	: Thymus-derived lymphocyte
TDD	: Thoracic duct drainage

CONTENTS

PREFACE

There is wide acceptance that in humans blood transfusions given to the recipient before grafting produce a strikingly beneficial effect on the post-transplant course of cadaveric or living related kidneys (Opelz *et al.*, 1981b; Salvatierra *et al.*, 1983).

Recently it has been reported by several authors that blood transfusions to the donor prior to nephrectomy positively influence graft survival (Jeekel *et al.*, 1980; 1981; Frisk *et al.*, 1981; 1983; Harder *et al.*, 1984). However, retrospective studies by Berg *et al.* (1982), Bock *et al.* (1984) and Opelz (1985a) could not confirm these reports. Many questions were raised by these retrospective observations and this made it necessary to experimentally investigate them in rigidly controlled studies. This thesis is the reflection of these experiments. Moreover, it contains an extensive introduction in the development of transplantation with special emphasis on the approaches to immune modulation in human kidney transplantation.

CHAPTER 1

GENERAL INTRODUCTION IN TRANSPLANTATION

1.1 A brief anthology of early transplantation history

The idea of grafting parts of the body from one person to another has inspired a number of legends. The following quotation is from a translation of a Chinese document written about 300 B.C.: "One day two men, Lu and Chao, called on the surgeon Pien Ch'iao. He gave them a toxic drink and they were unconscious for three days. Pien Ch'iao operated and opened their stomachs and explored the heart; after removing and interchanging their organs he gave a wonderful drug and the two men went home recovered" (Worshofsky, 1965). In the thirteenth century the Saints Cosmos and Damian transplanted a whole leg. According to this legend, the leg of a dead black man was successfully used to replace the cancerous leg of a white man (Worshofsky, 1965). In an attempt to save the life of Pope Innocent VIII by means of transplantation of young blood, two boys were bled to death in 1492 (Worshofsky, 1965).

The first description of a skin transplantation, performed to repair defects of the nose, is found in the Sushruta Sanhita, a document written by ancient Hindu surgeons about 700 B.C. (Bhisragratna, 1916).

Transplantation of teeth was described by Arabian writers about A.D. 1000, by Ambroise Paré in Paris in the sixteenth century and by John Hunter, a Scottish surgeon, in the eighteenth century (Woodruff, 1960; Calne, 1967). He wrote about the operation: "Success of this operation is founded on the disposition of all living substances to unite when brought in contact with one another, although they are of different structure and even though the circulation is carried in one of them".

In 1804, Baronio was the first to present a well-documented report of successful free autografts of skin of a sheep (Baronio, 1804). In 1823 Bünger (1823) reported the successful use of a free full-thickness human skin autograft to repair a nasal defect. In 1863, Paul Bert, a student of Claude Bernard, reported that autografts, allografts and xenografts behaved differently (Woodruff, 1960; Converse and Casson, 1968). The significance of these observations received little attention however. Nineteenth century authors generally failed to observe that the results of allografts and autografts of skin were different.

The development of transplantation during the twentieth century has depended on advances in surgical techniques, on the understanding of the problem of rejection and on graft preservation and storage and artificial life support. The first reports of consistently reliable vascular anastomoses by suturing were those of

Carrel and Guthrie between 1902 and 1912 (Carrel and Guthrie, 1905; Carrel, 1908). As earlly as 1905 they succeeded in transplanting an allogeneic dog kidney to the abdominal cavity.

As the technical problems had thus been overcome, transplantation studies would concentrate on the mechanism and prevention of rejection of the allograft. Many years elapsed before effective measures of prolonging allograft survival were reported.

Landsteiner (1901) was one of the pioneers in the detection of blood groups. He reported the genetic system which later proved to be important for histocompatibility. In studies with transplantable tumors during the first two decades of this century Jensen (1903) and Little (1914) reported the influence of genetic factors in transplantation. This oncological research led to important developments in transplantation immunology. Williamson (1923) concluded from renal transplantation studies in the dog that failure of transplants was attributable to a biologic incompatibility between donor and recipient. Numerous authors studied grafts of tumors and of normal tissue in highly inbred strains of mice. Gorer (1936) demonstrated that antigenic determinants found on normal and neoplastic tissues in mice are genetically controlled. In 1948 his group described H-2 as a genetic locus controlling strong histocompatibility antigens in the mouse; subsequently this locus and numerous minor histocompatibility loci were characterized in great detail (Snell *et al.*, 1953). The detection of the major histocompatibility complex of man (Dausset and van Rood, 1965), rhesus monkeys (Balner *et al.*, 1965), chimpanzee (Balner *et al.*, 1974), dog (Vriesendorp, 1976) and rat (Gill *et al.*, 1978) followed.

In 1903 Jensen observed that a second graft did not survive as long as the first when a mouse received two grafts of a tumor separated by an interval of several days, and he suggested that immunity accounted for the difference (Jensen, 1903). More than 40 years later Medawar reported similar observations in controlled experiments with rabbits (Medawar, 1944; 1945). He demonstrated the immunologic specificity of the phenomenon, which was observed only when the same donor was used for both first and second sets of grafts. The specificity of the second set phenomenon had been described by Shinoi in Japan in 1932 (Stickel and Seigler, 1981). This finding probably provided the most convincing evidence for the notion that the allograft rejection is based on immunological reactivity originating in the lymphatic system of the host and is induced by the disparity of the so-called histocompatibility antigens.

Voronoy, a Russian surgeon living in Mexico, performed in 1936 the first renal allograft transplantation in man. The patient died the second day posttransplant (Voronoy, 1936). Successful use of hemodialysis to substitute for renal function temporarily during a period of renal failure was developed by Kolff (1946). In 1954 Murray performed the first successful clinical transplantation using a monozygotic twin donor-host combination (Murray *et al.*, 1955). The long term success of this and subsequent renal transplants between monozygotic twins was followed by a great increase in clinical studies with renal allografts. Initially some patients received ACTH or cortisone postoperatively, but adequate immunosuppression

was not applied until 1958 when graft rejection was favourably influenced by total body irradiaton (Murray *et al.*, 1960).

Since the sixties transplantation has gained momentum. By now, over 100.000 kidney transplantations have been performed in man. It is the generally accepted treatment of chronic renal failure or end-stage renal disease. One year survival of cadaver renal grafts has reached 70-80% (Rapaport, 1981; Monaco, 1985). After the first heart transplantation by Barnard (1967), clinical heart transplantation is at present in its second decade. At Stanford the one year survival is more than 70% (Jamieson, 1985). In 1963, Starzl performed the first orthotopic liver graft in man. Results have dramatically improved since the use of cyclosporin A as immunosuppressant. One year graft survival is at present about 70% (Starzl, 1985). The same can be stated concerning pancreas transplantation, although the success rate is still relatively low. One year graft survival is reported to be about 36% (Sutherland and Kendall, 1985).

At the end of this very brief review of the history of transplantation it can be stated that due to an increasingly effective donor/host selection (HLA matching) and an ever growing successful immune modulation of the host (transfusions, azathioprine, prednisolone, antilymphocyte globulins, monoclonal antibodies, cyclosporin A) substantial progress in transplantation during the last two decades has been accomplished. An extensive introduction in the development of transplantation with special emphasis on these modern approaches to immune modulation in human kidney transplantation will be presented in the next part of this chapter.

1.2 **Approaches to immune modulation in human kidney transplantation**

1.2.1 *Introduction*

After most of the surgical problems of organ grafting had been solved it was understood that the graft should be protected against the inevitable rejection. In this chapter a bird's eye view of the major clinical approaches to avoid graft rejection will be presented.

First of all tissue matching was employed as a way of improving graft survival (see section 1.2.2). Soon the employment of drugs acting indiscriminately, blocking or damaging all cells that happened to be in mytosis followed (see section 1.2.3). The use of these nonspecific drugs e.g. azathioprine was not entirely satisfactory and ways were explored to develop lymhocytotoxic drugs or procedures which were restricted to the elimination of the immunocompetent cells. This goal could partly be achieved by the use of fractionated total lymphoid irradiation (see section 1.2.4), thoracic duct drainage (see section 1.2.5), antilymphocyte globulin (see section 1.2.6), steroids (see section 1.2.3) and splenectomy (see section 1.2.7).

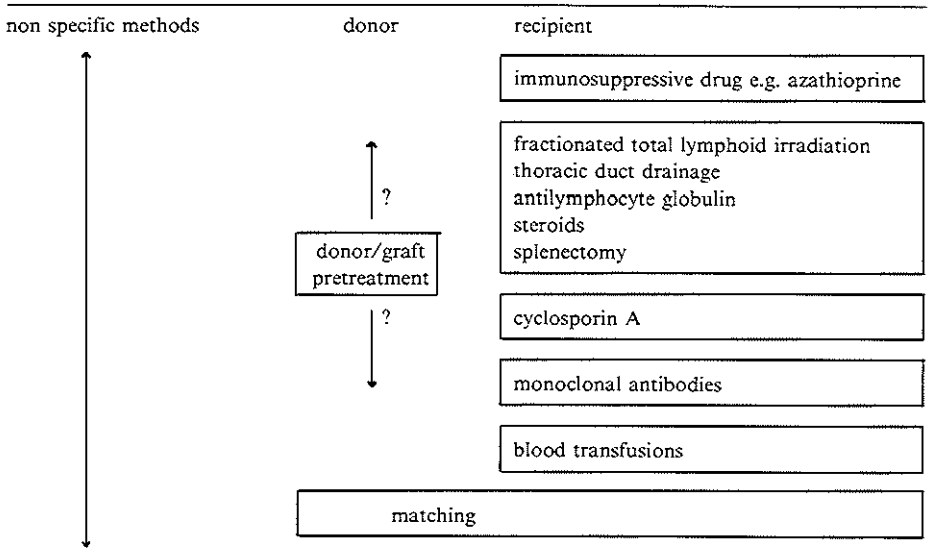
Currently a new, more biological, phase of immune modulation has been

entered. This phase is characterized by selective immune regulation using compounds or methods that specifically modulate defined subpopulations of immunocompetent cells. It is suggested that cyclosporin A (CsA) is such a drug (see section 1.2.8). Also monoclonal antibodies directed towards lymphocyte subsets may add to the therapeutic arsenal (see section 1.2.9). Certainly the discovery that blood and blood products given to the recipient do enhance allograft survival has resulted in such a biological approach to immune modulation (see section 1.2.10).

Finally, a different approach towards achieving non-responsiveness has been modification of the graft by donor pretreatment and culture procedures (see section 1.2.11).

These different clinical approaches to avoid graft rejection have been summarized in Table 1.1 and will now be discussed in more detail.

Table 1.1. Approaches to immune modulation in human kidney transplantation.



1.2.2 *Tissue typing*

1.2.2.1 Introduction

The genetic basis for the rejection of allografts lies in recipient recognition of foreign histocompatibility antigens coded for by multiple histocompatibility loci. Furthermore cells belonging to the donor immune system in the transplanted organ

(passenger leukocytes), which recognize the recipient antigens as "non-self", may also contribute to graft rejection (Elkins and Guttmann, 1968; van Schilfgaarde *et al.*, 1980).

In every mammalian species that has been examined so far, one complex of loci is of overwhelming importance in determining the fate of allografts. This complex has been termed the major histocompatibility complex (MHC). There is a striking similarity with regard to the organization of the MHC of the mouse (the H-2 system; Snell *et al.*, 1953), of man (the HLA system; Dausset and van Rood, 1965), of the rhesus monkey (the RhLA system; Balner *et al.*, 1965), of the dog (the DLA system; Vriesendorp 1976) and of the rat (the RT-1 system; Gill *et al.*, 1978). These homologies indicate conservation of these gene families during evolution. From a practical standpoint, the similarity in structure and function of MHC gene products between species make all of the experimental mammalian systems mentioned valuable models for understanding the MHC of man (Sachs, 1983).

The MHC complex in human beings is localized on the short arm of chromosome six (Jongsma *et al.*, 1973) where several distinct loci code for the highly polymorphic glycoprotein HLA antigens. The MHC can be subdivided into three such classes of genes, which all have specific functions in control of the different molecules involved in immunological responsiveness.

Class I antigens (HLA-A, -B, -C) are thought to be expressed on the cell surfaces of all nucleated somatic cells (Berah *et al.*, 1970). This idea has been based on cytotoxic assays of single cell suspensions or absorption assays of alloantisera or heteroantisera. Such assays have not been adequate in defining the detailed distribution of class I antigens within the various organs of the body. A monoclonal antibody together with a sensitive immunoperoxidase staining technique has recently been used to examine the detailed distribution of HLA class I antigens throughout the body. This study suggests that HLA class I antigens can be detected on most, but not all the nucleated cells in the body and that these antigens are thus not ubiquitous in their distribution, as previously suggested (Daar *et al.*, 1984a).

Class II antigens (HLA-D/DR and other Ia like antigens as well as products of the Ir or immune response genes) were initially thought to be mainly restricted to cells of the immune system. They have been described on B lymphocytes (Kissmeyer-Nielsen, 1975), epidermal Langerhans cells (Klareskog *et al.*, 1977), endothelial cells (Hirschberg *et al.*, 1979), activated T-cells (Fu *et al.*, 1978) and during different stages of differentiation of both myeloid and erythroid precursors (Wincester, 1980). More recently it has been shown in guinea pigs (Wiman *et al.*, 1978), mice (Parr and McKenzie, 1979), rats (Hart and Fabre, 1981a) and humans (Hart *et al.*, 1981; Natali *et al.*, 1981) that class II antigens have a wider distribution. Daar *et al.* (1984b) have recently examined the detailed tissue distribution of class II antigens using a monoclonal antibody and the peroxidase-antiperoxidase technique on freshly frozen normal human tissues. Their results indicate that this class of antigen has indeed a far wider distribution than was previously appreciated. Furthermore, there is increasing evidence in various species that class II antigen expression can be induced under physiological and pathological conditions

(Klareskog *et al.*, 1980; Lampert *et al.*, 1981; Mason *et al.*, 1981a; Daar *et al.*, 1982; de Waal *et al.*, 1983). Fuggle *et al.* (1983) have observed an individual variation of expression of class II antigens on renal tubular epithelium which might be due to an induction of these antigens under certain circumstances. In other situations, for example on capillary endothelium (Fuggle *et al.*, 1983) and dendritic cells (Daar *et al.*, 1983), however, class II antigen expression is more likely to be constitutive (Daar *et al.*, 1984b).

It is reported that endothelial cells of rats lack these class II antigens (Hart and Fabre, 1981a; Hart and Fabre, 1981b). However this finding of the absence of class II antigens from rat vascular endothelial cells conflicts with data published by Paul (Paul *et al.*, 1982; Fabre *et al.*, 1983; Paul and Carpenter, 1983).

Finally, class III antigens of the MHC control complement components (Fu *et al.*, 1974; Awdeh and Alper, 1980).

Aside from histocompatibility antigens defined by the MHC there are antigens defined by systems other than the MHC. The difference between the MHC and non-MHC antigens was first described in mice by Counce *et al.* (1956). The blood-group system ABO can be considered as such a non-MHC system. There are also other red cell antigens such as rhesus factors, the Lewis system etc. that belong to it (Oriol *et al.*, 1978).

Furthermore an antigen system not detectable on lymphocytes, but expressed on renal capillary endothelium and monocytes (EM-antigens) has been described (Paul *et al.*, 1979). The system is polymorphic and has been identified recently (Baldwin *et al.*, 1981).

1.2.2.2 The effect of tissue matching on graft survival

One of the dogmas of immunology dictates that the severity of allograft rejection is significantly influenced by the degree of histocompatibility between donor and recipient. Experimental and clinical experience has verified the dogma and corroborated the evidence.

The importance of compatibility of donor and recipient for the MHC antigens in transplantation has been shown in organ grafts in various animal species. Borleffs has reviewed this subject extensively in his thesis (Borleffs, 1982). Here the experiences in human kidney transplantation will be reviewed.

Transplants from HLA-identical siblings have a superior survival compared with all other donor-recipient combinations. The survival of HLA identical sibling grafts is significantly better than that of one-haplotype mismatched grafts and unrelated grafts (Terasaki *et al.*, 1981; Albrechtsen *et al.*, 1983).

The impact of matching for separate products of the HLA complex is less clear. The effect of HLA-A and -B locus matching on the survival of cadaver kidney grafts is disputed (Opelz and Terasaki, 1982), although several large series show a significant influence (van Rood *et al.*, 1977; Opelz *et al.*, 1977; Albrechtsen *et al.*, 1981; Festenstein *et al.*, 1981; Moen *et al.*, 1983; Sanfilippo *et al.*, 1984c; Festenstein *et al.*, 1985; 1986). Several authors have observed that matching for HLA-B is much

more effective in improving graft prognosis than matching for HLA-A (Festenstein *et al.*, 1976; d'Apice *et al.*, 1984b; Busson *et al.*, 1984).

Matching for HLA-C antigens has no significant influence on cadaver graft survival (Albrechtsen *et al.*, 1981; 1983).

Bach *et al.* (1970) have suggested that mixed leukocyte culture (MLC) non-responsiveness (matching for class II antigens) might be more important than matching for class I antigens. Indeed a superior graft survival has been reported in patients receiving grafts either from haploidentical family donors or from cadaver donors against which a low or non-response was recorded in MLC (indicating relative compatibility for HLA-D region products) (Ringdèn and Berg, 1977; Walker *et al.*, 1978; Berg and Ringdèn, 1982).

The cellular techniques used for defining HLA-D region products have been too time consuming to be useful for prospective matching of cadaver transplants. However, since serologic typing for HLA-DR has become available, the characterization of cadaver donors for HLA-DR is possible. A significant influence on cadaver graft survival of HLA-DR matching has been reported (Persijn *et al.*, 1981; Albrechtsen *et al.*, 1981; Festenstein *et al.*, 1981; d'Apice *et al.*, 1981; Goeken *et al.*, 1982; Ayoub and Terasaki, 1982; Albrechtsen *et al.*, 1983; Moen *et al.*, 1983; Berg *et al.*, 1983; Madsen *et al.*, 1983; d'Apice *et al.*, 1984b; Busson *et al.*, 1984; Madsen *et al.*, 1985; Festenstein *et al.*, 1985; Lucas *et al.*, 1985; Festenstein *et al.*, 1986).

Most authors have found that the positive effect of HLA-DR matching on graft survival is independent of the recipient having received pretransplant blood transfusions or not (Ting and Morris, 1980; Albrechtsen *et al.*, 1981; Goeken *et al.*, 1982; Moen *et al.*, 1983; Madsen *et al.*, 1985). However, some have reported a less pronounced effect either in transfused (Persijn *et al.*, 1981; Opelz, 1985a) or in non-transfused (d'Apice *et al.*, 1981) patients. Vanrenterghem *et al.* (1983a) have found that HLA-DR matching can significantly improve the survival of cadaveric kidney allografts in polytransfused recipients. Furthermore they found that the cumulative dose of corticosteroids during the first year after transplantation was significantly lower in transfused patients with no DR incompatibilities. These data have been confirmed by Madsen *et al.* (1983).

Excellent survival times have been reported in HLA-A, -B and -DR matched unrelated renal transplants (Persijn *et al.*, 1981). The effect of mismatching for DR antigens on the survival of renal allografts from related donors has been studied much less extensively (Watanabe *et al.*, 1983). It has been reported that avoiding DR mismatches in recipients of renal allografts from living-related donors sharing one HLA-ABC haplotype may provide only a slight improvement in graft survival rate, but that the need for anti-rejection treatment is significantly reduced (Sutherland *et al.*, 1983a).

A special status as a marker for "high responsiveness" in renal transplantation was ascribed to the HLA-DRw6 antigen by Hendriks *et al.* (1983). They found that recipients of HLA type DRw6 had a significantly lower kidney graft survival rate than DRw6-negative recipients. Furthermore, if DRw6-positive recipients were given a DRw6-positive kidney, graft survival was good, whereas survival was poor

if DRw6-negative kidneys were transplanted into DRw6-positive recipients. These findings were confirmed by some investigators (Kaplan *et al.*, 1983; Pohanka *et al.*, 1983), others (Vanrenterghem *et al.*, 1983c; Opelz, 1984), however, could not confirm the report of Hendriks *et al.* (1983).

As stated in the introduction of this section it is reported that apart from the MHC antigens also non-MHC histocompatibility antigens do exist. There is clear evidence that these non-MHC antigens are also involved in the rejection of kidney grafts, since kidney graft rejection has been reported in a number of transplantations between HLA identical siblings, despite immunosuppressive therapy (Salaman *et al.*, 1976; Cheigh *et al.*, 1977). The involvement of antibodies to non-MHC antigens in relation to kidney transplantation in man, has been published by Paul *et al.* (1979). They found that the presence of EM antibodies is associated with poor kidney graft prognosis. Thus donor-host incompatibility for EM-antigens may also be involved in the rejection process.

For the ABO blood group antigens, it is known that incompatibility shortens allograft survival (Dausset and Rapaport, 1966). Rh incompatibility has long been thought not to be important in organ rejection in an unsensitized host (Gleason and Murray, 1967), but recently a case of renal graft rejection possibly attributable to Rh incompatibility, has been reported (Gluckman *et al.*, 1981).

Brynger *et al.* (1984) have transplanted A2 donors into 0 recipients with good results. Recently it has been suggested that the use of ABO incompatible kidneys in closely HLA-DRw matched living related recipients may be successful (Slapak *et al.*, 1984). Alexandre *et al.* (1985) have reported that ABO-incompatible live donor renal homografting may be achieved successfully, provided a protocol is followed including splenectomy of the recipients.

The Lewis system (Oriol *et al.*, 1978) is reported to be of importance as well. Transplantation of Lewis-positive kidneys into Lewis-negative, transfused recipients has been uniformely unsuccessful, whereas matching gave excellent results (Pfaff *et al.*, 1983).

1.2.3 *Azathioprine and Prednisolone*

Attempts to transplant organs between individuals who were not identical twins were disappointing (Hume *et al.*, 1952) until experimental studies in dogs showed that 6-mercaptopurine and later its derivative azathioprine, could prevent rejection of renal allografts (Calne, 1960; Zukoski *et al.*, 1960). The clinical application of azathioprine as an immunosuppressive agent in human kidney transplantation soon followed (Murray *et al.*, 1963).

Billingham *et al.* (1951) have laid the experimental basis for the use of corticosteroids as immunosuppressive therapy in transplantation. An extensive review of the mode of action of glucocorticosteroids has been published recently (Dupont *et al.*, 1984).

The combined use of glucocorticosteroids and azathioprine as pharmacological immunosuppression in man dates from 1963 when Starzl *et al.* (1963a) reported excellent results in recipients treated with the combination of azathiprine and prednisolone.

Azathioprine and prednisolone have remained the mainstay of immunosuppressive therapy in renal transplantation for over twenty years and they have proved to be a reasonable effective combination. However the side-effects of azathioprine and corticosteroids represent a major problem.

Hypertension, Cushingoid face and body habitus, aseptic necrosis of the femoral head, posterior subcapsular cataracts, upper gastro-intestinal ulcerations and haemorrhage, psychosis, diabetes mellitus, growth retardation in children, osteoporosis and increased frequency of infection have clinically been the most troublesome side-effects of steroids (McDonald *et al.*, 1976; Starzl *et al.*, 1977).

Hepatic toxicity and teratogenicity are side-effects of azathioprine (Schein and Winokur, 1975); bone marrow depression is also a dangerous complication (Fisher *et al.*, 1976).

Furthermore there is an increased incidence of lymphomas and other malignancies in immunosuppressed transplant recipients (Penn, 1983; Sheil *et al.*, 1985; Blohmé and Brynger, 1985) as well as an increased incidence of cancer in patients on renal dialysis (Sheil *et al.*, 1985).

Despite the numerous complications associated with chronic immunosuppressive therapy, it is generally believed that these agents must be continued indefinitely to ensure graft survival (Naik *et al.*, 1980; Zoller *et al.*, 1980). The suggestion that continued administration of azathioprine and/or prednisolone may not be necessary in renal transplantation (Sheriff *et al.*, 1978; Dandavino *et al.*, 1978; Di Padova *et al.*, 1979) has been rejected by data obtained in a retrospective study in 165 renal transplant units in the USA (Zoller *et al.*, 1980). Forty-eight patients were identified who had discontinued all immunosuppressive therapy in a total transplant population of over 6000. The data learned that at no point after transplantation it is prudent to stop all immunosuppressive therapy barring in case of serious drug toxicity or infection. Furthermore, in patients who stop immunosuppressive therapy surreptitiously and in whom renal function remains normal, reinstitution of therapy is indicated. These results are consistent with isolated case reports of graft failure in humans, following cessation of therapy (Owens *et al.*, 1975; Najarian, 1975, Hussey, 1976; Uehling *et al.*, 1976). However, Campos *et al.* (1984) observed that graft survival rates in a group of patients where azathioprine therapy had to be discontinued as compared to a well-matched control group, showed no significant difference after a considerable follow-up, indicating that azathioprine may be discontinued when required. Recently Parfrey *et al.* (1985) could not confirm this.

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.

Alternate day steroid regimens have been proposed to reduce long term complications and side-effects of the drug. In three randomized prospective studies this has

been evaluated (McDonald *et al.*, 1976; De Vecchi *et al.*, 1980; Dumler *et al.*, 1982). McDonald *et al.* (1976) did not observe any advantage of alternate day prednisolone treatment, whereas the other authors (De Vecchi *et al.*, 1980; Dumler *et al.*, 1982) did find a reduction of long term complications and side-effects of the drug. Dumler *et al.* (1982) reported a significant decrease of the prevalence of clinical osteonecrosis and the rate of infectious complications requiring hospitalization in patients on alternate day methylprednisolone. Furthermore the therapy was as effective as daily steroids for the maintenance of graft function.
McGeown *et al.* (1980) have shown in a retrospective study that the use of lower steroid doses in kidney transplantation can reduce morbidity considerably without decreasing the graft survival rate. These results have been confirmed by several other groups (Chan *et al.*, 1980; Morris *et al.*, 1982; Cheigh *et al.*, 1982). However recent data suggest that the combination of low-dose steroids and low-dose azathioprine provides inadequate immunosuppression in renal transplantation, although higher doses of azathioprine (>1.75 mg/kg/day) allow the use of low-dose steroids without significantly more graft losses than without high-dose steroids (d'Apice *et al.*, 1984a).

1.2.4 *Total lymphoid irradiation*

Hamburger *et al.* (1962) explored nearly 25 years ago the immunosuppressive effects of ionizing irradiation on transplantation by treatment of the prospective renal allograft recipient with whole body irradiation. The dose required to prolong allograft survival did also produce severe bone marrow and gastrointestinal toxicity which prevented further clinical use. Other less toxic applications of X-irradiation were directed at immunocompetent lymphoid cells. Experimental and clinical use of local graft irradiation (Hume and Wolf, 1967), intralymphatic administration of radioisotopes and extracorporeal irradiation of blood (Cronkit *et al.*, 1965) and lymph (Monden *et al.*, 1981) were all tested experimentally and clinically. However, the clinical applicability has so far remained limited.

It has been found that patients with Hodgkin's disease who have been treated with fractionated total nodal irradiation sustain a long lasting impairment of cell mediated immune functions (Fuks *et al.*, 1976). These results have initiated a series of experimental studies in several animals and it has been demonstrated that after fractionated total lymphoid irradiation (FTLI) of the recipient skin and organ grafts follow a delayed rejection course (Slavin *et al.*, 1976; 1978; Bieber *et al.*, 1979; Howard *et al.*, 1981a). Furthermore it has been observed that skin allografts in FTLI-treated rodents given donor bone marrow simultaneously with the graft survive permanently. The animals were chimeric and graft-versus-host disease did not occur (Slavin *et al.*, 1977). Such chimeras have also been produced in mongrel dogs (Slavin *et al.*, 1979). The lack of toxicity to bone marrow and gastrointestinal tract using this protocol, while maintaining a strong immunosuppressive effect encouraged a clinical trial of FTLI in a group of renal transplant patients at high risk of rejection of their grafts.

Najarian *et al.* (1982) found in a group of twenty-two high risk patients all treated with FTLI a 72% two-year graft survival compared with 38% graft survival in a historical control group of recipients receiving secondary or tertiary grafts and treated with conventional immunosuppression. It was also shown that the immunosuppressive effects of pretransplant FTLI were diminished when transplantation was delayed. The administration of donor bone marrow at the time of transplantation did not produce chimerism. Seventeen patients had significant complications, including one death and two lymphomas (Najarian *et al.*, 1982). Nevertheless the side effects, the results suggest that, when properly utilized, FTLI can produce effective immunosuppression for clinical transplantation in high risk patients. This was further emphasized in a recent report of Levin *et al.* (1985) where cadaveric renal transplant recipients were treated with total lymphoid irradiation, antithymocyte globulin, and low-dose prednisone. Comparison of patients given FTLI with a group given cyclosporin A showed similar graft survival but better graft function in the FTLI group. Sutherland *et al.* (1983b) have shown that cyclosporin A treatment in recipients who have rapidly rejected previous renal allografts, is as effective as FTLI and less cumbersome. It can be concluded that FTLI might definitely be of use as an alternative treatment for those patients who reject a renal allograft while on cyclosporin A.

Finally, it should be noticed that local graft irradiation has been proposed to reverse rejection (Fidler *et al.*, 1973). However, in a recently reported randomized study no beneficial effect of such a treatment of acute rejection of renal transplants when used in combination with high-dose steroids was found (Pilepich *et al.*, 1983).

1.2.5 *Thoracic duct drainage*

Removal of circulating lymphocytes by thoracic duct drainage (TDD) depresses the immune response to tissue transplants. As early as 1964 McGregor and Gowans demonstrated a beneficial effect of TDD on skin graft survival in rats (McGregor and Gowans, 1964). TDD has been infrequently used for immunosuppression in tissue transplantation in man. This may be attributed to the technically cumbersome procedure as well as the need for a month hospitalization prior to transplantation (Klintmalm *et al.*, 1981c). However, several experienced clinics have reported a beneficial effect on renal graft survival (Sarles *et al.*, 1970; Tilney *et al.*, 1970; Franksson *et al.*, 1976; Starzl *et al.*, 1979; Fish *et al.*, 1981) without a reciprocal increase in patient mortality.

Despite the fact that the effectiveness in preventing rejection and prolonging allograft survival has been demonstrated, the exact mechanism of this beneficial effect is not clearly understood. In a recent study Bell *et al.* (1983) have shown by using monoclonal antibodies, which characterize specific human T-lymphocyte subpopulations, that significant alterations occur in both thoracic duct and peripheral blood lymphocytes during TDD. The data showed that as TDD progressed, a significant fall occurred in the percentage of thoracic lymph and peripheral

blood lymphocytes with surface antigens typical of most peripheral T-cells (OKT 3). Additionally they showed a reduction in the T-helper (OKT 4) to T-suppressor/cytotoxic (OKT 8) cell ratio in TDD. Finally, Bell *et al.* (1983) noted a significant increase in cells bearing a surface antigen typical for lymphocytes undergoing intrathymic maturation (OKT 10). This led to the suggestion that TDD produces a state of immunologic immaturity with a relative increase in suppressor T cells and thus creates an optimal condition of nonresponsiveness in the host at the time of grafting.

1.2.6 *Antilymphocyte globulin*

The injection of lymphocytes, thymocytes or other lymphoid cells into a non-compatible recipient leads to the production of a serum, which gives a depression of the number of circulating T-cells when injected into the donor of the lymphoid cells (Woodruff and Anderson, 1963). The antilymphocyte (ALG) or antithymocyte globulins (ATG) have proved to be remarkable successful in prolonging allograft survival in most animal models (Levey and Medawar, 1966; Monaco *et al.*, 1966; Starzl *et al.*, 1967).

However the effectiveness of these agents for clinical immunosuppression has been more difficult to evaluate. Only recently evidence for true clinical effectiveness in organ transplantation has been achieved. The reasons for this were numerous, including variability among individual batches, inadequate dosage schedules, lack of randomly selected control data, limited numbers of evaluable patients leading to multicenter trials with all their attendant problems. Furthermore, during the early years of ALG testing, numerous factors (age, blood transfusions, HLA typing etc.) were identified to influence kidney transplantation outcome. Many early ALG trials were not appropriately stratified for these factors in test and control groups. Finally, early trials were restricted to ALG use as an additional immunosuppressive agent administered with conventional doses of azathioprine and prednisone.

The early ALG trials invariably showed reduced incidence and severity of early rejections with greater ease of reversibility (Turcotte *et al.*, 1973; Sheil *et al.*, 1973; Cosimi *et al.*, 1976; Wechter *et al.*, 1979). ALG treatment favoured long term survival (Taylor *et al.*, 1976; Monaco and Codish, 1976; Levey, 1979), but usually not significantly (Groth, 1981). Cosimi (1981) found that ATG added to prednisone and azathioprine reduced early rejection without an increased incidence of infections, decreased the overall steroid requirement and reduced the incidence of avascular necrosis. Patient survival was the same with or without ATG but graft survival was 10-15% better with adjunctive ATG.

Recently, Novick *et al.* (1983) compared adjunctive antilymphoblast globulin used with prednisone and azathioprine versus prednisone and azathioprine alone in a prospective randomized study of cadaver kidney transplants. ALG recipients had no major side effects secondary to ALG, had delayed onset of rejection, reduced number of rejection episodes, fewer in hospital days, reduced cost of transplantation, improved graft survival and equivalent patient survival. These authors

concluded that antilymphoblast globulin was safe, cost-effective, and of definite benefit in cadaver renal transplantation.

Despite the increasing evidence for the efficacy of this type of protocol, many groups now prefer to reserve ATG for treatment of established rejection. Shield et al. (1979) compared in recipients of living related donor allografts the effectiveness of ATG alone with high dose steroids as the treatment of choice of rejection episodes. This randomized trial favoured the ATG treated group. Similar results have been found in the treatment of established rejection in recipients of cadaver donor renal allografts. Hoitsma *et al.* (1985) have compared rabbit antithymocyte globulin (RATG) with high dose prednisone. They found RATG to be at least as good as steroid treatment, with the RATG protocol obviously adding a major steroid sparing effect. Other groups have combined ATG with high dose steroids and found this to be more effective than steroids alone (Filo *et al.*, 1980; Howard *et al.*, 1981b; Nowygrod *et al.*, 1981; Simonian *et al.*, 1983).

It can be concluded that the experience now suggests that addition of ATG at the time of rejection may be the most efficacious way to use the agent. Further refinements of this therapy are being explored (see section 1.2.9: monoclonal antibodies).

1.2.7 *Splenectomy*

The value of splenectomy as an adjunctive procedure to improve renal allograft survival has been controversial since its introduction by Starzl *et al.* (1963b). Retrospective analysis by several transplant groups have shown a beneficial effect of splenectomy (Pierce and Hume, 1968; Kauffman *et al.*, 1974; Schulak *et al.*, 1978); others however have not shown a difference (Opelz and Terasaki, 1973) or have suggested an adverse effect of splenectomy on patient survival (Rai *et al.*, 1978).

These early retrospective analyses were performed before numerous other factors like age, blood transfusions, HLA typing etc. were identified to influence kidney graft survival. Most of these studies were not appropriately stratified for these factors and thus the conclusions drawn bear limited weight.

There is a general agreement that splenectomy corrects the leukopenia associated with the hypersplenism of chronic renal failure and minimizes the leukopenic effect of the immunosuppressive regimen, and thus allows a higher dose of azathioprine to be administered (Woods *et al.*, 1971; Kauffman *et al.*, 1974; Schulak *et al.*, 1978). On the other hand, splenectomy is a major operation with potential complications. Especially the risk for death due to overwhelming bacterial infection is generally feared (Krivit *et al.*, 1979; Sherman, 1980). Schröter *et al.* (1977) have observed overwhelming postsplenectomy sepsis in transplant patients.

Two prospective studies have shown that splenectomy had a beneficial effect for at least the first two years posttransplant without a detrimental effect on patient survival (Stuart *et al.*, 1980; Fryd *et al.*, 1981). These studies have been followed-up by a reanalysis of the long-term effect of splenectomy versus no splenectomy on renal allograft survival. Sutherland *et al.* (1985a) showed that the differences in

graft survival rates between splenectomized and non-splenectomized recipients were no longer significant. Furthermore there were more late deaths from sepsis in the splenectomized group, although the overall patient survival rates were similar in splenectomized and non-splenectomized recipients. Rohrer *et al.* (1985) have reported a continuing overall benefit of splenectomy even after five years. However, after certain subgroups, e.g. patients over 50 years of age, were analyzed, it became apparent that splenectomy might have a detrimental effect on patient and graft survival.

Recently it has been proposed to perform only splenectomy after transplantation in a selected group of patients which are characterized by the development of a persistent leukopenia that requires discontinuation of or a significant reduction in cytotoxic drug therapy (Lewis *et al.*, 1983). The preliminary results of these authors are encouraging and indicate that selective posttransplant splenectomy may be a reasonable alternative to routine prophylactic splenectomy in recipients of cadaveric renal allografts. Very much in favour of such a policy are the data as reported by Alexander *et al.* (1984). They have published the late adverse effect of splenectomy on patient survival following cadaveric renal transplantation. Their conclusion is that splenectomy should not be performed routinely in the preparation for a cadaveric transplant because of an unacceptably high late mortality rate that is primarily caused by sepsis. These findings are consistent with those of Peters *et al.* (1983).

Finally, it should be mentioned that we recently found in an experimental dog transplantation model that splenectomy can interfere with immunoregulatory responses induced by blood transfusions (Marquet *et al.*, 1984). Shelby *et al.* (1985) have confirmed this finding. This forms an other reason to support a conservative attitude towards splenectomy in transplant patients.

1.2.8 *Cyclosporin A*

Cyclosporin A (CsA) is a fungal peptide first isolated from mycelia of two strains of fungi imperfecti of the species Tolypocladium inflatum Gams in a soil sample from Hardanger Vidda, a high, treeless plateau in southern Norway (Kahan *et al.*, 1985). Its immunosuppressive activity was first discovered in 1972 by Borel (Borel *et al.*, 1976). Subsequent experimental studies revealed a strong immunosuppressive activity without myelosuppression. Experimental organ transplantation in the rat (Kostakis *et al.*, 1979), rabbit (Green and Allison, 1978), dog (Homan *et al.*, 1980), pig (Calne *et al.*, 1978a) and non-human primate (Reitz *et al.*, 1981) have demonstrated the potent influence of CsA in preventing acute allograft rejection.

The remarkable experimental results led Calne *et al.* (1978b) to first initiate a clinical trial using CsA as the sole immunosuppressive agent in renal transplantation. Seven patients on dialysis with renal failure received transplants from mismatched cadaver donors. Rejection prophylaxis did occur, however an unexpected impairment of renal function in oliguric patients was noted. This led to additional immunosuppression with steroids and Cytimun® in the mistaken belief that this

impairment of renal function was a manifestation of rejection, though histological changes did not indicate severe immune reactions. The result of this excessive immunosuppression was a high incidence of bacterial, viral and fungal infections, and the development of several lymphomas.

A revised protocol of CsA dosage was introduced that included the routine use of mannitol at operation and the administration of CsA, beginning at 6 hr after the end of the operation and then only if an adequate diuresis was present. Subsequently, CsA improved the 1-year actuarial graft survival from a previous 50-55% to over 80% in recipients of renal grafts from unmatched cadaveric donors (Calne *et al.*, 1981). Furthermore, it was observed that CsA can be steroid-sparing (Calne *et al.*, 1981).

Starzl *et al.* (1980) evaluated the combination of early CsA therapy alone followed by the delayed addition of corticosteroids. They obtained excellent results: graft survival being almost 80% at 1 year in a series of 66 consecutive recipients of 67 cadaver renal grafts (Starzl *et al.*, 1981). This combination yielded fewer rejection episodes and allowed a decreased dose of CsA to be used, thus decreasing the nephrotoxicity observed. This happened without an increase in infectious complications.

Recently the results of many controlled clinical trials have been reported (Morris, 1981; Ferguson *et al.*, 1982; Morris *et al.*, 1983; Report on the European Multicentre Trial, 1982; 1983; Sells, 1983; The Canadian Multicentre Transplant Study Group, 1983; Stiller, 1983; Najarian *et al.*, 1983; Sheil *et al.*, 1983; Kahan *et al.*, 1983; Rosenthal *et al.*, 1983; Wood RFM *et al.*, 1985; Johnson *et al.*, 1985; Sutherland *et al.*, 1985b; Squifflet *et al.*, 1985; Calne and Wood, 1985; The Canadian Multicentre Transplant Study Group, 1986).

Morris *et al.* (1983), and Wood RFM *et al.* (1985) have compared the use of CsA in the first three months after transplantation with conventional immunosuppression (azathioprine and low-dose prednisone). In the first trial, including only patients with diuresing kidneys and HLA-DR incompatible grafts, they found essentially no difference in graft survival between the two relatively small groups (Morris *et al.*, 1983). In the second trial including all patients who had received a cadaver kidney, it was demonstrated that short term CsA with conversion to conventional immunosuppression resulted in a significant improvement in graft survival compared to conventional therapy (Wood RMF *et al.*, 1985). In two trials CsA as a monotherapy has been compared with conventional therapy (Report on the European Multicentre Trial, 1983; Sheil *et al.*, 1983). The one year patient survival was in neither of the two trials significantly different in the two treatment groups (respectively 98.3% vs. 93.9% in the European trial and 92% vs. 100% in the Australian trial). In the European trial the one year graft survival was 72% in the CsA group and 52% in the control group. In the Australian trial the one year graft survival was 80% in the CsA group and 76% in the control group, where in contrast with the European trial, ALG was added to the conventional azathioprine and prednisone therapy regimen. Recently, Calne and Wood (1985) reported that in the European trial, the better graft survival in the CsA group, which was apparent at

one year, has been maintained for more than three years, without an increase in morbidity and mortality.

In the other trials CsA has been combined with low-dose prednisone (Ferguson *et al.*, 1982; The Canadian Multicentre Transplant Study Group, 1983; Stiller, 1983; Najarian *et al.*, 1983; Kahan *et al.*, 1983; Rosenthal *et al.*, 1983). This seems not to have affected patient survival negatively, for none of the studies shows a detrimental influence of the addition of prednisone to the CsA treatment as compared to the conventional treatment scheme. On the contrary, in two studies a significant difference in patient survival in favour of the CsA groups was found (The Canadian Multicentre Transplant Study Group, 1983; Stiller, 1983; Kahan *et al.*, 1983). The result of CsA plus prednisone treatment as compared to conventional therapy showed an equally good (Najarian *et al.*, 1983; Sutherland *et al.*, 1985b; Squifflet *et al.*, 1985) or significantly better (Stiller, 1983; Kahan *et al.*, 1983; Rosenthal *et al.*, 1983) graft survival. Very recently, the Canadian Multicentre Transplant Study Group (1986) reported that analysis at three years still showed better graft and patient survival in recipients of cadaveric renal transplants treated with CsA plus low-dose prednisone as compared with alternative forms of immunosuppressive therapy.

The prevalence of infections was in most of the forementioned studies evenly distributed between the two treatment groups or significantly lower in the CsA group (Najarian *et al.*, 1983; Sutherland *et al.*, 1985b). In a recently published randomized study of early infections in transplant patients on CsA it was found that CsA treated patients had significantly less overall infections and less nonviral infections as compared to azathioprine treated patients (Dummer *et al.*, 1983; Ho *et al.*, 1983).

In some studies a trend towards a lower occurrence of rejection episodes in CsA treated patients has been noticed (Najarian *et al.*, 1983; Sheil *et al.*, 1983; Sutherland *et al.*, 1985b).

Conflicting data concerning the effect of pretransplant blood transfusions to the recipient in CsA treated patients have appeared. In the European Multicentre Trial previously non-transfused patients in the CsA group had a better graft survival than multiple transfused patients (Sells, 1983). Gardner *et al.* (1985) have confirmed this finding. Other studies have shown no positive correlation between the number of pretransplant blood transfusions and improved graft survival in the CsA group (Kahan *et al.*, 1983; Klintmalm *et al.*, 1985). Opelz (1985a) and Woods RFM *et al.* (1985) reported a significant benefit from blood transfusions in the CsA group. Thus, claims that CsA abrogate the transfusion effect must be interpreted with caution.

In the European Multicentre Trial there was a negative relationship noted between the degree of HLA-A and -B match between donor and recipient and graft survival in the CsA treated patients (Sells, 1983). Kahan *et al.* (1983) could not confirm these findings.

Opelz for the Collaborative Transplant Study (1985b) has analyzed the correlation of HLA matching with kidney graft survival in patients with or without CsA

treatment. Matching for HLA-B and HLA-DR loci was shown to have an additive positive effect on graft survival in CsA treated recipients of cadaver kidney grafts. However, several single centre retrospective analyses of DR matching with CsA immunosuppression has demonstrated that CsA seems to negate the effect of DR matching in cadaveric renal transplantation (Taylor *et al.*, 1985; Harris *et al.*, 1985). If these results are confirmed by others, the role of prospective DR matching in cadaveric renal transplantation treated with CsA may be of little importance.

The most frequently reported side-effects in CsA treated patients are nephrotoxicity and hepatotoxicity. In order to clarify both, renal function has been studied in liver transplant recipients (Klintmalm *et al.*, 1981a; Hamilton *et al.*, 1981; Powell-Jackson *et al.*, 1983) and liver function in renal transplant patients (Klintmalm *et al.*, 1981b; Rodger *et al.*, 1983). In liver transplant recipients nephrotoxicity was noted but could be reversed easily with dosage reduction (Klintmalm *et al.*, 1981a). Most authors in the previously mentioned randomized clincial trials have found a poorer renal function in the CsA treated patients as compared to the conventional treated patient group. However, the renal functions did not deteriorate further and remained stable at a subnormal level.

Frequently, in renal transplantation, it is extremely difficult to distinguish between CsA-induced nephrotoxicity and rejection. Several methods have been described to differentiate between these two clinical entities (Klintmalm *et al.*, 1983).

Initially it was thought that anuria posttransplant was a contraindication to use CsA (Calne *et al.*, 1981). However there are now a number of reports in the literature suggesting that CsA can be safely used in renal transplant recipients with early nonfunction (Rynasiewicz *et al.*, 1981; Flechner *et al.*, 1983; Castro *et al.*, 1983).

Other major side-effects mentioned in the literature include tremor, hirsutism, gum hypertrophy, hyperaesthesia, central nervous system toxicity, and gastrointestinal intolerance. All side-effects have shown to be dose-related and reversible on stopping the drug (Sells, 1983; Najarian *et al.*, 1983; Wilczek *et al.*, 1985). Soon after the introduction of CsA considerable concern arose that lymphomas might be more frequently observed after CsA treatment than with conventional immunosuppression (Calne *et al.*, 1981). In subsequent studies of patients treated with CsA such a trend could not be substantiated (Penn, 1983), but long-term follow-up of large numbers of patients treated exclusively with CsA are needed to clarify the carcinogenic potential of this agent in man.

Studies have indicated that the immunosuppressive properties of CsA are mostly limited to T-lymphocyte-dependent immune responses (Borel *et al.*, 1977; Green and Allison 1978; Calne *et al.*, 1978a). The specific mechanism by which CsA exerts its immunosuppressive effects on lymphocytes remains unclear. Recent studies have demonstrated that this agent, in vitro, inhibits the induction of cytolytic lymphocytes in primary mixed lymphocyte response (MLR) while permitting the activation and expression of suppressor cells, a process that leads to a state of specific allo-antigen tolerance that is maintained by a nylon-wool-adherent

suppressor cell (Hess *et al.*, 1982). Recently, the inhibitory effect of CsA on the production of interleukin 2 (IL-2) and on the development of responsiveness to this lymphokine have been implicated as some of the major reasons accounting for CsA mediated immunosuppression (Hess, 1985). Yoshimura and Kahan (1985) have examined the effect of CsA administered in vivo on the capacity of kidney transplant recipient lymphocytes to generate IL-2 after mitogen (phytohemagglutinin [PHA]) stimulation. They found that CsA treatment impairs the generation of IL-2 by patient lymphocytes and that failure to display this response is associated with a poor level of immunosuppression and allograft rejection.
Several centres have used monoclonal antibodies to monitor human T-lymphocyte subsets in renal allograft recipients receiving CsA-prednisone immunosuppression. Some authors were unable to demonstrate a change in OKT 4 +/ OKT 8 + ratios (Hakala *et al.*, 1983), whereas others did find a decrease in OKT 4 +/OKT 8 + lymphocyte ratio in the peripheral blood of these patients (Vanburen *et al.*, 1983). The decreased ratio was mainly due to a drop in the percentage of OKT 4 + lymphocytes following transplantation. This alteration in OKT 4 +/OKT 8 + ratio was not noted in azathioprine-treated patients with stable allograft function (Vanburen *et al.*, 1982). However, CsA combined with prednisone therapy may have a different influence upon lymphocyte subpopulations than CsA alone (Vanburen *et al.*, 1983). This remains to be clarified.

1.2.9 *Monoclonal antibodies*

In section 1.2.6 the results obtained with antilymphocyte globulin have been reviewed. Despite the steroid sparing effect and the increased specificity offered by polyclonal antilymphocyte preparations, they still fail to provide optimal suppression. It has recently become possible to distinguish a number of antigens on T lymphocytes by producing monoclonal antibodies against them using the hybridoma technique of Köhler and Milstein (1975). Thus, a second generation of antilymphocyte antibodies has become available (Cosimi *et al.*, 1981a). A number of studies of these reagents have begun to indicate where there future application may be most useful.

Preclinical studies in non-human primates are very promising. Renal allograft survival was significantly prolonged after treatment of Cynomolgus with OKT 4 monoclonal antibody (Cosimi *et al.*, 1981b). Similar results have been recorded in skin grafting in Rhesus monkeys (Jonker *et al.*, 1983). Cosimi *et al.* (1981c) reported the first clinical trial of OKT 3 monoclonal antibody directed against the antigen specific T-cell receptor for rejection therapy after cadaver kidney transplantation. Established rejection episodes were reversed within two to seven days in all eight cases given OKT 3 antibody without other treatment and with concomitant lowering of steroid treatment. In the following 3-13 months period, further rejection episodes occurred in five of these patients, two of which were irreversible, although six remained well with excellent function. Many patients in this first group treated with a monoclonal antibody, developed antibodies to the monoclonal

reagent, a factor which, in most patients, would apparently limit the duration of this therapy (Jaffers *et al.*, 1983). However it has recently been reported that modifications in the protocol have led to still very successful treatment of rejection episodes with limited sensitization to the monoclonal reagent (Thistlethwaite *et al.*, 1984).
Other authors have confirmed the effectiveness of monoclonal antibody therapy for rejection episodes (Norman *et al.*, 1985; Takahashi *et al.*, 1985).
At present a prospective randomized trial is being performed comparing OKT 3 therapy with high dose steroids, and ATG if necessary, for reversal of rejection in cadaver donor allograft recipients. It is reported that the results are encouraging in the OKT 3 treated group (Cosimi, 1983; Goldstein *et al.*, 1985; Ortho Multicenter Transplant Study Group, 1985).

It is clear that this second generation of antilymphocyte antibodies constitutes a major potential advance in antilymphocyte antibody therapy. The future prospects for the treatment of rejection episodes appear to be exciting.

The use of monoclonal antibodies for the prophylaxis, but not the treatment, of cadaver kidney rejection is still experimental and it is difficult to predict their future in renal transplantation (Kreis *et al.*, 1985). However, the availability of monoclonal antibodies against the human interleukin-2 (IL-2) receptor on activated T cells and the favourable results on allograft survival in mice of anti-IL-2 receptor monoclonal antibodies (Kirkman *et al.*, 1985), raises hope of achieving specific immunosuppression.

1.2.10 *Blood transfusions to the recipient*

There is now wide acceptance that pretransplant blood transfusions to the recipient produce a strikingly beneficial effect on transplant survival, whether the kidney is from cadaveric or living related sources.

Dosseter *et al.* (1967) were the first to state a beneficial effect of blood transfusion on cadaver renal allograft survival. Opelz *et al.* (1973) did enhance the interest in this phenomenon and since then there has been confirmation in many separate studies (Opelz and Terasaki, 1980). There are, however, significant discrepancies among the results published by different investigators (Opelz *et al.*, 1981a). A recent analysis of the long-term effects of blood transfusions suggests that the graft survival rate in transfused recipients is not permanently better than that in non-transfused recipients. However the patient survival rate is much better in these transfused recipients (Fehrman *et al.*, 1985).

Regarding the number of transfusions it has been reported that multiple transfusions more potently produce the transfusion effect (Opelz *et al.*, 1981b; Fehrman, 1982; Kerman *et al.*, 1983; Horimi *et al.*, 1983; Opelz, 1985a). However, a single transfusion from a random donor is reported to be effective as well (Persijn *et al.*, 1979; Opelz, 1985a). In one study the difference in survival rates between grafts in patients with no transfusions and those with one transfusion is reported to be about 5% at one year with a progressive increase in graft survival up to 10-15 transfusions.

Additional transfusions did not increase the survival rate (Horimi *et al.*, 1983). Opelz (1985a) for the Collaborative Transplant Study has reported that patients with 6-10 pretransplant transfusions had the highest success rate, whereas the rate was lower again in patients with more than 10 transfusions. Several authors have reported a lack of correlation between cadaver kidney transplant survival and the number of pretransplant transfusions (Feduska *et al.*, 1982; Zeicher *et al.*, 1983).

It is suggested that the active cells in blood transfusions required to elicit the blood transfusion phenomenon are probably leukocytes (Rapaport and Dausset, 1983). This suggestion is supported by the finding that white blood cell free blood is ineffective in producing the transfusion effect (Persijn *et al.*, 1979). The use of frozen, washed or HLA-A and B matched blood has been advocated by some groups in an attempt to reduce the risk of patient sensitization or disease transmission. Several studies have suggested that recipients receiving only frozen blood have no significant benefit in terms of graft survival (Opelz and Terasaki, 1974; Persijn *et al.*, 1979), but others found that frozen blood was effective in providing increased graft survival (Fuller *et al.*, 1982; Horimi *et al.*, 1983). In a recently published retrospective study examining the effect of transfusions with packed, washed, frozen and mixed blood products on graft survival, an association of any of these blood products with a significant increase in graft survival was found (Sanfilippo *et al.*, 1984a). Furthermore, the same authors reported that the effect on graft survival was not significantly different among the various blood product groups. Finally, they found that only minor differences in the degree of patient sensitization were associated with the type of blood product (Sanfilippo *et al.*, 1984a). These results are in agreement with the earlier report of Opelz and Terasaki (1980), who compared the effect of whole blood, packed cell and washed packed cell transfusions on graft survival.

Nubé *et al.* (1983) have shown that prospective administration of HLA-A and B matched identical or compatible pretransplant blood transfusions have a beneficial effect on graft survival while avoiding sensitization as indicated by a significant reduction of lymphocytotoxic antibodies. However, reports from other centers have suggested that this treatment is ineffective (Albert *et al.*, 1981; Vanrenterghem *et al.*, 1983b).

One group has employed randomly selected thrombocyte- and leukocyte-enriched buffy coat transfusions in potential cadaver recipients. They have reported an improved renal allograft survival over that of whole blood transfused recipients (Okazaki *et al.*, 1985). Borleffs *et al.* (1982) have reported that platelet transfusions improve kidney allograft survival in Rhesus monkeys without inducing cytotoxic antibodies. This report has generated considerable interest because of its great potential for clinical transplantation. In dogs however, platelet transfusions were found to be ineffective (Marquet *et al.*, 1983; Bijnen *et al.*, 1984). Furthermore Opelz (1985a) and Chapman *et al.* (1985; 1986) have observed in humans that platelet transfusions given to nontransfused recipients do not simulate the transfusion effect.

Transfusion are reported to be effective only when given prior to transplantation rather than at the time of transplantation (Hourmant *et al.*, 1979; Opelz and

Terasaki, 1981; Glass *et al.*, 1982; Opelz, 1985a). It has been reported that the interval beween transfusion and transplantation is not critical as long as enough time has elapsed for the effect to be induced (Opelz *et al.*, 1981a). For the Collaborative Transplant Study Opelz (1985a) has reported that patients who received transfusions during transplantation surgery did somewhat better than patients who were not transfused at all; however, they did much worse than patients with pretransplant transfusions.

Glass *et al.* (1982) have reported that peroperative blood transfusions are without significant benefit in previously untransfused patients, and significantly lower allograft survival in previously transfused patients. However, this is a matter of debate as several studies, including one prospective study, have been published suggesting a beneficial effect of peroperative transfusions (Stiller *et al.*, 1978; Williams *et al.*, 1980). The matter has become even more complicated after the recent publication of a report which indicates that peroperative transfusions alone were beneficial in decreasing allograft rejection, did not provide an apparent risk for patients who had already received pretransplant transfusions and finally seemed to influence beneficially graft survival in sensitized regrafted patients who received pretransplant transfusions (Sanfilippo *et al.*, 1984b).

As mentioned earlier the greatest objection against transfusions has been the development of cytotoxic antibodies, which has generally been thought to mean that the patient is sensitized with as a result a poor graft survival (Terasaki *et al.*, 1971). This view is logical. However, evidence has accumulated that transfused patients with antibodies have a higher graft survival than those without antibodies who have not been transfused (Werner-Favre *et al.*, 1979; Spees *et al.*, 1980; Horimi *et al.*, 1983). It has been suggested that highly sensitized patients with cytotoxins can be successfully grafted and that it is only necessary to avoid transplants in patients who have cytotoxins directly reactive to the donor's cells, that is, patients who have positive crossmatches (Horimi *et al.*, 1983).

Several mechanisms have been proposed to explain the beneficial effect of pretransplant transfusion to recipients of cadaveric renal allografts. It has been proposed that the transfusion effect may be attributable to donor preselection (Opelz *et al.*, 1972). This means that the degree of presensitization, and thus the transplantability, of recipients by blood transfusions distinguishes "responders" with a high level of cytotoxic antibodies and a higher rejection rate from "non-responders" with a negative antibody response and a lower rejection rate (Opelz *et al.*, 1972). It has also been proposed that blood transfusions may play an active role by inducing anti-idiotypic or anti-Fab antibodies (Singal *et al.*, 1982; Fagnilli and Singal, 1982; Chia *et al.*, 1982; Singal *et al.*, 1983). Others favour the hypothesis that blood transfusions modify the immune response by the induction of suppressor T cells (Maki *et al.*, 1981; Lenhard *et al.*, 1982; Smith *et al.*, 1983). According to Bianchi *et al.* (1983) the alloantigen-specific suppressor T cells can also suppress the in vivo immune response to unrelated alloantigens. Finally, recently Terasaki (1984) has proposed the "clonal deletion theory" to explain the beneficial effect of transfusions. The decloning hypothesis states that a blood transfusion activates

alloreactive clones causing a secondary antidonor response shortly after transplantation. The patient is thought to be "decloned" by high doses of steroids or other immunosuppressive agents, either deliberately administered at the time of grafting or released endogenously in response to surgical stress.

In an attempt to extent the beneficial effect of random blood transfusions on cadaveric renal allograft survival, Salvatierra *et al.* (1980) did start a deliberate donor specific blood transfusion (DST) scheme prior to living related renal transplantation. Since donor blood transfusions given to recipients before renal allografting might result in sensitization to the donor and subsequent hyperacute rejection, clinicians previously have been very hesitant to employ this technique in human renal transplantation. However, reported results have thus far been very encouraging, and patients receiving related kidneys from their crossmatch-negative blood donors appeared to have an outcome and post-transplant course similar to that achieved with HLA identical siblings (Salvatierra *et al.*, 1983). The graft survival rates at one, two and three year in the DST and non-DST one haplotype match recipient groups are reported to be 94%, 91% and 87%, and 67%, 62% and 56% respectively (Salvatierra *et al.*, 1983). Other centres that have adopted the protocol have reported similar results (Whelchel *et al.*, 1982; Mendez *et al.*, 1982; Takahashi *et al.*, 1982; Yamauchi *et al.*, 1984; Glass *et al.*, 1985).

The DST protocol has been extended with good results to two-haplotype-mismatch siblings, distant relatives, and unrelated individuals (Sollinger *et al.*, 1984).

As mentioned earlier the sensitization of a recipient to his prospective related donor is a major concern when using DST. Salvatierra *et al.* (1980) reported that 31% of the patients receiving fresh DST's developed positive white blood cell crossmatches with their blood donor. However, other groups have reported much lower sensitization rates (Mendez *et al.*, 1982; Yamauchi *et al.*, 1983). The sensitization rate is noted to decrease when stored blood is used without a detrimental influence on the beneficial DST effect (Whelchel *et al.*, 1985). Anderson *et al.* (1985) have reported that the administration of azathioprine to patients during the period of transfusions from the donor did reduce the rate of sensitization. This has been confirmed by Glass *et al.* (1985). They found that the importance of the azathioprine plus DST protocol lays in the reduced incidence of sensitization and the increase in the number of transplants that can be done.

Recently Okazaki *et al.* (1985) have reported significant reduction of sensitization and improved allograft outcome after donor-specific buffy coat transfusion of the recipient.

Bijnen *et al.* (1985) have tried to diminish the incidence of sensitization by using donor-specific thrombocyte transfusions in one-haplotype related dogs. It was found that donor-specific thrombocyte transfusions did not sensitize the recipients but also had no favourable effect on kidney graft survival. It is generally felt that the excellent graft survival in DST-treated recipients does far outweigh the risk of sensitization (Salvatierra *et al.*, 1981).

The mechanism of action of DST's which allows for improved graft survival, is not totally clear. Several alternative hypotheses have been proposed to explain this phenomenon. These include: recipient selection, decloning, and autoregulation. Salvatierre *et al.* (1980) postulated that both recipient selection of "responders" and "non-responders" to a potential donor and the modification of the host immune response may occur with DST's. The experience with stored blood and the azathioprine plus DST protocol seem to indicate that recipient selection might only be a minor contributing factor (Whelchel *et al.*, 1982; Anderson *et al.*, 1985; Glass *et al.*, 1985).

The decloning hypothesis, recently proposed by Terasaki (1984), states that DST activates alloreactive clones causing a secondary antidonor response shortly after transplantation. As stated in a previous part of this chapter, the patient is thought to be "decloned" by high doses of steroids or other immunosuppressive agents, either deliberately administered at the time of grafting or released endogenously in response to surgical stress.

In contrast, the various autoregulation hypotheses, based on experimental and clinical studies, state that DST induces inhibition of antidonor immune response rather than stimulation. Specifically, the proposed regulatory mechanisms include induction of suppressor cells (Marquet and Heystek, 1981; Marquet *et al.*, 1982) that specifically inhibit MLC or CML responses to donor alloantigens (Leivestad *et al.*, 1982; Nagarkatti and Singal, 1983; Wood *et al.*, 1984; Leivestad and Thorsby, 1984; Cheigh *et al.*, 1984) and induction of antibodies to host T cell antigen receptors for donor alloantigens as measured by inhibition of MLC or CML responses (Nagarkatti and Singal, 1983; Nagarkatti *et al.*, 1983; Sollinger *et al.*, 1984; Cheigh *et al.*, 1984; Burlingham *et al.*, 1985).

It can be concluded that there is now wide acceptance that pretransplant blood transfusions to the recipient produce a favourable effect on graft survival. Only few studies have appeared on the effect on graft survival of transfusions given to the donor. The available literature concerning transfusions to the donor will be reviewed in chapter 2.

1.2.11 *Reduction of immunogenicity of the graft*

According to the classical view of allograft rejection, the transplanted tissue is the source of antigen that stimulates a T-cell and B-cell response of the host which in turn leads to the rejection process. Central to this concept about graft rejection is the idea that antigen recognition is a sufficient requirement for lymphocyte activation (Medawar, 1944). In recent years however evidence has accumulated that one signal is not sufficient and that two signals are required for lymphocyte activation (Lafferty, 1980).

Snell (1957) has proposed that donor leukocytes in grafts play a major role in this process because of their capacity to carry along draining lymphatics antigen to the

host lymphoid system. Steinmuller (1967) and Elkins and Guttmann (1968) actually showed in rodents that sensitization of the host after transplantation might be attributable to non-parenchymal hemopoietic cells. Elkins and Guttmann (1968) did also suggest that the rejection of organ allografts might be initiated by the immune interaction of host lymphocytes with donor leukocytes carried as passengers within grafts. They introduced the term "passenger leukocytes".

Furthermore, in an elegant model, Guttmann *et al.* (1969) and Guttmann and Lindquist (1969) used rat bone marrow chimeras to demonstrate that the trigger site of immunogenicity seems to reside on donor cells of hemopoietic origin and that these cells seem to be sensitive to cytotoxic agents which might prolong allograft survival by reducing allograft immunogenicity. Steinmuller and Hart (1971) have confirmed that bone marrow derived leukocytes make a significant contribution to graft immunogenicity in rodents.

Until recently these antigen presenting passenger leukocytes have remained a theoretical entity. A candidate for the passenger leukocyte has appeared when it was shown that all tissues, except brain, are populated with a migrant population of bone marrow derived Ia positive dendritic cells (Hart and Fabre, 1981c) and that these cells are extremely potent stimulators of the mixed lymphocyte reaction (Steinman and Witmer, 1978; Mason *et al.*, 1981b). Dendritic cells were recently shown to be a major immunogenic stimulus of a kidney allograft. Lechler and Batchelor (1982) were able to restore immunogenicity with as few as 10^4–5×10^4 dendritic cells in long surviving renal allograft bearing rats.

It is thought that in rodents the ability to present antigens is confined to these specialist cells and that it is unlikely that the graft parenchymal cells themselves induce the rejection process. The transplantation antigens of the graft are presumably processed and presented by antigen presenting cells (dendritic cells/macrophages) of the host (Mason, 1983). Batchelor *et al.* (1978) have suggested that this indirect antigen presentation to the recipient's immune system is much less efficient than the process of direct antigen presentation. Lechler and Batchelor (1982) have added the observation that the immune response evoked by MHC-incompatible tissues devoid of dendritic cells more closely resembles that produced by a minor histocompatibility antigen disparate graft than one with major histocompatibility differences.

As a result of these observations Batchelor (1983) has proposed two routes of allo-immunization. In route 1, which is a very powerful form of immunization, allodendritic cells present in graft tissue directly present major histocompatibility antigens of donor type to recipient T cells. If the donor and recipient differ at the major histocompatibility complex, T cells will be specifically activated against the major histocompatibility antigens carried by the transplant. This direct stimulation of recipient T cells will thus lead to the generation of cells reactive to both class I and class II antigens of the donor. The route 2 involves the breakdown, phagocytosis and handling of alloantigen by the recipient's own antigen presenting cells. The crux of this route is that alloantigen is finally presented to the recipient's T helper cells by the recipient's own dendritic cells/macrophages, which route in principle is

the same as that involved for minor histocompatibility antigens. This process, which is termed indirect antigen presentation, will activate T cells in the recipient that are specific for graft antigens presented in association with the restricting element of the host antigen-presenting cell, the class II antigen of the host (Schwartz *et al.*, 1978). This indirect antigen presentation will also lead to a graft-specific T-cell response; these T cells, however, do recognize the graft antigens in association with recipient type class II antigens, and thus are unable to attack cells of the graft directly. Although the responding T-cells are not specific for the transplanted tissue, they could act as helper cells for B cells that have picked up antigens from the transplant. Such responses might as a result lead to the production of an amount of graft specific antibody. Such antibodies could potentially act as enhancing antibodies.

Lafferty (1980) has proposed a different model concerning lymphocyte activation. This model is called the stimulator cell model and he makes two postulates concerning the process of immunocyte activation. The first is the "Bretscher-Cohn" postulate (Bretscher and Cohn, 1970): two signals are required for lymphocyte activation. Signal 1 is provided by antigen binding to the potentially responsive lymphocyte. Signal 2 is provided by an inductive molecule, which is said to possess costimulator activity. According to the "Bretscher-Cohn" postulate, signal 1 alone is a negative signal which leads to the development of tolerance (Bretscher and Cohn, 1970). A corollary of this postulate is the notion that a stimulator cell is required for lymphocyte activation. The stimulator cell is the cell that provides the source of costimulator activity. According to this model, antigen-presenting cells are stimulator cells.

The second postulate is "the control postulate": a control molecule on the surface of the stimulator cell regulates production and/or release of costimulator activity. Costimulator activity is released only when the control structure is engaged by the potentially responsive lymphocyte. The implications of this stimulator cell concept for the understanding of alloreactivity are three fold (Lafferty *et al.*, 1983a, Lafferty and Prowse, 1984). The first implication of the model is that only cells expressing the stimulator phenotype will stimulate allogeneic T cells in vitro, and that this capacity of stimulator cells to stimulate is dependent on their metabolic activity. Indeed metabolic inactivation of the stimulator cell population with ultraviolet destroyed the capacity to activate an antigen-specific T-cell response (Lafferty *et al.*, 1978). It is suggested that the most likely candidate for the physiologically relevant stimulator cell is the Ia-rich dendritic cell (Steinman and Witmer, 1978).

The second implication derived from the stimulator cell model is that there will be two distinct classes of alloantigen. One will be highly immunogeneic for allogeneic T cells in vitro and the other will be non immunogeneic. Experimentally, it has been shown that major histocompatibility molecules behave as the control structures defined by this model. Other cell surface antigens behave as minor histocompatibility antigens (Bevan, 1975).

The third implication of the model is that alloantigen carried on the surface of donor stimulator cells in the graft constitutes the major source of tissue immuno-

genicity. To summarize, the antigen presenting cell plays an active role in the immune induction because this cell, in addition to presenting antigen (a source of signal 1), provides a source of the second signal (costimulator activity) required for the lymphocyte activation (Lafferty and Prowse, 1984).

It can be concluded that the antigen presenting cell, whether it be called passenger leukocyte or dendritic cell, carried in the grafted tissue is a major source of tissue immunogenicity and it is suggested that an efficient way of ensuming minimum sensitization is to eliminate these functionally active cells from the allograft before it is transplanted (Batchelor, 1983; Lafferty *et al.*, 1983b).

Indeed, attempts at elimination of these antigen-presenting cells by treatment of the donor with irradiation, antilymphocyte globulin or cytotoxic drugs (Guttmann and Lindquist, 1969; Freeman *et al.*, 1971; Steinmuller *et al.*, 1971; Stuart *et al.* 1971a; 1971b; Zincke and Woods, 1974) as well as by in vitro culture (Lafferty *et al.*, 1975; Naji *et al.*, 1979; Lacy *et al.*, 1982) have resulted in prolongation of allograft survival in animals. In 1973 Guttmann *et al.* (1973) were the first to describe a decrease in the number of graft rejections in man after pretreatment of the donor with methylprednisolone and cyclophosphamide before nephrectomy. Studies reporting an improved kidney graft survival after donor drug pretreatment in man followed (Beaudoin *et al.*, 1973; Guttmann *et al.*, 1975; Zincke and Woods, 1977; Zincke *et al.*, 1978; Corry *et al.*, 1980; Guttmann *et al.*, 1980). These studies did not definitely prove its beneficial effect, since most of them compared the survival of donor drug pretreated kidney grafts with historical controls and all dealt with a small number of patients.

Prospective studies on the effectiveness of donor drug pretreatment on renal allograft survival could not establish its effectiveness with the exception of the study of Corry *et al.* (Chatterjee *et al.*, 1977; Dienst, 1977; Barry and Bennett, 1978; Jeffery *et al.*, 1978; Soulillou *et al.*, 1979; Corry *et al.*, 1980). Thus, the clinical results of donor drug pretreatment are inconclusive.

One reason might be the inadequate removal of interstitial dendritic cells by the clinically used pretreatment protocols. McKenzie *et al.* (1984a; 1984b) recently examined the effect of donor pretreatment on the interstitial dendritic cell (or passenger leukocyte) content of rat heart grafts. In rat heart, where class II major histocompatibility antigens are most likely restricted to interstitial dendritic cells (Hart and Fabre, 1981c), direct cell counts were made on frozen sections stained with monoclonal antibodies. It was possible for the first time to compare the effect of a pretreatment regimen on the target cell population and subsequently to observe the survival of pretreated heart grafts transplanted into untreated recipients. It was found that pretreatment (cyclophosphamide and/or total-body irradiation) of the donor only resulted in prolonged graft survival when more than 95% of the interstitial dendritic cells were removed. Very small numbers were still sufficient to cause rapid rejection. Furthermore, to be effective, donor pretreatment schedules had to be initiated 5 days prior to transplantation. Pretreatment 6 hr prior to transplantation failed to deplete the graft interstitial dendritic cells or prolong graft survival.

An other reason for the different results of donor pretreatment between animals and men might be more structural. In man it has been suggested that in addition to the presentation of antigens by dendritic cells, endothelial cells of vascular endothelium may also perform this function (Hirschberg *et al.*, 1980). In contrast with the antigen presenting dendritic cells which are a migrant population with a limited lifespan, the vascular endothelial cells of an allograft are fixed and persist the lifetime of the graft. Thus, it follows that, if indeed vascular endothelium can serve as an antigen presenting cell, a human organ allograft contains a permanent population of antigen presenting cells of donor origin. This difference in antigen presenting cell population between rodents and man might explain the divergent results in both groups seen after the elimination of passenger leukocytes as a result of donor or graft pretreatment.

CHAPTER 2

TRANSFUSION TO THE DONOR: RATIONALE, OBJECTIVES AND DESIGN OF THE EXPERIMENTS PERFORMED

Blood transfusions may have a profound effect on the immune response, as established by many investigators, in renal transplant recipients (Chapter 1.2.10). In this chapter data will be reviewed which indicate that blood transfusions given to the transplant donor shortly before transplantation may affect graft survival in dog and man.

The first authors who described an effect of blood transfusions to the donor on kidney graft survival have been Jeekel *et al.* (1980). They described that in dogs the prolonged kidney graft survival achieved by one peroperative blood transfusion could be abolished by transfusion of the donor on day-1 with 100 ml third party blood.

Subsequently in 7 retrospective studies the effect of blood transfusions given to the donor on cadaver kidney graft survival in man has been evaluated. In 4 studies a significant beneficial effect has been found.

In Leiden 44 patients, who never received blood transfusions before transplantation, but were transfused at the time of transplantation, were studied (Jeekel *et al.*, 1980; 1981; 1982; 1983). The overall one year graft survival in these 44 patients was 27.5%, which is comparable to the 32% one year graft survival in nontransfused recipients from Eurotransplant, reported in a previous study (Persijn *et al.*, 1979). Of the cadaveric donors of these 44 kidneys, 26 donors were not transfused during the period immediately preceeding their death, whereas 18 donors did receive at least one blood transfusion. The 26 recipients of kidney grafts from nontransfused donors rejected their grafts significantly earlier than the group of 18 patients with renal grafts from transfused donors, the corresponding one year graft survival being 11.5% and 50%, respectively. Comparison of the recipients in the two groups for sex, age and dialysis period did not reveal significant differences. The number of HLA-A and B locus mismatches were comparable in the two groups.

In Basel the study covered 96 first kidney grafts transplanted between 1971 and 1979 (Jeekel *et al.*, 1980; 1981; 1982; 1983). Of these 96 grafts, 79 were transplanted as a first graft to transfused recipients, and 17 to nontransfused recipients. The group of 79 patients was divided into subgroups, depending on whether the donor had been transfused or not in the period immediately preceeding death. The 38 patients who received a graft from transfused donors fared better. Graft survival in these patients at one year was 84% versus 53.1% for the 41 nontransfused grafts. This is a statistically significant difference. In the 17 nontransfused recipients, the

survival of 12 nontransfused grafts was 42%, whereas the survival of 5 transfused grafts was 60%.
The transfused donors received from 1 till more than 10 units of blood. No clear dose-effect relationship was noted. The amount of corticosteroids and mannitol given to the donor in the period between admission and nephrectomy was not different in transfused and nontransfused donors. The recipient groups were comparable for sex, age, dialysis period and HLA-A and B mismatch.

A retrospective study performed in Gothenburg on the effect of blood transfusion to the cadaveric kidney donor and graft survival in 129 primary transplantations has been reported (Frisk *et al.*, 1981; 1983; Jeekel *et al.*, 1983). Of these 129 grafts, 109 were transplanted to transfused recipients and 20 to nontransfused recipients. Of 109 transfused recipients of kidney grafts, 55 received kidneys from transfused donors and 54 from nontransfused donors. A significantly better graft survival was found up to 36 months after grafting in transfused recipients when the kidneys came from transfused as compared to nontransfused donors. Graft survival at one year was 76.3% versus 55.4% respectively. In nontransfused recipients no difference in graft survival was found between kidneys from transfused and nontransfused donors; graft survival among these recipients was very poor with either type of kidney (8% and 25% one year graft survival respectively). HLA-A, B matching, age and number of transfusions in the recipient did not differ between the two groups. There was a difference in sex distribution among the recipients who were given kidneys from transfused and nontransfused donors. However, the six months graft survival was similar in male and female recipients. Furthermore a significant difference in the distribution of diagnosis of fatal disease of the donors was found. More traumatic injuries and fewer intracerebral hemorrhage were recorded among transfused than among nontransfused donors. However, further analysis did not reveal a relation with graft survival in the two groups. Finally the amount of steroids given was similar in the two groups of donors, and there was no significant difference in graft survival of kidneys harvested from donors treated with steroids.

Recently Harder *et al.* (1984) have reported the results of a prospective collection of relevant donor- and recipient-data in 6 Swiss transplantation centers. They found that at 2 months, 90% of the 30 transfused kidneys were functioning versus 67% of the 70 nontransfused. At 1 year 77% of the transfused kidneys and 57% of the nontransfused kidneys did function in transfused first graft recipients. In the two groups, recipients did not differ in sex, age, HLA-A,B matching and cytotoxic antibodies and the donors did not differ in steroid dose, vasoactive drugs, blood pressure and urine production until nephrectomy. However, there were 73% trauma patients in the transfused versus 45% in the nontransfused donor group.

Three reports have been published that do not support the forementioned data which suggest that there is a beneficial effect of transfusion of the donor prior to nephrectomy.

Berg *et al.* (1982) from Iowa did a retrospective analysis of 293 kidney recipients of whom 110 received kidneys from nontransfused donors. Actuarial analysis

revealed no significant differences in graft survival rates between nontransfused kidney donors and transfused kidney donors. One year graft survival in both groups was 59%. Considering only first transplant recipients, there was no difference in graft survival rates between nontransfused (n=151) and transfused (n=80) donor kidneys. One year graft survival was 64% versus 65%. When only preoperatively transfused recipients receiving first transplants were examined, there was no difference in graft survival rates between nontransfused kidney donors (n=81) and transfused kidney donors (n=43). One year graft survival in both groups was 68%. The recipient groups were comparable for HLA-A,B and DR mismatch.

Bock *et al.* (1984) from Detroit did a retrospective analysis of 47 kidney recipients of whom 21 received kidneys from transfused donors. Actuarial analysis revealed no significant differences in graft survival rates between the nontransfused and transfused kidney donors, 41.9% and 47.6% at one year respectively. It was reported that when transfused recipients receiving grafts were examined, there was not a difference in graft survival rates between nontransfused and transfused kidney donors either. The number of HLA mismatches was comparable between the two groups.

Opelz for the Collaborative Transplant Study (1985a) has analyzed the effect of transfusions given to kidney donors on first cadaver transplants. According to this analysis, it did not matter whether the kidney donor was transfused (n=1452) or not (n=1937). Neither in the overall material, nor in a subset of patients without pretransplant transfusions, was there an influence of the donor's transfusion status on graft outcome.

In Table 2.1 the relevant clinical data are summarized.

With regard to the mechanism underlying the donor transfusion phenomenon, it has been suggested that the presence of passenger leukocytes of two origins in the kidney at the time of transplantation, i.e. donor leukocytes and third party cells from the blood donor, may be responsible for the altered immunologic reactivity either in the graft or in the host (Frisk *et al.*, 1983; Jeekel *et al.*, 1983).

It has also been speculated that, assuming there is an effect of peroperative recipient blood transfusion, transplantation of a transfused donor kidney might represent additional peroperative transfusion of blood-donor leukocytes (Berg *et al.*, 1982).

Batchelor has offered the hypothesis that dendritic cells in the donor kidney might be mobilized in response to a transfusion, thereby rendering the kidney depleted of dendritic cells and less immunogenic (Opelz and van Rood, 1983).

It is clear that many questions have been raised by the retrospective finding of a possibly beneficial effect of blood transfusions given to the donor. Therefore, it was thought to be important to investigate some of these questions in rigidly controlled experimental studies.

Table 2.1. Effect of blood transfusions to the donor on (first) kidney graft survival.

Center	Donor	No.	Recipient	One year graft survival	p-value (chi square test)
Leiden	Transfused	18	per-op. transf.	50 %	$p < 0.05$
(Jeekel)	—	26	per-op. transf.	11.5%	
Basel	Transfused	38	pre-op. transf.	84 %	$p < 0.05$
(Jeekel)	—	41	pre-op. transf.	53.1%	
	Transfused	5	—	60 %	$p < 0.05$
	—	12	—	42 %	
Swiss Transplant	Transfused	30	pre-op. transf.	77 %	$p < 0.05$
Centers (Harder)	—	70	pre-op. transf.	57 %	
Gothenburg	Transfused	55	pre-op. transf.	76.3%	$p < 0.05$
(Frisk)	—	54	pre-op. transf.	55.4%	
	Transfused	12	—	8 %	$p > 0.05$
	—	8	—	25 %	
Iowa	Transfused	43	pre-op. transf.	68 %	$p > 0.05$
(Berg)	—	81	pre-op. transf.	68 %	
Detroit	Transfused	21	?	47.6%	$p > 0.05$
(Bock)	—	26	?	41.9%	
Collaborative	Transfused	1452	?	±68%	$p > 0.05$
Transplant Study	—	1937	?	±68%	
(Opelz)					

Data from four different retrospective studies do show that blood transfusions to the donor prior to nephrectomy positively influence graft survival in transfused recipients (Jeekel *et al.*, 1980, 1981; 1982; 1983; Frisk *et al.*, 1981; 1983; Harder *et al.*, 1984). Three other retrospective studies could not confirm these results (Berg *et al.*, 1982; Bock *et al.*, 1984; Opelz for the Collaborative Transplant Study, 1985a).

The experiments described in this thesis had the following three objectives:

1. to study the effect of different whole blood transfusions and transfusions of blood components to the donor on graft survival.
2. to determine, in case such an effect was apparent, the underlying mechanism(s).
3. to contemplate possible implications for clinical transplantation.

Since the introduction of microvascular surgical techniques for organ grafting in the mid-1960's it has been possible to study questions relevant to clinical organ transplantation in inbred rats (Abbott *et al.*, 1964; Fisher and Lee, 1965).

Many investigators have used inbred rat transplantation models over the past 15 years. It has become increasingly clear that one should proceed with caution in the interpretation of the data obtained in rats as to their potential importance for clinical transplantation. This subject has been lucidly reviewed by Fabre in his article titled "Rat kidney allograft model. Was it all too good to be true?" (Fabre, 1982).

According to Hart and Fabre (1981a; 1981b) the essential difference between rat and man in this context is that the rat lacks Ia antigens on its endothelial cells, whereas man has abundant Ia (DR) antigens on endothelial cells, at least in the capillaries of the kidney (Hart *et al.*, 1981). However, it should be mentioned that this finding of the absence of class II antigens from rat vascular endothelial cells conflicts with data as published by Paul *et al.* (Paul *et al.*, 1982; Paul and Carpenter 1983; Fabre *et al.*, 1983).
In both man and rat this Ia positive cell has been demonstrated in the interstitial connective tissue (Hart and Fabre, 1981c; Hart *et al.*, 1981), although definite identification in human connective tissue is more difficult because of the Ia positive endothelium of the capillaries. The Ia positive cell isolated from the lymhoid tissues of rodents has as its most prominent characteristics that it is the major cell involved in antigen presentation (Nussenzweig and Steinman, 1980) and mixed lymphocyte culture (MLC) stimulation (Nussenzweig *et al.*, 1980). In man the Ia positive endothelial cells have been shown to be capable of MLC stimulation and antigen presentation (Hirschberg *et al.*, 1980), capacities which, according to Fabre (1982), the Ia negative endothelium of the rat (Hart and Fabre, 1981a; 1981b) is most unlikely to possess.

In summary, the fresh rat allograft is likely to have only one cell type, the interstitial dendritic cell, with a specialized capacity for stimulation of host lymphocytes and for antigen presentation, whereas the fresh human graft has in addition the vascular endothelial cell which has these properties. As a result the data obtained in the rat must be interpreted with caution, but doing this, experiments can be done fruitfully with potentially important implications for clinical transplantation (Fabre, 1982).

Taking the above mentioned contemplations into account we decided to use the rat heterotopic heart- and kidney-transplantation models for our investigations.

A part of the results of these experiments in rats have already been published in the literature and presented at scientific meetings (Jeekel *et al.*, 1980; 1981; 1982; Heineman *et al.*, 1983a-b; 1985; in press a-b). However, quite a substantial number of experiments has not yet been reported. Taking this into consideration it was decided to rewrite the articles and abstracts already published and to complete the material with new data.

CHAPTER 3

MATERIALS AND METHODS

3.1 Animals

Animals of six highly inbred rat strains were used. These were male Brown-Norway (BN), male WAG, male Brofo, male Lewis (LEW), female RI and male (WAG × LEW) F_1 hybrid rats.

Between the BN, WAG, Brofo and LEW strains a major RT-1 histoincompatibility exists. The BN strain is homozygous for the RT-1^n haplotype, the WAG strain for the RT-1^u haplotype, the Brofo strain for the RT-1^c haplotype and the LEW strain for the RT-1^l haplotype (Palm and Black, 1971; Festing and Staats, 1973). The R1 (RT-1^p) has a recombination within its major histocompatibility complex. For class I antigens, the R1 is similar with the WAG strain (RT-1^u), whereas for class II antigens it is associated with the LEW strain (RT-1^l) (Vaessen *et al.*, 1979).

The animals were obtained from the Laboratory Animals Center of the Erasmus University Rotterdam, The Netherlands. They were kept clean under conventional conditions. The strains are tested regularly by skin transplant in order to demonstrate the absence of genetic drift.

In all transplantation experiments (330 heterotopic heart transplantations and 37 heterotopic kidney transplantations), animals of 12-16 weeks of age, weighing 220-250 grams were used. In the popliteal lymph node assay 32 (WAG × LEW) F_1 hybrids were used; they were six weeks old and weighed about 100 grams.

3.2 Heart and kidney transplantation

3.2.1 *General*

Operations were performed under standardized conditions. The majority of transplantations was performed by the author, but especially in the first group of experiments highly appreciated support was received from other microsurgeons.

In all transplantations the interruption of the blood flow (ischemic period) lasted forty minutes or less on the average, which did not produce lasting functional damage to the hind legs of the animals. Ether anesthesia was used throughout the operation.

The cardiac grafting was done according to the technique described by Abbott *et al.* (1964) and by Bui-Mong-Hung and Vigano (1966) and as modified by Ono and Lindsey (1969). Kidney transplantation was performed according to the technique of Fisher and Lee (1965) and Lee (1967) with the modifications of Lameijer *et al.*

(1972) for the ureter-bladder anastomosis. All heart and kidney donors were heparinized with 500 units of Na-heparine (Thromboliquine®) dissolved in 1 ml of saline injected into the tail or penile vein.

No food limitations were imposed before and after grafting and no antibiotic treatment was given.

3.2.2 *Heart transplantation*

3.2.2.1 Surgical technique

Donor: after shaving and desinfection with chlorohexidine (hibitane) the thoracic cavity is opened. The anterior chest wall is separated from the diaphragm and the anterior rib cage is divided with heavy scissors on both sides of the sternum from the lower rib margin to the clavicles. The whole cage is then reflected superiorly in order to provide maximum exposure. The pericardium is incised and the heart is depressed inferiorly. The ascending aorta and the pulmonary trunk are freed from one another by careful dissection. The ascending aorta is cut with irridectomy scissors close to the origin of the innominate artery. Next the pulmonary trunk is cut close to the bifurcation into the left and right pulmonary arteries. This transsection of the main arterial vessels leaves enough length attached to the heart to allow for the performance of micro anastomoses. The heart is lifted up and one ligature is placed around the caval veins and pulmonary veins. After cutting these veins distal to the ligature, the heart is removed. Finally the heart is perfused with 4 ml of Hank's balanced salt solution (HBSS) through the ascending aorta in a retrograde direction using a blunt needle, in order to clean the coronary vessels from blood. The heart is stored in HBSS at 4°C.

Recipient: after shaving and desinfection with chlorohexidine (hibitane) a long abdominal mid-line incision is made to expose the great vessels. First the intestines are wrapped in a moistered gauze and are retracted to the left. Next the aorta and caval vein are dissected and freed from surrounding tissues with the use of cotton wool tipped applicators. Longitudinal openings of 2-3 mm are made in both the aorta and caval vein after being clamped together below the renal vessels with a curved hemostat. Through the openings the vessels are flushed with HBSS to remove thrombi.

The donor heart is placed transversely in the right side of the abdominal cavity of the recipient. Stay sutures (7.0 silk, Ethicon) are placed at the proximal and distal ends in the recipient and donor aorta and are used for continuous suturing. After completion of the left side of the aortic anastomosis, the heart is flipped-over to allow placement of the right half of the suture line. The pulmonary artery is connected end-to-side to the inferior caval vein in a similar manner. The aortic anastomosis restricts access to the left side of the caval vein, thus it is necessary to place the first half (left side) of the pulmonary artery-caval vein suture line from within the lumen. In the author's experience it is easier to make the longitudinal opening in the caval vein more proximal to avoid the restriction caused by the aortic

anastomosis. Torsion of the vessels is carefully avoided. After completion of the anastomoses the hemostat is removed and a gauze is gently pressed onto the anastomoses until hemostasis is complete. Restoration of graft circulation is followed within a few seconds by a resumption of myocardial contractions. The mechanics of circulation are essentially those described by Mann *et al.* (1933). The transplanted heart is perfused through its coronary vessels. Blood is returned to the recipient's inferior caval vein through the right atrium, right ventricle and pulmonary artery of the donor heart. In this fashion the left side of the heart is bypassed, although perfusion of the left myocardium is maintained.

If the heart does not attain its normal colour and pulsations fail to show the appropriate rate, the graft is considered technically unsuccessful and discarded. The heart is placed retroperitoneally on the right side of the abdominal cavity, the intestines are replaced and the abdominal wall is closed in one layer.

3.2.2.2 Evaluation of function

It has been found that cessation of electrical activity of the heart as determined by electrocardiographic monitoring fits in very well with the cessation of palpable heart beats (van Schilfgaarde, 1978). The electrocardiographic monitoring does not yield more or better information (van Schilfgaarde, 1978). As palpation is technically the easiest way of monitoring cardiac function, we assumed that complete rejection had taken place when pulsations could no longer be felt.

Technical failures, mainly due to thrombosis were only occasionally encountered. In these cases, the hearts stopped beating within one or two days and were excluded from the experimental groups.

3.2.3 *Kidney transplantation*

3.2.3.1 Surgical technique

Donor: After shaving and desinfection with chlorohexidine (hibitane) the abdominal cavity is opened through an abdominal mid-line incision. The intestines are wrapped in moistened gauze and retracted laterally to the left side. The right kidney is used preferably, provided it does not show double arteries, evident hydronephrosis or dilatation of the ureter. The abdominal aorta and inferior caval vein, adrenal, and left renal arteries are identified and the latter three vessels are double ligated close to the aorta and are divided. The kidney is dissected from the perirenal fatty tissue and retracted medially to free the right renal artery from the renal vein in retrograde fashion. After the dissection is completed the aorta is clamped proximal to the origin of the renal artery. Next the aorta is cut, leaving a segment with the renal artery. This segment is made into a patch. The renal vein is cut with an elliptical excision of the caval vein. The ureter is easily mobilized, but care must be taken not to strip it. It is transected near the bladder. The kidney is

removed and placed in HBSS at 4°C. A blunt neddle is inserted into the renal artery and the kidney is very gently flushed with 4 ml of HBSS.

Recipient: The procedure is similar as described for the heterotopic heart transplantation. Torsion of the vessels is carefully avoided. After re-establishing the blood flow, the kidney should show its normal colour within a few moments; if not, either an inflow or an outflow tract obstruction (stenosis, torsion) should be suspected. In these cases the graft is considered technically unsuccessful and discarded.

The ureter is pulled through the posterior wall of the dome of the bladder having first opened the bladder from the front. A stich of 7.0 silk is passed from the outside through the bladder wall and one wall of the ureter and to the outside again. The stich is tied outside the bladder and the bladder is closed.

Bilateral nephrectomy is performed at the time of operation. The graft is placed retroperitoneally in the right half of the abdominal cavity. Intestines are replaced and the abdominal wall is closed in one layer.

3.2.3.2 Evaluation of function

Renal function was evaluated by serial determination (at days 3, 7, 10, 14, 17 and 21 posttransplantation) of blood urea nitrogen level (BUN). BUN levels in rat serum were determined by the photometric method nr 2410 of the Netherlands Normalisatie-Instituut (published by the Rijks Instituut voor de Volksgezondheid, Utrecht, 1966). Concentrations are expressed in mmol/l.

Blood samples were taken by bleeding the rats from the tail or orbita and were centrifuged at 1500 rpm for ten minutes. Sera for BUN determination were kept at 4°C until the tests were performed. Per test a quantity of 20 μl serum was required. All serum BUN concentrations were assessed in duplicate.

Rejection was defined by the BUN value exceeding 60 mmol/l value (Lameijer *et al.*, 1972; van Schilfgaarde, 1978). At autopsy the diagnosis was confirmed macroscopically. If hydronephrosis was present the graft was excluded.

3.3 **Transfusions**

3.3.1 *Whole blood*

Donors and recipients in several experimental groups received intravenously (i.v.) via the penile vein transfusions of whole blood. These consisted of different amounts of fresh pooled heparinized blood and were given at different intervals before transplantation. Blood donors were not used as heart or kidney donors. Blood was obtained by cardiac puncture or puncture of the abdominal aorta.

3.3.2 *Irradiated whole blood*

In one experimental group donors received fresh whole blood irradiated in vitro with 10 Gy just prior to transfusion (see 3.5).

3.3.3 *Erythrocytes*

Erythrocyte transfusates were prepared by using the cotton wool column technique as described by Diepenhorst *et al.* (1972). The equivalent number of erythrocytes present in 1 ml of whole blood (5×10^9) was given i.v. The final contamination with leukocytes was less than 1% of the number found in normal blood.

3.3.4 *Leukocytes*

Leukocyte suspensions were prepared by three successive incubations of buffy coat suspensions, with hemolytic buffer (tris-buffered ammonium chloride 0.17 m) and subsequent washing in HBSS. A marked reduction in the number of erythrocytes (< 0.1%) could be achieved by this procedure. The viability of the remaining leukocytes was determined by trypan blue exclusion, and this was usually more than 95%. About $5\text{-}7 \times 10^6$ leukocytes suspended in 2 ml of HBSS were injected i.v.

3.4 **Cyclosporin A**

In several experimental groups the recipients were treated postoperatively with Cyclosporin A (CsA). The CsA was supplied as a white powder and was a gift from the Sandoz Corp., Basle, Switzerland. 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 as described by Niessen *et al.* (1982). Recipients of heart transplants received a single dose of 15 mg/kg body weight which was administered after transplantation on the day of grafting. Kidney graft recipients were given a single dose of 5 mg/kg body weight on the day of grafting.

3.5 **Irradiation**

A ^{137}Cs (γ) source (Atomic Energy of Canada, Ltd.) was used in the radiation experiments. Animals were irradiated with a whole body dose of 10 Gy.

The period of time between donor irradiation and transplantation was either 5 days or less than 1 day. The peripheral blood leukocyte counts were uniformly less than 500 cells per cubic millimeter in donors irradiated 5 days before grafting. Donors irradiated on the day of grafting did not show any decrease in peripheral blood leukocyte counts as compared to non-irradiated controls.

Similar conditions were used for ex vivo irradiation of fresh whole blood.

3.6 Popliteal lymph node (PLN) assay

The basis of the popliteal lymph node (PLN) assay has been described by Ford *et al.* (1970). Parental lymphoid cells injected into the hind footpad of F_1 hybrids will induce a Graft versus Host (GvH) reaction in the draining popliteal lymph node, leading to an increase in weight as well as cellularity. The enlargement of the popliteal node, which peaks at 5-7 days after inoculation, is related to the number of parental cells injected and can be quantified by simple weighing. The main immunological attack is exhibited by the injected donor T cells against the histocompatibility antigens of the host. The local GvH reaction can be used to quantify specific cell mediated immunity, if the relevant parenteral strain and F_1 hybrids are used. States of sensitization as well as immunosuppression can be monitored in a relatively simple manner.

In the present experiments, (WAG × LEW) F_1 hybrids were used to monitor specific cell mediated immunity of WAG rats subjected to various treatments and allografting. Spleen cells of the WAG rats under investigation were pooled and a cell suspension was made in Hank's balanced salt solution (HBSS). Erythrocytes were lysed by hemolytic buffer. In all cases the different numbers of spleen cells from the WAG rats were injected in a constant volume of 0.2 ml into the hind footpads of F_1 rats. Two hybrids were used per parental strain under investigation and the spleen cells were injected into both hind footpads, thus four weights were recorded per animal to be monitored. One week after injection, the popliteal nodes were excised, cleaned on filter paper and weighed to the nearest 0.1 mg.

Dose response curves were obtained with normal and treated WAG spleen cells and were used to assess the degree of GvH activity. The significance of differences in node weights was determined by using the Wilcoxon-rank sum test.

3.7 Statistical analysis

For statistical analysis of the results obtained in the various experiments the Wilcoxon-rank sum test (Wilcoxon, 1945) and chi square test (Yates, 1934; Armitage, 1971) were used. P values are given in the text.

CHAPTER 4

MODIFICATION OF RAT CARDIAC ALLOGRAFT SURVIVAL BY ADMINISTRATION OF THIRD PARTY BLOOD TRANSFUSION(S) TO THE DONOR

This chapter was published in a modified form in Transplant. Proc. 1983; 15: 994 and Transplantation 1983; 36: 362.

4.1 Introduction

There is now wide acceptance that in humans blood transfusions given to the recipient before grafting produce a strikingly beneficial effect on the post-transplant course of cadaveric or living related kidneys (Opelz *et al.*, 1981b; Salvatierra *et al.*, 1983).

Recently it has been reported by several authors that blood transfusions to the donor prior to nephrectomy positively influence graft survival in transfused recipients (Jeekel *et al.*, 1980; 1981; Frisk *et al.*, 1981; 1983; Harder *et al.*, 1984). However, retrospective studies by Berg *et al.* (1982), Bock *et al.* (1984) and Opelz (1985a) could not confirm these reports.

The effect of third party blood transfusions given to the donor has been further evaluated in experimental studies in animals.

In the dog it was found that the beneficial effect of a peroperative transfusion was abolished by transfusion of the donor one day before grafting (Jeekel *et al.* 1980).

Berg *et al.* (1982) reported animal studies in (Lewis × Brown Norway) F_1 (LBN F_1) hybrid rat hearts transplanted heterotopically to LEW recipients. Non-transfused LBN F_1 heart grafts had a mean survival time of 8.0 ± 1.1 days. LBN F_1 hearts from rats receiving 2 ml of heparinized whole blood from Charles River (CD) rats 24 hr before heart transplantation, had a mean survival time of 6.6 ± 0.9 days, which was reported to be significantly less than the survival times of untransfused donor hearts (Berg *et al.*, 1982).

This chapter describes the effect of third party whole blood transfusion(s) of the donor on heterotopic heart allograft survival.

The following variables were studied:

1. The effect of donor transfusion in different donor-host combinations
2. The effect of donor transfusion after different types of immune modulation of the host.
3. The effect of varying number and timing of donor transfusion.

4.2 Experimental protocol

Five main experimental groups can be distinguished, they are shown in Table 4.1.

In the first experimental group the effect of third party (Brofo) whole blood transfusion(s) to the BN and RI donor on heart allograft survival in the unmodified WAG host was studied. The BN donor differs at the major histocompatibility complex from the WAG host, whereas the R1 strain has a recombination within its major histocompatibility complex. For class I antigens, the R1 is similar with the WAG strain and for class II antigens, the R1 is similar with the LEW strain (Vaessen *et al.*, 1979).

In the second experimental group the effect of third party (Brofo or LEW) whole blood transfusion(s) to the BN donor on heart allograft survival in WAG hosts pretreated with one donor-specific transfusion (DST) was studied. Furthermore, the effect of varying number and timing of transfusions was investigated.

In the third experimental group it was studied whether third party (Brofo) whole blood transfusion of the WAG donor would influence heart allograft survival in BN hosts pretreated with one DST.

In the fourth experimental group the effect of third party (Brofo) whole blood transfusion(s) to the BN donor on heart allograft survival in LEW hosts pretreated with one DST was studied.

In the fifth experimental group the effect of third party (Brofo of LEW) whole blood transfusion(s) to the BN donor on heart allograft survival in the Cyclosporin A (CsA) treated WAG host was studied. The recipients received 15 mg/kg bodyweight CsA intramuscularly after the operation on the day of transplantation.

In all experiments each blood transfusion consisted of 1 ml fresh pooled heparinized whole blood. Cardiac function was assessed as evaluated in chapter 3.2.2.2. The results were analyzed using the chi-square and Wilcoxon-rank sum tests.

4.3 Results

4.3.1 *The effect of third party whole blood transfusion to the BN and R1 donor on heart allograft survival in the unmodified WAG host*

The survival times observed in the different experimental groups are shown in Table 4.2 and Figure 4.1. No significant difference was found between survival times achieved in nontransfused and transfused donors in the BN/WAG and R1/WAG donor/host combinations when the WAG host was not treated. A single Brofo transfusion to the BN donor at 2 days before transplantation had no effect on graft survival. As in the control animals the hearts were rejected in 8-9 days (groups 1 and 2). In the R1/WAG donor/host combination a graft survival between 8 and 13 days was found when donor and host were not treated. When the R1 donor was treated with two Brofo transfusions at 2 days and 1 day before grafting the survival times did not change and were of the same magnitude as in the nontransfused animals (groups 3 and 4).

Table 4.1. Experimental protocol.

Experiment	Group	Donor/Host combination	Donor transfusion	Host treatment
I	1	BN/WAG	—	—
	2	BN/WAG	day -2, Brofo 1 ml	—
	3	RI/WAG	—	—
	4	RI/WAG	day -2, Brofo 1 ml	—
II	1	BN/WAG	—	day -7, BN 1 ml
	2	BN/WAG	day -2, Brofo 1 ml	day -7, BN 1 ml
	3	BN/WAG	day -2, LEW 1 ml	day -7, BN 1 ml
	4	BN/WAG	day -2,-1, Brofo 1 ml	day -7, BN 1 ml
	5	BN/WAG	day -2,-1, LEW 1 ml	day -7, BN 1 ml
	6	BN/WAG	day -7, LEW 1 ml	day -7, BN 1 ml
III	1	WAG/BN	—	—
	2	WAG/BN	—	day -7, WAG 1 ml
	3	WAG/BN	day -2, Brofo 1 ml	day -7, WAG 1 ml
IV	1	BN/LEW	—	—
	2	BN/LEW	—	day -7, BN 1 ml
	3	BN/LEW	day -2,-1, Brofo 1 ml	day -7, BN 1 ml
V	1	BN/WAG	—	day 0, CsA 15 mg/kg
	2	BN/WAG	day -2,-1, Brofo 1 ml	day 0, CsA 15 mg/kg
	3	BN/WAG	day -2,-1, LEW 1 ml	day 0, CsA 15 mg/kg

4.3.2 *The effect of third party whole blood transfusion to the BN donor on heart allograft survival in WAG hosts pretreated with DST*

The survival times observed in the different experimental groups are shown in Table 4.3 and Figure 4.2. It was found that grafts from nontransfused donors did survive indefinitely in recipients which were transfused with a donor-specific transfusion 7 days before grafting (group 1). These animals served as controls for the experimental groups 2-6. A single Brofo or LEW transfusion given to the donor 2 days before grafting shortened graft survival significantly. A Brofo transfusion led to a shortened survival time in 73% of the transplantations (group 2, $p < 0.001$), a LEW transfusion led to the same result in 66% of the cases (group 3, $p < 0.05$). Brofo or LEW transfusions given to the donor at 2 days and 1 day before grafting had the same effect. Shortened graft survival times were found in 75% and 40% of the animals, respectively (group 4 and group 5, $p < 0.05$). A single LEW transfusion given to the donor 7 days before grafting also shortened graft survival time. The grafts were rejected in 42% of the cases (group 6, $p < 0.05$).

Table 4.2. The effect of third party whole blood transfusion to the BN and RI donor on heart allograft survival in the unmodified WAG host.

Group	Donor	Donor treatment	Recipient	Recipient treatment	Number	Survival in days
1	BN	—	WAG	—	10	8,8,8,8,8,8,8,8,9,9
2	BN	day –2, Brofo 1 ml	WAG	—	6	8,8,8,9,9,9
3	RI	—	WAG	—	10	8,10,10,10,11,11,11,11,13,13
4	RI	day –2, and –1, Brofo 1 ml	WAG	—	8	11,11,11,11,11,12,12,12

The donors were transfused i.v. at different intervals (day –1 and/or –2) with 1 ml of whole Brofo blood. The recipients were not treated.

Table 4.3. The effect of third party whole blood transfusion to the BN donor on heart allograft survival in transfused WAG hosts.

Group	Donor	Donor treatment	Recipient	Recipient treatment	Number	Survival in days
1	BN	—	WAG	day –7, BN 1 ml	25	permanent
2	BN	day –2, Brofo 1 ml	WAG	day –7, BN 1 ml	15	5,7,7,8,8,8,9,9,10,21,28, permanent 4×
3	BN	day –2, LEW 1 ml	WAG	day –7, BN 1 ml	9	7,10,14,17,18,26, permanent 3×
4	BN	day –2,–1, Brofo 1 ml	WAG	day –7, BN 1 ml	4	15,16,20, permanent 1×
5	BN	day –2,–1, LEW 1 ml	WAG	day –7, BN 1 ml	10	11,14,16,16,17,30, permanent 4×
6	BN	day –7, LEW 1 ml	WAG	day –7, BN 1 ml	7	26,28,30, permanent 4×

The donors were transfused i.v. at different intervals (day –1 and/or –2 or –7) with 1 ml of whole third party (Brofo or LEW) blood.
The recipients were transfused at day –7 with 1 ml of BN blood.

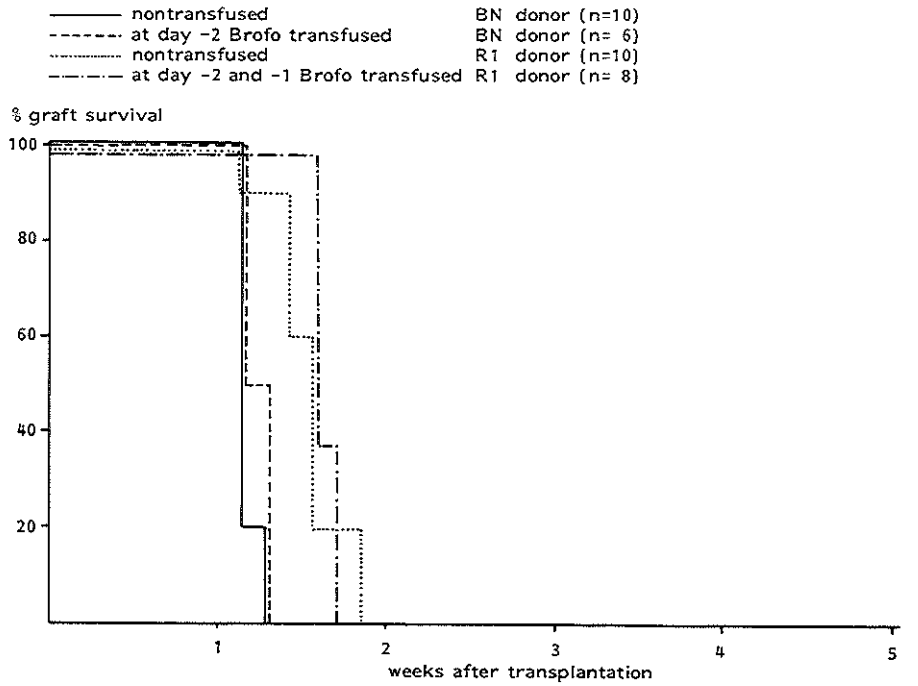


Figure 4.1. The effect of third party whole blood transfusion to the BN and R1 donor on heart allograft survival in the unmodified WAG hosts (see Table 4.2).

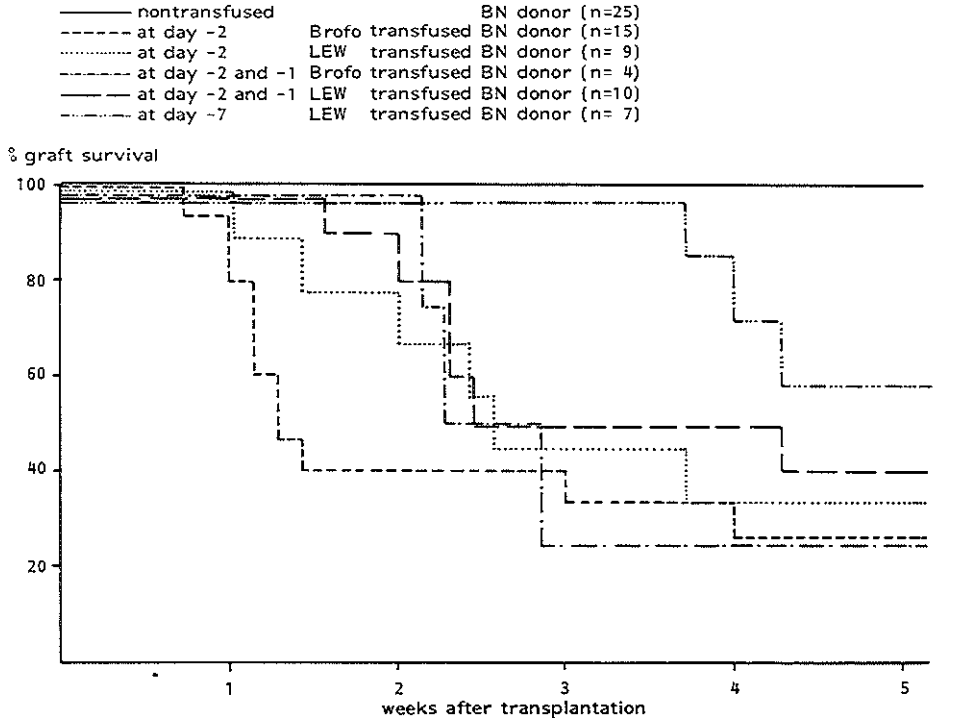


Figure 4.2. The effect of third party whole blood transfusion(s) to the BN donor on heart allograft survival in transfused WAG hosts (see Table 4.3).

4.3.3 *The effect of third party whole blood transfusion to the WAG donor on heart allograft survival in BN hosts pretreated with DST*

The survival times observed in the different experimental groups are shown in Table 4.4 and Figure 4.3. In the WAG to BN donor/host model a donor-type transfusion to the recipient always led to accelerated rejection in 5 days compared to the 8-9 days graft survival in the controls (groups 1 and 2). A single Brofo transfusion given to the donor 2 days before grafting resulted in a further acceleration of rejection in 66% of the cases (group 3, $p < 0.05$).

4.3.4 *The effect of third party whole blood transfusions to the BN donor on heart allograft survival in LEW hosts pretreated with DST*

The survival times observed in the different experimental groups are shown in Table 4.5 and Figure 4.4. In the unmodified BN/LEW donor/host combination cardiac grafts survived 6-7 days (group 1), which is similar to the survival times reported by van Schilfgaarde (1979). Grafts from nontransfused BN donors did survive significantly longer in LEW hosts pretreated with DST. Rejection was observed after 12-13 days (group 2). These animals served as controls for group 3. In this experimental group it was found that Brofo transfusions given to the donor at 2 days and 1 day before grafting abrogated the beneficial DST effect in 85% of the cases (group 3, $p < 0.05$).

4.3.5 *The effect of third party whole blood transfusion to the BN donor on heart allograft survival in WAG hosts treated with Cyclosporin A (CsA)*

The survival times observed in the different experimental groups are shown in Table 4.6 and Figure 4.5. In all experimental groups the WAG recipients were treated intramuscularly on the day of operation with 15 mg/kg CsA. When using an unmodified BN donor, a single injection of CsA led to a graft survival of 9-15 days (group 1). This group forms the control group. If the donor was modified by transfusion with Brofo or with LEW blood at 2 days and 1 day before transplantation, the effect of CsA treatment on graft survival was enhanced. Brofo transfusions to the donor led to prolongation of graft survival in 75% of the transplantations; LEW transfusions had this effect in 100% of the transplantations (groups 3 and 4, $p < 0.05$).

4.4 Discussion

In rats, it was investigated whether third party transfusion(s) to the donor would influence the survival of heterotopically transplanted hearts in nontransfused, transfused and CsA treated recipients.

The effect of third party transfusion(s) to the donor in the unmodified WAG host was tested in the first experiment. Two donor/host combinations, the BN to WAG and the R1 to WAG combination were employed. Graft survival in the BN to WAG model was 8-9 days (group 1) and was not influenced by donor pretreatment (group 2). In the R1 to WAG model, where there is compatibility for class I antigens between donor and recipient (Vaessen *et al.*, 1979), graft survival was 8-13 days (group 3). It was tested whether in this "weaker" donor/host combination blood transfusions to the donor would influence graft survival. However, even twice as many Brofo transfusions as employed in the BN to WAG combination, did not show any effect on graft survival. Grafts were rejected in 11-12 days (group 4). It can be concluded that there is no measurable effect of third party transfusion to the donor on heart allograft survival in the unmodified WAG hosts, even if there is compatibility for class I antigens. Our results are in contrast with those obtained by Berg *et al.* (1982). These authors reported a significant detrimental influence of blood transfusion to the donor on heart allograft survival in unmodified hosts. However, they used a different donor/host combination, the (LEW × BN) F_1 to LEW combination.

In the next three experiments we investigated the effect of third party transfusion(s) to the donor on heart allograft survival in DST transfused recipients. Three different donor-host combinations were employed, the BN to WAG, the reverse WAG to BN, and the BN to LEW combination.

It was found that in the BN to WAG combination, in which a donor-type transfusion to the recipient always leads to permanent graft acceptance (group 1) (Marquet *et al.*, 1971), a single third party transfusion to the donor 2 days before grafting resulted in a significant abrogation of the beneficial DST effect (groups 2 and 3). Two third party transfusions to the donor 2 days and 1 day before grafting had the same effect (groups 4 and 5). Thus, no dose-effect relation was noticed. Further it was found that both Brofo and LEW transfusion to the donor did diminish the favourable effect of a donor-type transfusion to the same degree. Consequently, the expression of the donor transfusion phenomenon is not confined to blood from a single strain.

Finally we tested in the BN to WAG combination the effect of a single donor transfusion at 7 days before transplantation. Graft survival in transfused recipients was reduced in a significant number of animals (group 6). The results were similar as those obtained for donors transfused shortly before transplantation. It can be concluded that there was no effect of timing.

In the third group of experiments it was tested whether donor transfusion had an effect in the WAG/BN donor-host combination. In this model a donor-type transfusion to the recipient always results in accelerated rejection of cardiac grafts in 5

Table 4.4. The effect of third party whole blood transfusion to the WAG donor on heart allograft survival in transfused BN hosts.

Group	Donor	Donor treatment	Recipient	Recipient treatment	Number	Survival in days
1	WAG	—	BN	—	10	8,8,8,8,8,8,8,8,9,9
2	WAG	—	BN	day –7, WAG 1 ml	25	5 25×
3	WAG	day –2, Brofo 1 ml	BN	day –7, WAG 1 ml	9	2,3,3,3,3,4,5,7,8

The donors were transfused at day –2 with 1 ml of whole Brofo blood. The recipients were transfused at day –7 with 1 ml of BN blood.

Table 4.5. The effect of third party whole blood transfusions to the BN donor on heart allograft survival in the transfused LEW hosts.

Group	Donor	Donor treatment	Recipient	Recipient treatment	Number	Survival in days
1	BN	—	LEW	—	6	6,7,7,7,7,7
2	BN	—	LEW	day –7, BN 1 ml	8	12,12,12,13,13,13,13,13
3	BN	day –2,–1, Brofo 1 ml	LEW	day –7, BN 1 ml	7	6,7,10,10,10,11,12

The donors were transfused at day –2 and –1 with 1 ml of whole Brofo blood. The recipients were transfused at day –7 with 1 ml of BN blood.

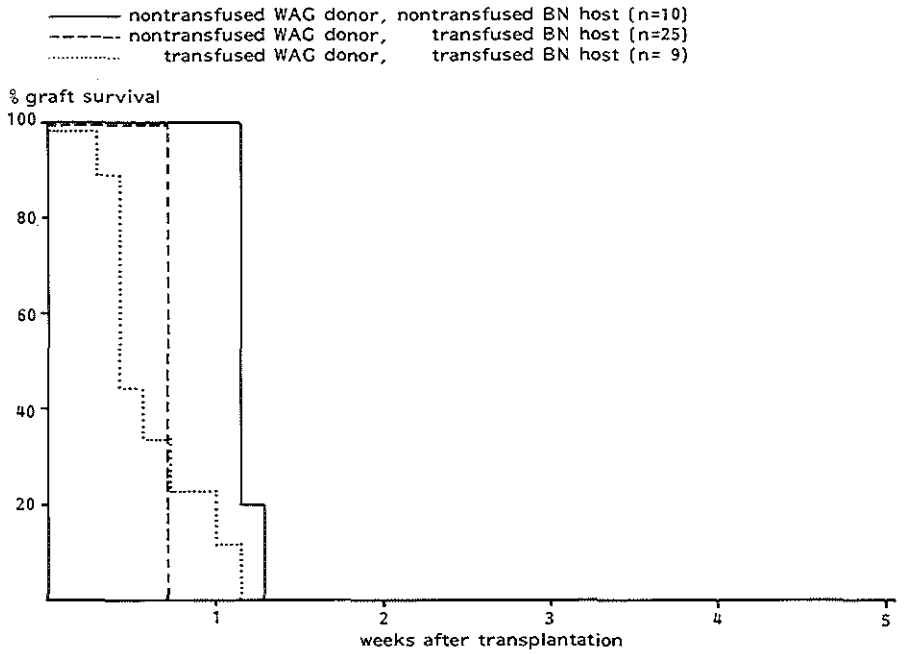


Figure 4.3. The effect of third party whole blood transfusion to the WAG donor on heart allograft survival in transfused BN hosts (see Table 4.4).

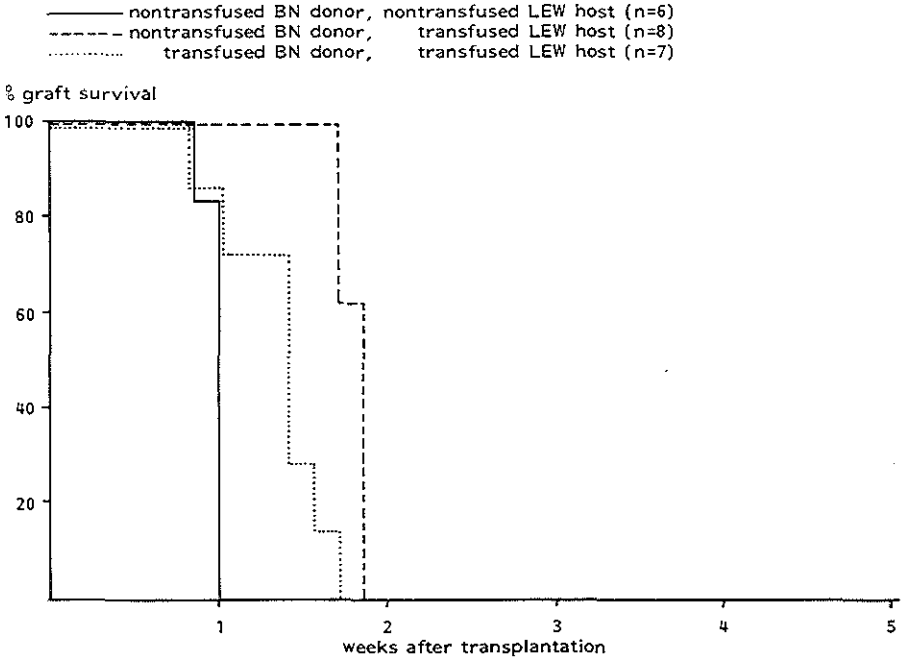


Figure 4.4. The effect of third party whole blood transfusions to the BN donor on heart allograft survival in the transfused LEW host (see Table 4.5).

Table 4.6. The effect of third party whole blood transfusions to the BN donor on heart allograft survival in Cyclosporin A (CsA) treated WAG hosts.

Group	Donor	Donor treatment	Recipient	Recipient treatment	Number	Survival in days
1	BN	—	WAG	day 0, CsA 15 mg/kg	12	9,10,11,12,13,13,13,14,14, 14,15,15
2	BN	day −2,−1, Brofo 1 ml	WAG	day 0, CsA 15 mg/kg	8	11,12,18,19,22,26,28, permanent 1×
3	BN	day −2,−1, LEW 1 ml	WAG	day 0, CsA 15 mg/kg	10	16,16,16,21,23,29,29,34,35, permanent 1×

The donors were transfused i.v. at day −2 and −1 with 1 ml whole blood from Brofo or LEW rats. The recipients were injected on the day of grafting i.m. in the hind leg with CsA in a dose of 15 mg/kg body weight in a volume of 0.1 ml.

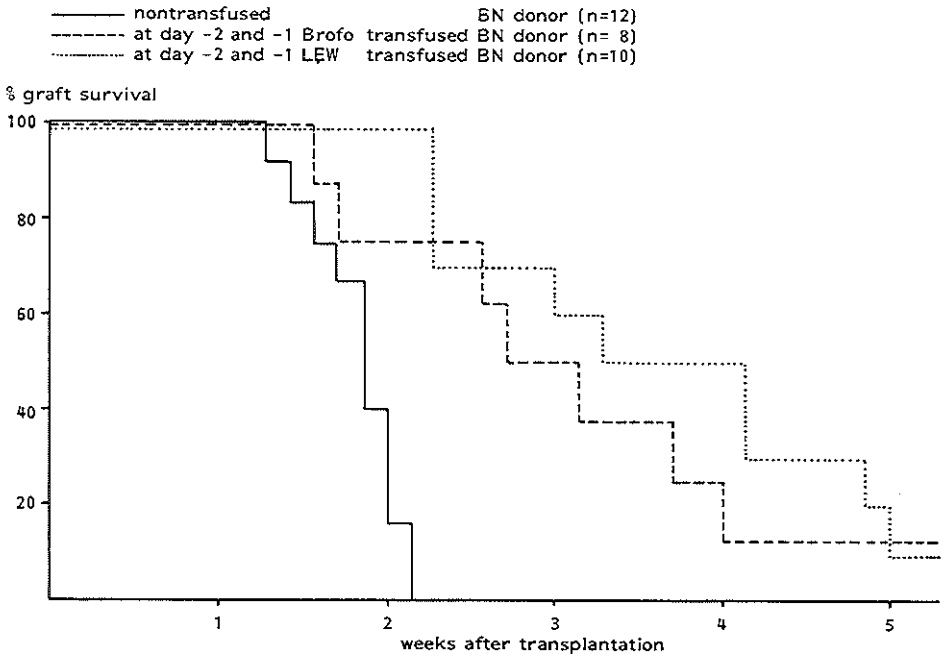


Figure 4.5. The effect of third party whole blood transfusions to the BN donor on heart allograft survival in Cyclosporin A (CsA) treated WAG hosts (see Table 4.6).

days (group 2) (Marquet and van Bekkum, 1973) as compared to the 8-9 days in controls (group 1). It was found that donor transfusions with third party blood resulted in a further acceleration of rejection in a significant number of the cases (group 3).

In the fourth group of experiments it was tested whether third party transfusion of the BN donor had an effect on allograft survival in the DST transfused LEW host. First it was observed that donor-type blood transfusion to the recipient led to a significantly prolonged cardiac graft survival. In the unmodified donor/host combination grafts were rejected after 6-7 days (group 1), whereas in the transfused hosts grafts were only rejected after 12-13 days (group 2). Third party transfusion of the donor had a detrimental influence on graft survival in a significant number of the cases (group 3).

From the results obtained in the second, third and fourth group of experiments it can be concluded that third party transfusion of the donor has an effect on cardiac allograft survival in hosts pretreated with DST. It is clear that this effect is not confined to one single donor/host combination, but that it is manifest in various donor-recipient combinations. Furthermore, in all these combinations it was found that in DST transfused recipients grafts from transfused donors fared worse than grafts from non transfused donors. Thus, a detrimental influence of third party

donor transfusion on graft survival was observed. However, the results obtained in the fifth group of experiments show that third party blood transfusion to the donor can have a beneficial effect on graft survival when the recipient has been treated with CsA. Brofo transfusions to the donor led to prolongation of allograft survival in 75% of the cases (group 2) whereas LEW transfusions to the donor had this effect in even 100% of the animals (group 3).

4.5 Summary

In a rat heterotopic heart transplantation model blood transfusion(s) given to the donor did markedly influence graft survival in transfused or immunosuppressed recipients.

When the recipient was pretreated with a donor-specific transfusion, which leads, depending on the donor-host combination employed, to a shortened, prolonged or even permanent graft survival, the effect of blood transfusions to the donor on graft survival was predominantly detrimental. When the recipient had been treated with a single injection of 15 mg/kg CsA on the day of operation, the effect of blood transfusions to the donor was beneficial.

The donor transfusion phenomenon was not linked to one particular donor-host combination.

Furthermore, the donor transfusion phenomenon was not linked to one single type of blood. Different sources of third party derived blood did have the same influence on graft survival.

Finally, there was no difference in graft survival after a single or multiple transfusions of the donor, nor was there an effect of timing.

CHAPTER 5

MODIFICATION OF RAT KIDNEY ALLOGRAFT SURVIVAL BY ADMINISTRATION OF THIRD PARTY BLOOD TRANSFUSIONS TO THE DONOR

This chapter has been accepted for publication in Transplant. Proc.

5.1 Introduction

It has been reported by several authors that in the clinic transfusions to the donor prior to nephrectomy have a positive influence on graft survival (Jeekel et al., 1980; 1981; Frisk *et al.*, 1981; 1983; Harder *et al.*, 1984). Retrospective studies by other authors however, could not confirm these reports (Berg *et al.*, 1982; Bock *et al.*, 1984; Opelz, 1985a).

The effect of third party blood transfusions given to the donor has been further evaluated in experimental studies in animals. We showed in the BN to WAG donor-host combination, in which a donor-specific transfusion to the recipient always leads to permanent cardiac graft acceptance (Marquet *et al.*, 1971), that a single transfusion to the donor resulted in abrogation of the beneficial transfusion effect in 75% of all cases (Jeekel *et al.*, 1982; Heineman *et al.*, 1983a). In a further study we showed that third party blood transfusions to the donor can have a beneficial effect on cardiac graft survival too, when the recipient has been treated with Cyclosporin A (CsA) (Heineman *et al.*, 1983b).

This chapter describes the effect of third party whole blood transfusion of the donor on the function and survival of kidney allografts.

5.2 Experimental protocol

Two different groups of experiments can be distinguished, as shown in Table 5.1.

In the first experimental group the effect of third party blood transfusions to the BN donor on kidney graft function in the unmodified WAG host was studied. The donors were transfused intravenously at day -1 and -2 with 1 ml of whole Brofo blood.

In the second experimental group the effect of third party blood transfusions to the BN donor on kidney graft function in the Cyclosporin A (CsA) treated WAG host was studied. The donors were transfused intravenously at day -1 and -2 with either 1 ml or 3 ml of whole Brofo blood. The recipients received 5 mg/kg body weight CsA intramuscularly after the operation on the day of transplantation.

Table 5.1. Experimental protocol.

Experiment	Group	Donor/Host combination	Donor treatment	Host treatment
I	1	BN/WAG	—	—
	2	BN/WAG	day -2,-1, Brofo 1 ml	—
II	3	BN/WAG	—	day 0, CsA 5 mg/kg
	4	BN/WAG	day -2,-1, Brofo 1 ml	day 0, CsA 5 mg/kg
	5	BN/WAG	day -2,-1, Brofo 3 ml	day 0, CsA 5 mg/kg

Renal function was evaluated as described in chapter 3.2.3.2. Rejection was defined by the blood urea nitrogen (BUN) level exceeding the 60 mmol/l value. The renal function and moment of rejection were analyzed using the Wilcoxon-rank sum test and chi square test respectively.

5.3 Results

The renal function in the various experimental groups is presented in Tables 5.2 and 5.3 and in Figures 5.1, 5.2 and 5.3.

Table 5.2. The effect of third party whole blood transfusions (1 ml) at day -2 and -1 to the BN donor on kidney graft function in the unmodified WAG host.

Rat no.	Group	Blood urea nitrogen (BUN) mmol/l		
		day 3	day 7	day 10
9		6.1	*93.4*	-
10		5.8	*62.0*	*175.2*
12	1	5.8	*85.3*	*123.6*
13	(control)	15.2	*67.4*	*175.4*
14		11.1	*73.4*	*198.0*
16		13.0	*61.2*	*80.8*
17		21.2	*92.2*	*188.2*
2 VL 1		17.8	*71.0*	*137.0*
2 VL 4		13.4	54.7	*125.6*
2 VL 5	2	20.2	*95.7*	*137.8*
2 VL 6	(experimental)	21.0	*61.2*	*123.7*
2 VL 7		13.7	*74.3*	*147.8*
2 VL 13		15.2	34.5	*157.5*
2 VL 14		23.4	49.5	*150.8*

Rejection was defined by the blood urea nitrogen (BUN) level exceeding the 60 mmol/l value. From the day the graft was considered to be rejected the BUN value is printed in italics.
There were no significant differences in either the renal function or the moment of rejection between the two groups.

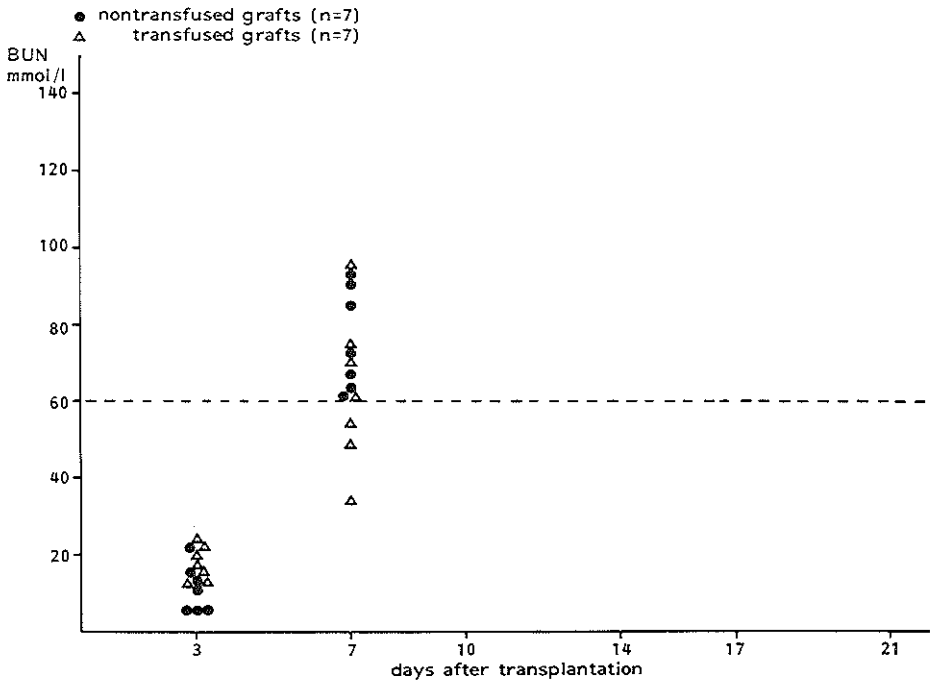


Figure 5.1. The effect of third party whole blood transfusions (1 ml) at dat –2 and –1 to the BN donor on kidney graft function in the unmodified WAG host (see Table 5.2).

In the first group of experiments it was found that there was no significant difference in renal function or the moment of rejection between transfused and non-transfused BN donors when the WAG hosts were not treated. The majority of the grafts lost their function after 7 days and all were rejected after 10 days (group 1 and 2) (Fig. 5.1).

In the second group of experiments the WAG hosts were postoperatively treated with a single intramuscular injection of 5 mg/kg CsA, which led to graft rejection in 64% of the recipients (group 3) during an observation period of 3 weeks. Blood transfusion to the donor of 1 ml did lead to graft rejection in 50% of the cases (group 4) (Fig. 5.2). However, transfusions to the donor of 3 ml did lead to graft rejection in only 17% of the animals (group 5). There was a significant difference in renal function and in the number of rejected grafts from day 14 posttransplant onwards between the non-transfused and 3 ml transfused animals (group 3 vs. group 5) (Wilcoxon-rank sum test, $p < 0.05$, and chi square test, $p < 0.05$, respectively) (Fig. 5.3).

Table 5.3. The effect of third party whole blood transfusions of 1 ml (group 4) or 3 ml (group 5) at day -2 and -1 to the BN donor on kidney graft function in the CsA treated (5 mg/kg i.m. inj.) WAG host.

Rat no.	Group	Blood urea nitrogen (BUN) mmol/l					
		day 3	day 7	day 10	day 14	day 17	day 21
C 1		9.2	16.4	40.6	*118.0*	*71.2*	—
C 3		17.5	16.8	34.6	*64.6*	*75.4*	*63.8*
C 4		11.0	10.6	19.6	38.6	36.8	40.2
C 6		27.8	18.6	17.8	33.8	44.0	43.6
C 15	3	14.2	31.4	36.1	*104.7*	*83.2*	*25.4*
E 1	(control)	13.8	16.4	25.2	*86.2*	*54.6*	*55.2*
E 4		12.6	14.2	15.0	*61.8*	*52.8*	*52.2*
E 5		15.8	14.0	19.2	37.2	43.0	31.8
E 6		11.4	17.2	32.2	*123.6*	*83.8*	*36.2*
E 9		24.4	16.2	27.0	57.4	*60.2*	—
E 10		16.2	16.4	16.2	41.6	48.6	44.8
EV 1		12.0	14.6	27.6	*71.2*	*65.6*	—
EV 2		11.0	15.6	20.0	48.0	48.2	46.4
EV 3	4	9.6	26.0	*68.4*	*93.2*	*55.6*	—
EV 4	(experimental)	20.0	19.2	23.4	51.4	59.2	52.0
EV 5		15.8	16.2	19.0	30.0	32.8	24.4
EV 6		23.0	38.0	*77.4*	*80.8*	*82.2*	—
EP 1		12.4	19.0	19.2	22.9	22.0	19.8
EP 3		15.8	18.2	12.4	25.6	19.3	16.4
EP 4	5	27.0	14.6	24.6	31.8	31.2	32.4
EP 5	(experimental)	19.2	20.2	17.4	20.6	19.6	19.8
EP 6		18.6	17.1	13.8	19.7	20.0	18.2
EP 7		15.4	*101.5*	*159.2*	—	—	—

Rejection was defined by the blood urea nitrogen (BUN) level exceeding the 60 mmol/l value. From the day the graft was considered to be rejected the BUN value is printed in italics.
There was a significant difference in renal function and in the number of rejected grafts from day 14 onwards between group 3 (control) and group 5 (experimental, 2×3 ml donor transfusion) (Wilcoxon-rank sum test, $p < 0.05$, and chi square test, $p < 0.05$, respectively).

5.4 Discussion

In rats, it was investigated whether third party transfusions to the donor would influence the function and survival of heterotopically transplanted kidneys in non-treated and CsA treated recipients.

It was found that neither graft function nor graft survival were influenced by donor pretreatment with third party blood when the recipient was not treated (group 1 and 2). This result in the kidney transplantation model is in accordance with the results obtained in the heart transplantation model, where we observed no effect of third party blood transfusion when the immune response of the recipient was not modulated (Heineman *et al.*, 1983a; 1983b).

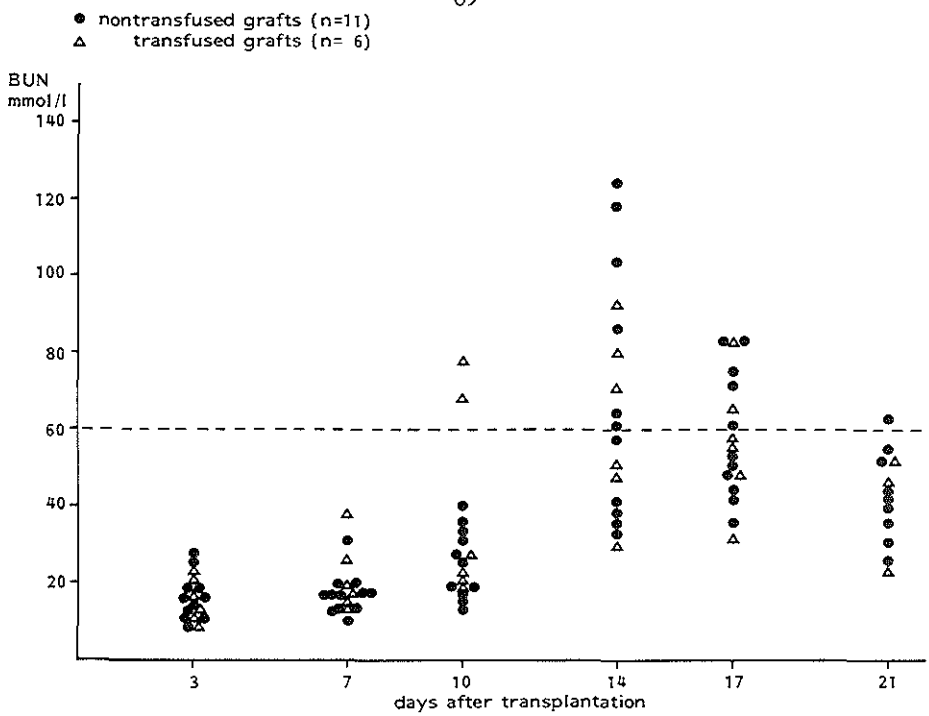


Figure 5.2. The effect of third party whole blood transfusions (1 ml) at day –2 and –1 to the BN donor on kidney graft function in the CsA treated (5 mg/kg i.m. inj.) WAG host (see Table 5.3).

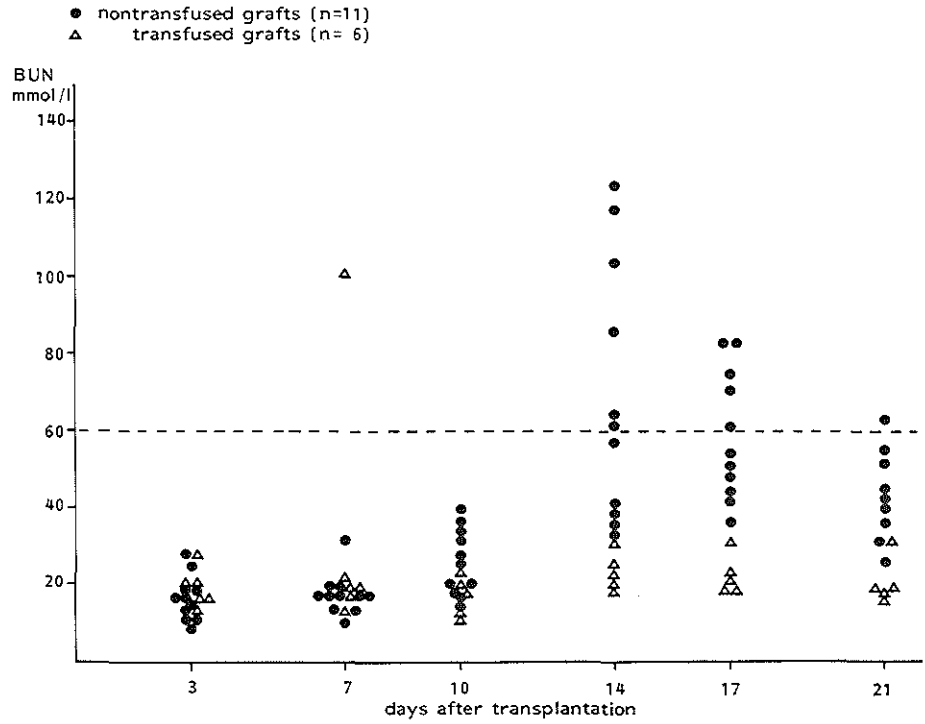


Figure 5.3. The effect of third party whole blood transfusions (3 ml) at day –2 and –1 to the BN donor on kidney graft function in the CsA treated (5 mg/kg i.m. inj.) WAG host (see Table 5.3).

Next it was tested whether CsA treatment of the host could facilitate the expression of the donor transfusion effect in the kidney transplantation model. It was found that two transfusions of 1 ml third party blood to the donor had no significant effect on renal function and graft survival (group 3 vs. group 4), however, when the transfused dose was tripled, a significant beneficial effect on graft function and survival could be noted (group 3 vs. group 5). From these results it can be concluded that there is a beneficial effect on kidney graft function and survival of third party transfusion to the donor in the CsA treated host like there is in the cardiac transplantation model (Heineman *et al.*, 1983b). Furthermore, it is demonstrated that there is a dose dependancy of this effect in this model. This is in contrast with our results in the heart transplantation model where we did not notice a difference in graft survival after different volumes of transfused third party blood. In those experiments however, we did not search for a critical minimum volume of third party blood required to elicit the phenomenon (Heineman *et al.*, 1983a; 1983b).

The mechanism responsible for the donor transfusion phenomenon is not clear. It has been suggested that the presence of cells of two origins in the graft at the time of transplantation, i.e. donor leukocytes (passenger leukocytes) and third party cells from the blood donor, may be responsible for an altered immunologic reactivity either in the graft or in the host (Frisk *et al.*, 1983; Jeekel *et al.*, 1983). We have suggested that there might be an interaction between leukocytes from the transfusate and the highly immunogenic Ia antigen carrying passenger cell population in the graft, leading to an altered immunogenicity of these cells (Heineman *et al.*, 1983b). (This is further discussed in chapter 9). If this hypothesis holds true than there might be a relation between the amount of transfused blood and the number of passenger leukocytes in the graft. Several authors have demonstrated that the BN kidney contains at least four times as many passenger leukocytes as the BN heart (van Schilfgaarde *et al.*, 1979; Bouwman, personal communication). Thus, assuming that transfused third party blood does in equal quantities reach the different organs and that there is indeed an interaction between third party blood derived cells and the passenger leukocytes in the graft, than it can be deduced that the kidney graft needs more third party blood than the cardiac graft to show the effect of donor transfusion. This is indeed what we observed and this finding lends support to the role of passenger leukocytes in the manifestation of the donor transfusion phenomenon.

5.5 Summary

In the present experiments the donor transfusion phenomenon was studied in a rat kidney allograft model. It was observed that a beneficial effect of third party blood transfusions to the donor on graft function and survival could be elicited under the condition that the recipient was moderately immunosuppressed with CsA. When the host was not treated no effect on graft function or survival could be

recorded. Furthermore, it was shown that the phenomenon was dose dependent. It is discussed that this finding lends support to the role of passenger leukocytes in the manifestation of the donor transfusion phenomenon, as the rat kidney contains at least four times as many passenger leukocytes as the rat heart.

CHAPTER 6

CHARACTERISTICS OF THE TRANSFUSATE RESPONSIBLE FOR THE DONOR TRANSFUSION PHENOMENON

This chapter was published in a modified form in Transplant. Proc. 1983; 15: 994 and Transplantation 1983; 36: 362.

6.1 Introduction

It has been described in the previous two chapters that third party whole blood transfusion of the donor does markedly influence cardiac graft and kidney graft survival in immunosuppressed recipients. When the recipient was pretreated with a donor-specific transfusion, which led, depending on the donor-host combination employed, to a shortened, prolonged or even permanent heart allograft survival, the effect of blood transfusions to the donor on graft survival was predominantly detrimental. When the recipient had been treated with a single injection of Cyclosporin A (CsA) on the day of operation, the effect of blood transfusions to the donor on heart and kidney graft survival was beneficial.

This chapter describes the characteristics of the transfusate required to elicit a donor transfusion effect in the heart transplantation model. More in particular the importance of the presence of leukocytes in the transfusate and of the degree of compatibility for MHC antigens between transfusate and host were studied.

6.2 Experimental protocol

Two different groups of experiments can be distinguished, as shown in Table 6.1.

In the first experimental group the importance of the composition of the third party transfusate was studied. The BN cardiac graft donors were transfused two days before grafting with either 1 ml fresh whole third party (Brofo) blood or with third party (Brofo) erythrocyte or leukocyte suspensions. The suspensions were prepared as described in chapter 3. The equivalent number of erythrocytes present in 1 ml of whole blood (5×10^9) was given intravenously (i.v.). The equivalent number of leukocytes present in 0.5-0.7 ml of whole blood (1 ml equals 10^7 leukocytes) was given i.v.. In one experimental group, donors received 1 ml fresh whole third party (Brofo) blood irradiated with 10 Gy just prior to transfusion. In the control group non transfused donors were used. The WAG recipients were injected i.v. with 1 ml of fresh whole donor (BN) blood 7 days before transplantation.

Table 6.1. Experimental protocol.

Experiment	Group	Donor/Host combination	Donor transfusion	Host treatment
I	1	BN/WAG	—	day -7, BN 1 ml
	2	BN/WAG	day -2, Brofo 1 ml	day -7, BN 1 ml
	3	BN/WAG	day -2, Brofo 1 ml irrad.	day -7, BN 1 ml
	4	BN/WAG	day -2, Brofo erythrocytes	day -7, BN 1 ml
	5	BN/WAG	day -2, Brofo leukocytes	day -7, BN 1 ml
IIa	1	BN/WAG	—	day -7, BN 1 ml
	2	BN/WAG	day -2, Brofo 1 ml	day -7, BN 1 ml
	3	BN/WAG	day -2, R1 1 ml	day -7, BN 1 ml
IIb	4	BN/LEW	—	day -7, BN 1 ml
	5	BN/LEW	day -2,-1, Brofo 1 ml	day -7, BN 1 ml
	6	BN/LEW	day -2,-1, R1 1 ml	day -7, BN 1 ml
IIc	1	BN/WAG	—	day -7, BN 1 ml
	2	BN/WAG	day -2, Brofo 1 ml	day -7, BN 1 ml
	7	BN/WAG	day -2, WAG 1 ml	day -7, BN 1 ml

In the second experimental group the effect of histocompatibility for class I and/or class II antigens between blood donor and graft recipient on the donor transfusion phenomenon in the heart transplantation model was studied.
Compatibility for class I and incompatibility for class II antigens between blood donor and host was studied in the BN/WAG donor-host combination where the BN donor received 1 ml of whole R1 blood two days before transplantation. The R1 strain is for class I antigens identical with the WAG strain (Vaessen *et al.*, 1979). The control animals consisted of a group of non transfused BN donors and of a group of third party (Brofo) transfused BN donors (Table 6.1 Experiment IIa).
Compatibility for class II and incompatibility for class I antigens between blood donor and host was studied in the BN/LEW donor-host combination where the BN donor received 1 ml of whole R1 blood two days and one day before transplantation. The R1 strain is for class II antigens identical with the LEW strain (Vaessen *et al.*, 1979). The control animals consisted of a group of non transfused BN-donors and of a group of third party (Brofo) transfused BN donors (Table 6.1 Experiment IIb).
Compatibility for both class I and class II antigens between blood donor and host was studied in the BN/WAG donor-host combination where the BN donor received 1 ml of whole WAG blood two days before transplantation. The control animals consisted of a group of non transfused BN donors and of a group of third party (Brofo) transfused BN donors (Table 6.1 Experiment IIc).

All recipients were injected intravenously with 1 ml of fresh whole donor (BN) blood 7 days before transplantation.

In all experiments cardiac function was assessed as evaluated in chapter 3.2.2.2. The results were analyzed using the chi square and Wilcoxon-rank sum tests.

6.3 Results

6.3.1 *The importance of the composition of the third party transfusate for the manifestation of the donor transfusion phenomenon*

The survival times observed in the first experimental group are shown in Table 6.2 and Figure 6.1.
It was found that grafts from nontransfused donors did survive indefinitely in recipients which were transfused with a donor-specific transfusion 7 days before grafting (group 1). These animals served as controls for the experimental groups 2-5. A single Brofo transfusion given to the donor 2 days before grafting significantly shortened graft survival. This transfusion led to a reduced graft survival in 73% of the transplantations (group 2, $p < 0.001$). The detrimental effect of a Brofo transfusion on graft survival was absent if irradiated (10 Gy) blood or purified erythrocytes were used. All grafts survived permanently (group 3 and 4). However, a Brofo leukocyte transfusion to the donor did lead to reduced graft survival in a small but significant number of cases. Here the grafts were rejected in 42% of the cases (group 5, $p < 0.01$).

6.3.2 *The importance of histocompatibility for class I and/or class II antigens between blood donor and graft recipient for the manifestation of the donor transfusion phenomenon*

The survival times observed in the second experimental group are shown in Table 6.3 and Figures 6.2, 6.3 and 6.4.
First the effect of donor transfusion with blood compatible for class I antigens with the WAG host was tested. It was found that the R1 blood transfusion did not influence graft survival in transfused recipients, whereas third party blood (Brofo) did significantly reduce graft survival (group 1 vs. group 3, $p > 0.05$; group 1 vs. group 2, $p < 0.001$) (Figure 6.2).
Second, the effect of donor transfusion with blood compatible for class II antigens with the LEW host was tested. It was found that the R1 blood transfusion did significantly reduce graft survival in transfused recipients (group 4 vs. group 6, $p < 0.05$). This effect was in the same order of magnitude as the effect observed in this model after third party blood (Brofo) transfusion (group 4 vs. group 5, $p < 0.05$) (Figure 6.3).
Finally the effect of donor transfusion with blood compatible for both class I and II antigens with the WAG host was tested. It was found that the WAG blood transfusion did not influence graft survival in transfused recipients, whereas third party blood (Brofo) transfusion did significantly reduce graft survival (group 1 vs. group 7, $p > 0.05$; group 1 vs. group 2, $p < 0.001$) (Figure 6.4).

Table 6.2. The effect of different composition of third party blood transfusion to the BN donor on heart allograft survival in transfused WAG hosts.

Group	Donor	Donor treatment	Recipient	Recipient treatment	Number	Survival in days
1	BN	—	WAG	day –7, BN 1 ml	25	permanent
2	BN	day –2, Brofo 1 ml	WAG	day –7, BN 1 ml	15	5,7,7,8,8,8,9,9,10,21,28, permanent 4×
3	BN	day –2, Brofo 1 ml irradiated (10 Gy)	WAG	day –7, BN 1 ml	6	permanent
4	BN	day –2, Brofo erythrocytes	WAG	day –7, BN 1 ml	5	permanent
5	BN	day –2, Brofo leukocytes	WAG	day –7, BN 1 ml	7	18,23,30, permanent 4×

The donors were transfused i.v. at day –2 with 1 ml of whole blood or irradiated (10 Gy) whole blood, or with erythrocyte or leukocyte suspensions. The recipients were transfused at day –7 with 1 ml of BN blood.

Table 6.3. The effect of histocompatibility for class I and/or class II antigens between blood donor and graft recipient on the donor transfusion phenomenon.

Group	Donor	Donor treatment	Recipient	Recipient treatment	Number	Survival in days
1	BN	—	WAG	day –7, BN 1 ml	25	permanent
2	BN	day –2, Brofo 1 ml	WAG	day –7, BN 1 ml	15	5,7,7,8,8,8,9,9,10,21,28, permanent 4×
3	BN	day –2, R1 1 ml (class I identic. class II differ.)	WAG	day –7, BN 1 ml	12	17,18, permanent 10×
4	BN	—	LEW	day –7, BN 1 ml	8	12,12,12,13,13,13,13,13
5	BN	day –2,–1, Brofo 1 ml	LEW	day –7, BN 1 ml	7	6,7,10,10,10,11,12
6	BN	day –2,–1, R1 1 ml (class I differ. class II identic.)	LEW	day –7, BN 1 ml	6	6,6,7,10,10,13
7	BN	day –2, WAG 1 ml (class I identic. class II identic.)	WAG	day –7, BN 1 ml	5	permanent

The donors were transfused i.v. at day –2 and/or –1 with 1 ml of whole blood which had different compatibility with the recipient. The recipients were transfused at day –7 with 1 ml of BN blood.

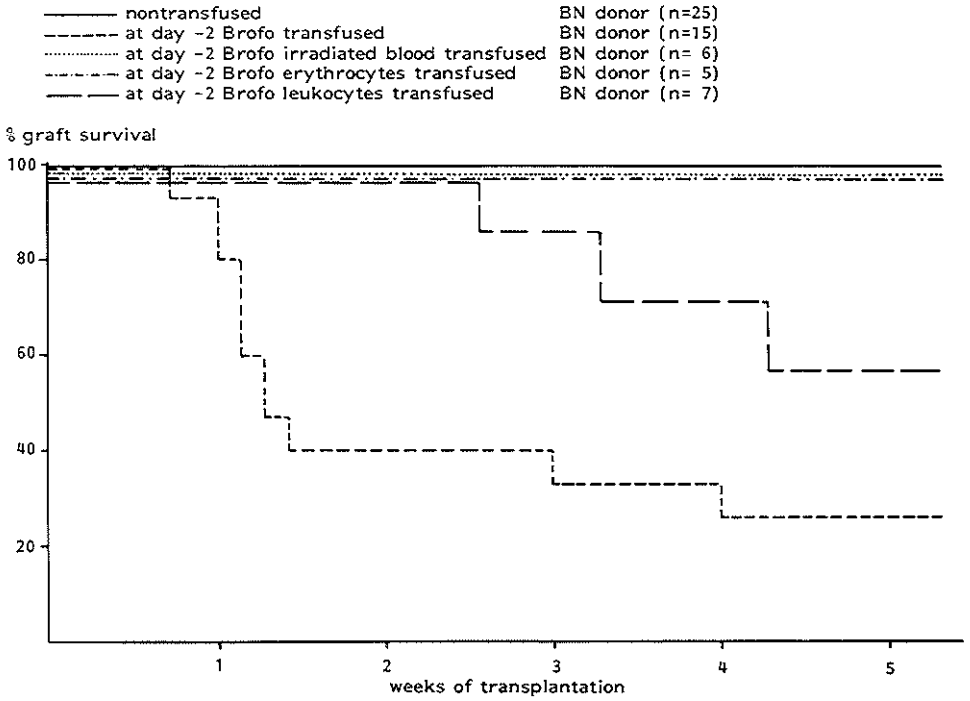


Figure 6.1. The effect of different composition of third party blood transfusion to the BN donor on heart allograft survival in transfused WAG hosts (see Table 6.2).

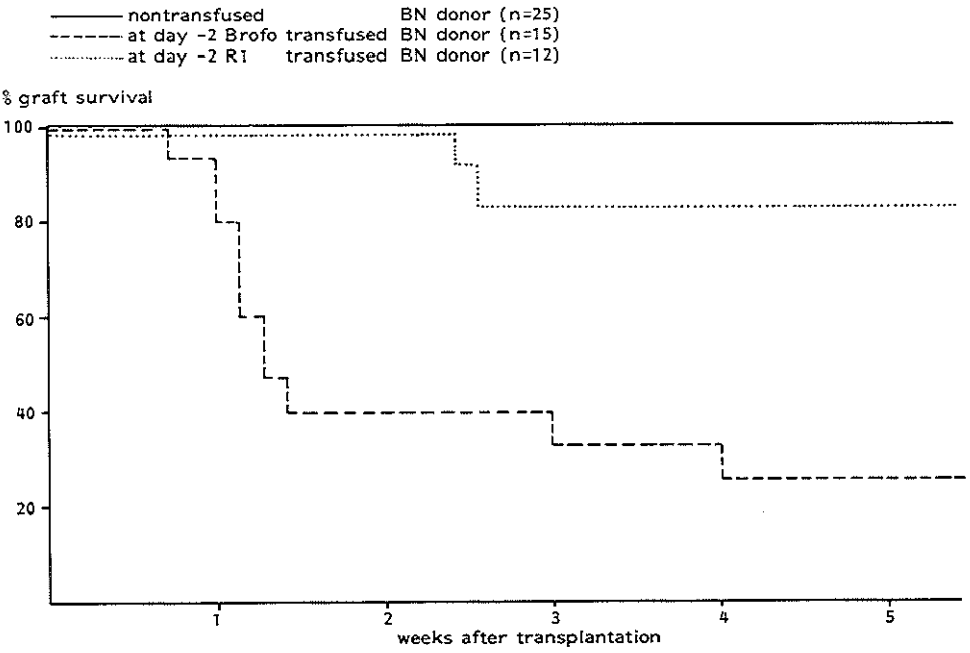


Figure 6.2. The effect of histocompatibility for class I antigens between R1 blood donor and WAG graft recipient on the donor transfusion phenomenon (see Table 6.3).

6.4 Discussion

This study shows that the donor transfusion phenomenon was manifest if viable third party leukocytes were part of the transfusate. Third party erythrocytes or irradiated third party blood did not have an effect on graft survival. For the induction of the phenomenon, mere third party antigen presentation was not sufficient; viable leukocytes appeared to be mandatory.

Furthermore it was observed that histoincompatibility for class I antigens between blood donor and graft recipient seemed to be of importance. The R1 and Brofo strains differ for class II antigens from the WAG strain, whereas only Brofo differs for class I antigens (Vaessen *et al.*, 1979). Brofo blood clearly induced the donor transfusion phenomenon, R1 blood did not. This is compatible with the fact that R1 blood did very significantly induce a transfusion effect when the host concerned a LEW rat, which only differs from the R1 for class I antigens (Vaessen *et al.*, 1979).

It appears from these experiments that viable leukocytes that differ from the host for class I antigens are responsible for the induction of the donor transfusion effect. It has been suggested that passenger leukocytes play a major role in the realization of the effect on graft survival (Frisk *et al.*, 1983; Jeekel *et al.*, 1983). It can be speculated that the third party derived leukocytes interact with the passenger leukocytes in such a way that the immunological reactivity of these cells and/or the host changes with subsequent consequences for graft survival. It should be noticed that class I difference between blood donor and graft recipient seemed to play a crucial role in this process, neither class II difference nor class I and class II compatibility showed the effect. Speculating further, it can be assumed that the passenger leukocyte, arriving with the graft in the recipient, presents to the recipient immune system all the antigens it has collected. However, only the presence of the "extra" third party class I antigens resulted in the described phenomenon. After its recognition by the recipient the route of immunization and subsequent graft rejection changed.

Concerning the "classical" transfusion effect, either donor-specific or third party, many studies have been done to determine the most effective composition of the transfusate. These analyses have produced conflicting results, although lymphocytes, erythrocytes, and platelets have all been shown to be effective in rodents (Marquet *et al.*, 1971; Jenkins and Woodruff, 1971; Fabre and Morris, 1972; Welsh *et al.*, 1977; Jeekel *et al.*, 1977; Heslop and Heslop, 1979; Lauchart *et al.*, 1980; Majoor and van Breda Vriesman, 1983; El-Malik *et al.*, 1984; Nagata *et al.*, 1984; Wood KJ *et al.*, 1985). In most of these studies the transfusion was only effective in influencing allograft survival if it was given at least a week before transplantation.

Concerning the genetics of the "classical" blood transfusion effect it has been demonstrated that the transfusion effect is MHC-specific and cannot be induced in every strain combination. Furthermore gene products of all three RT1 gene regions can be involved, but those of the RT1 B region (coding for class II antigens) are most prominent (Soulillou *et al.*, 1984).

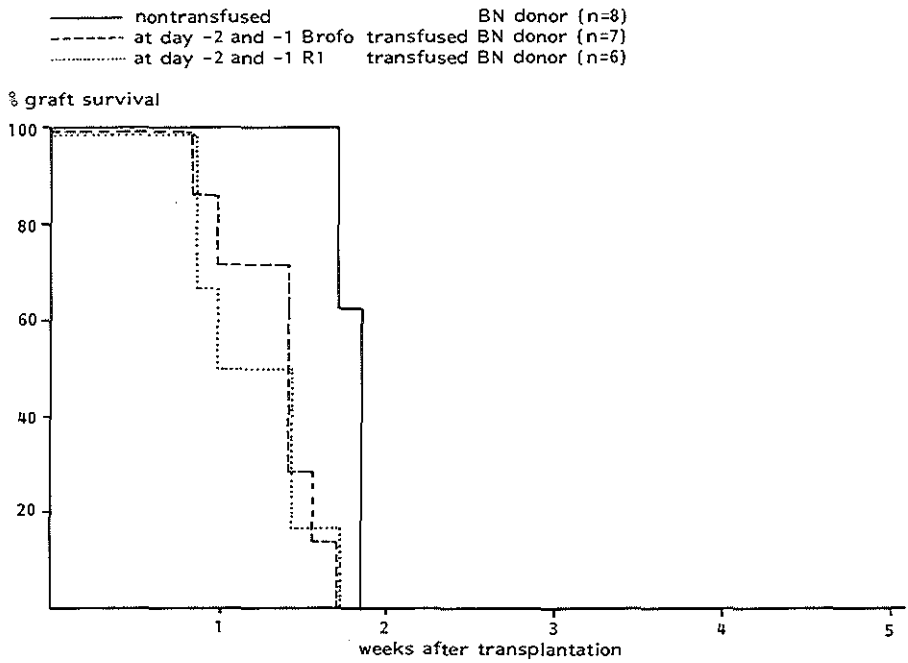


Figure 6.3. The effect of histocompatibility for class II antigens between R1 blood donor and LEW graft recipient on the donor transfusion phenomenon (see Table 6.3).

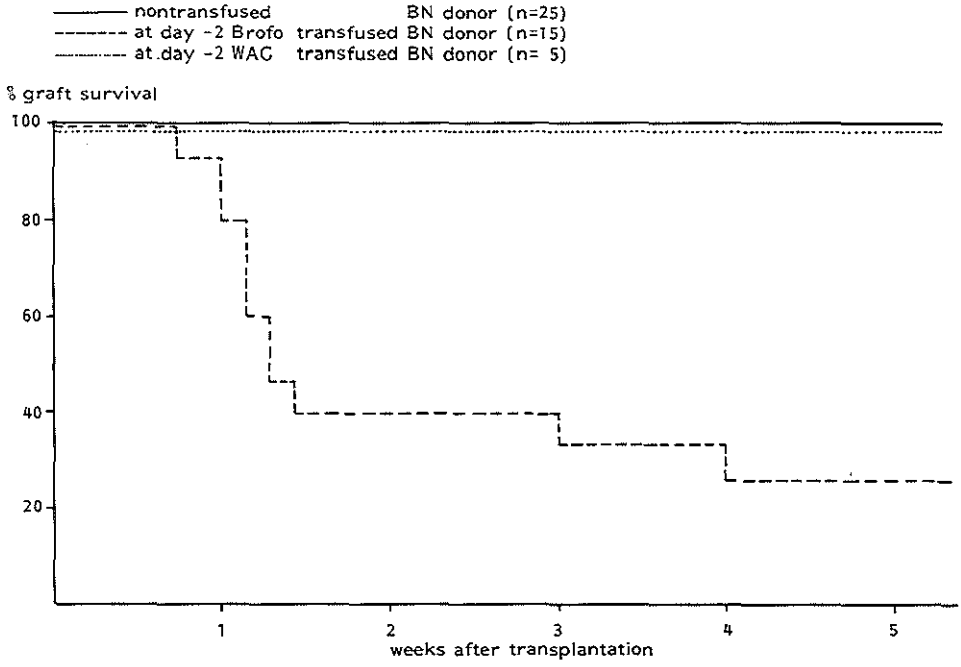


Figure 6.4. The effect of histocompatibility for class I and class II antigens between WAG blood donor and WAG graft recipient on the donor transfusion phenomenon (see Table 6.3).

7.2 Experimental protocol

Two different groups of experiments can be distinguished, as shown in Tables 7.1 and 7.2.

In the first experiment the effect on BN heart allograft survival of a peroperative transfusion of 1 ml of Brofo blood to the WAG recipient treated with Cyclosporin A (CsA) was studied. The recipients received 15 mg/kg bodyweight CsA intramuscularly after the operation on the day of transplantation.

Cardiac function was assessed as described in chapter 3.2.2.2. The results were analyzed using the Wilcoxon-rank sum test (Table 7.1 Experimental protocol I).

In the second group of experiments (WAG × LEW) F_1 hybrids were used to monitor in a PLN assay LEW-specific cell mediated immunity of WAG rats subjected to various treatments. The assay has been described in chapter 3.6.

First a dose response curve between popliteal lymph node weight and number of injected, normal, WAG spleen cells was obtained. Five WAG recipients were used. The F_1 hybrids received 2-60 × 10^5 spleen cells in both hind foot-pads.

Thereafter it was tested whether intravenous injection of LEW antigen into WAG recipients resulted in measurable "immunological memory". Five WAG recipients were injected with 1 ml of LEW blood. After 7 days the spleens were harvested. The F_1 hybrids received 5-20 × 10^5 spleen cells in both hind foot-pads (Table 7.2 Experiment IIa).

In the crucial experiments it was studied whether donor transfusion would lead to a specific immune response in the recipient. BN donors were transfused two days before heterotopic heart transplantation with 1 ml Brofo or LEW blood. WAG recipients were transfused with 1 ml BN blood on day −7. The spleens of the recipients (3 animals per group) were harvested 30 days after BN heart transplantation, thus in a situation where donor transfusion had not evoked abrogation of the donor specific transfusion effect (see chapter 4.3.2). The F_1 hybrids received 10-50 × 10^5 spleen cells in both hind foot-pads (Table 7.2 Experiment IIb).

In all these experiments two F_1 hybrids were used per dosis injected spleen cells. The significance of differences in node weights were analyzed by using the Wilcoxon-rank sum test.

7.3 Results

7.3.1 *The effect of a peroperative third party blood transfusion on BN heart allograft survival in WAG hosts treated with Cyclosporin A (CsA)*

The survival times observed in this experiment are shown in Table 7.3 and Figure 7.1. No significant difference was found between survival times achieved in non-transfused and peroperatively transfused recipients. The majority of the grafts was rejected after 9-15 days (group 1 and 2, $p > 0.05$).

Table 7.1. Experimental protocol I.

Experiment	Group	Donor/Host combination	Donor transfusion	Host treatment
I	1	BN/WAG	—	day 0, CsA 15 mg/kg
	2	BN/WAG	—	day 0, CsA 15 mg/kg day 0, Brofo 1 ml

Tabel 7.2. Experimental protocol II.

Experiment	Group	Spleen Cell Donor	Treatment Spleen Cell Donor	Number of cells ($\times 10^5$) injected in (WAG × LEW)F_1 hybrids
IIa	1	WAG	—	2,5,10,15,20,30,60
	2	WAG	day −7, LEW 1 ml	5, 10, 20
IIb	3	WAG	day −37, BN 1 ml day −30, BN heart pre-treated with Brofo 1 ml	10, 20, 50
	4	WAG	day −37, BN 1 ml day −30, BN heart pre-treated with LEW 1 ml	10, 20, 50

7.3.2 *The effect of transfusion of the donor on specific immune responsiveness of the recipient*

The dose response curves obtained with normal WAG spleen cells and WAG spleen cells from transfused animals are depicted in Figure 7.2. The corresponding lymph node weights and number of injected spleen cells are shown in Table 7.4.

It was found that there was a clear dose relationship between the number of injected normal WAG spleen cells or WAG spleen cells from transfused animals and the degree of popliteal node enlargement in (WAG × LEW) F_1 hybrids. Furthermore it was observed that intravenous injection of third party antigen (LEW transfusion) led to a significant reduced specific immune competence.

The dose response curves obtained with spleen cells from WAG rats bearing heart allografts from donors which were either Brofo or LEW transfused are depicted in Figure 7.3.

The corresponding lymph node weights and number of injected spleen cells are shown in Table 7.5.

It was found that again there was a clear dose response relationship between the number of injected WAG spleen cells from the two experimental groups and the degree of popliteal node enlargement in (WAG × LEW)F_1 hybrids. Furthermore, it was noticed that transfer of LEW antigen via the donor to the recipient led to immunological "memory" in the recipient as reflected by a reduced specific immune compentence to LEW antigens in the recipient.

Table 7.3. The effect of a peroperative third party transfusion on BN heart allograft survival in the Cyclosporin A (CsA) treated WAG host.

Group	Donor	Donor treatment	Recipient	Recipient treatment	Number	Survival in days
1	BN	—	WAG	day 0, CsA 15 mg/kg	12	9,10,11,12,13,13,13,14,14, 14,15,15
2	BN	—	WAG	day 0, CsA 15 mg/kg day 0, Brofo 1 ml	7	10,11,12,13,13,17,19

Third party blood (Brofo) transfusions of 1 ml were given intravenously peroperative to the WAG recipients. The recipients were injected on the day of grafting intramuscularly in the hind leg with Cyclosporin A (CsA) in a dose of 15 mg/kg body weight in a volume of 0.1 ml.

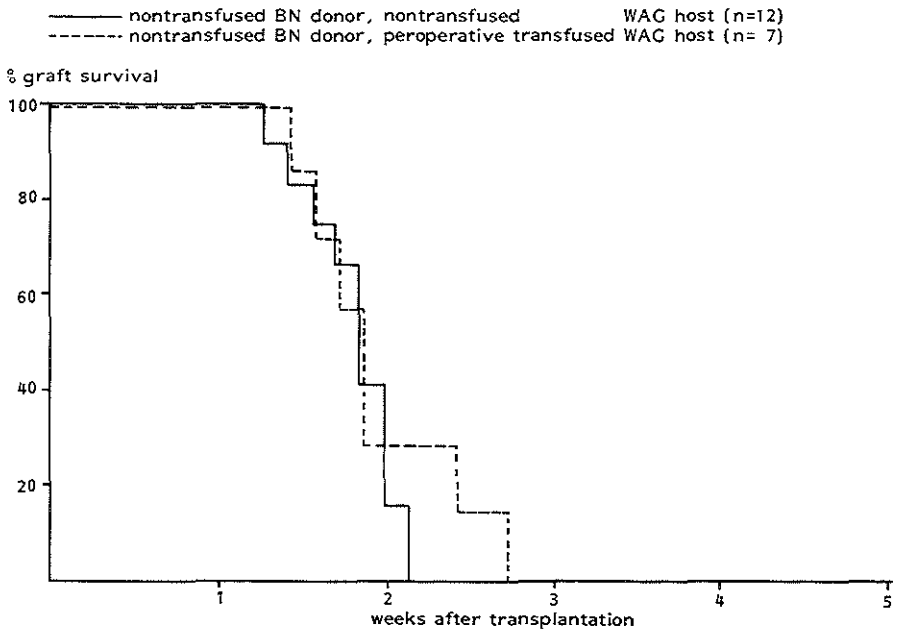


Figure 7.1. The effect on BN heart allograft survival of a peroperative third party (Brofo) transfusion in the Cyclosporin A (CsA) treated WAG recipient (see Table 7.3).

7.4 Discussion

The results obtained in this study show that a peroperative third party transfusion has no enhancing effect on graft survival when the recipient has been treated with CsA, whereas donor transfusion clearly resulted in a significant beneficial effect in the same model (Heineman *et al.*, 1983b). Thus, the suggestion of Berg *et al.* (1982) that transplantation of a graft from a transfused donor might represent an additional (peroperative) transfusion of random donor leukocytes, resulting in a beneficial effect on graft survival, does not seem to be the explanation for the donor transfusion phenomenon.

In the second part of the study it was tested whether transfusion of the donor leads to a specific immune response in the recipient which might be held responsible for the observed effect of donor transfusion. The test employed was a local graft versus host (GvH) assay, called the popliteal lymph node (PLN) assay, described by Ford *et al.* (1970). The basis of this regional lymph node assay is the finding that parental spleen cells injected into the hind footpad of F_1 hybrids induce a GvH reaction in the draining popliteal lymph node, leading to an increase in weight as well as cellularity. The enlargement of the popliteal node, which peaks at 5-7 days after inoculation, is related to the number of parental cells injected and can be quantified by simple weighing. The main immunological attack is exhibited by the

Table 7.4. Popliteal lymph node weights in (WAG × LEW)F_1 hybrids after injection of different dosages of normal WAG spleen cells and WAG spleen cells after a LEW blood transfusion.

Number of injected cells ($\times 10^5$)	mean lymph node weight (mg ± SD)	
	Non-transfused	LEW-transfused
2	7.25 ± 0.9	
5	8.0 ± 0.7	5.5 ± 0.6
10	11.8 ± 1.5	9.5 ± 0.6
15	13.0 ± 2.0	
20	15.0 ± 2.7	11.5 ± 3.0
30	18.0 ± 0.8	
60	22.0 ± 2.8	

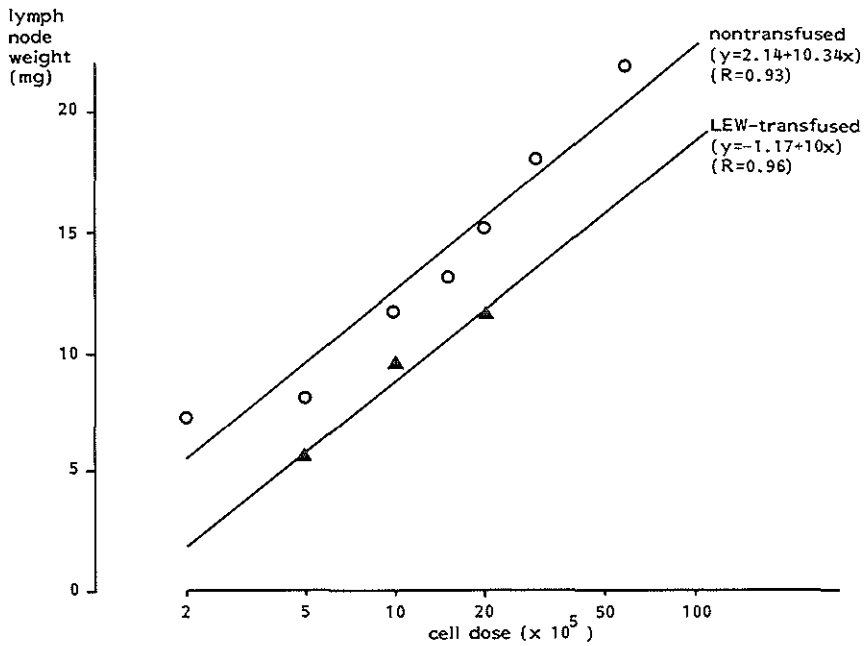


Figure 7.2. Relation between the number of normal WAG spleen cells and LEW-transfused WAG spleen cells and the degree of popliteal node enlargement in (WAG×LEW)F_1 hybrids (see Table 7.4).

injected donor T cells against the histocompatibility antigens of the host. This local GvH assay can be used to quantify specific cell mediated immunity, if the relevant parental strain and F_1 hybrids are used. States of sensitization as well as immunosuppression can be monitored in a relatively simple manner.

In the present experiments (WAG × LEW) F_1 hybrids were used to monitor LEW-specific cell mediated immunity of WAG rats subjected to various treatments and allografting. Spleen cells of the animals to be tested were injected into the hind footpads of F_1 rats.

Table 7.5. Popliteal lymph node weights in (WAG × LEW)F_1 hybrids after injection of different dosages of spleen cells obtained from WAG rats bearing either Brofo- or LEW-transfused BN heart allografts.

Number of injected cells ($\times 10^5$)	mean lymph node weight (mg ± SD)	
	Brofo-transfused graft	LEW-transfused graft
10	10.75 ± 0.95	8.0 ± 0.8
20	14.0 ± 1.63	10.75 ± 1.25
50	18.75 ± 1.50	15.0 ± 2.5

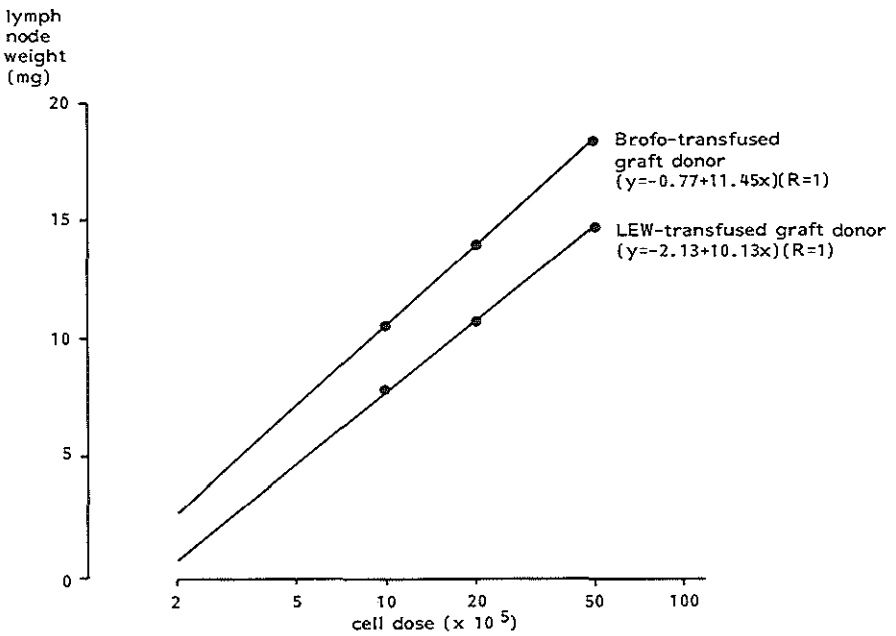


Figure 7.3. Relation between the number of spleen cells obtained from WAG rats bearing either Brofo or LEW transfused BN heart allografts and the degree of popliteal node enlargement in (WAG×LEW)F_1 hybrids (see Table 7.5).

The results showed that the assay is indeed suitable to monitor WAG-anti-LEW immunity and that intravenous injection of LEW antigen leads to a significantly reduced specific immune competence. Furthermore, it was found that transfer of relevant antigens (LEW) via donor to recipient leads to a significantly reduced specific immune competence as compared to the results obtained after transfer of irrelevant antigen (Brofo). These results convincingly show that the WAG recipient has a "memory" for the third party transfusion given to the graft donor. However, the transfer of antigen via the graft, resulting in this "memory" in the recipient, can not be hold responsible for the donor transfusion phenomenon, as the recipients employed did not show the donor transfusion effect (the grafts were not rejected).

In conclusion, it was found that transfusion of the donor leads to a specific immune response in the recipient, which is not responsible for the donor transfusion phenomenon. This finding is corroborated by the results in the cardiac transplantation study where peroperative transfusion did not enhance graft survival in the model employed.

7.5 Summary

In the first part of the present experiments it was studied in rats whether a peroperative transfusion with third party blood did influence heart allograft survival in a model in which donor transfusion clearly showed to be effective. It was found that this was not the case. In the second part it was tested in rats in a popliteal lymph node (PLN) assay whether transfusion of the donor leads to a specific immune response in the recipient. It was observed that transfer of third party antigen via the donor to the recipient led to a reduced specific immune competence, however it was also noticed that this tranfer of antigen via the graft was not responsible for the donor transfusion phenomenon.

CHAPTER 8

THE ROLE OF PASSENGER LEUKOCYTES IN THE MANIFESTATION OF THE DONOR-SPECIFIC TRANSFUSION (DST) PHENOMENON

This chapter has been published in Transplant. Proc. 1985; 17: 821.

8.1 Introduction

The beneficial effect of third party and donor-specific blood transfusions on organ graft survival has been confirmed in numerous studies in animals and man (Marquet *et al.*, 1971; van Es *et al.*, 1977; Opelz and Terasaki, 1980; Salvatierra *et al.*, 1980).

The mechanism underlying the transfusion phenomenon is not yet clarified. Evidence for the involvement of suppressor cells in prolonged survival by donor-specific transfusions (DST) was obtained (Marquet and Heystek, 1981; Marquet *et al.*, 1982). Unresponsive recipients with long-term surviving hearts did develop T-suppressor cells which could upon adoptive transfer, impose the unresponsiveness to naive WAG hosts. Furthermore, retransplantation of long-surviving BN hearts to normal WAG recipients resulted in permanent survival in the secondary hosts. These findings suggested that the unresponsive state is maintained by suppressor cells and graft modification. This hypothesis was tested and found valid (Marquet *et al.*, 1985).

A different approach to improve graft survival is to reduce the immunogenicity of the graft. In several earlier and recent studies in animals it has been shown that a major component of the sensitizing stimulus for allograft rejection is the presence and release from the parenchyma of bone-marrow derived cells, the so called passenger leukocytes or dendritic cells (Steinmuller, 1967; Elkins and Guttmann, 1968; Guttmann and Lindquist 1969; Steinmuller and Hart, 1971; Hart and Fabre, 1981c, Leichler and Bachelor, 1982). Attempts to eliminate these antigen-presenting cells by treatment of the donor with irradiation, antilymphocyte globulin or cytotoxic drugs (Guttmann and Lindquist, 1969; Freeman *et al.*, 1971; Steinmuller *et al.*, 1971; Stuart *et al.*, 1971a; 1971b; Zincke and Woods, 1974) as well as by in vitro culture (Lafferty *et al.*, 1975; Naji *et al.*, 1979; Lacy *et al.*, 1982) have resulted in prolongation of allograft survival in animals. The clinical results of donor pretreatment are, however, inconclusive (Guttmann *et al.*, 1975; Dienst, 1977; Zincke *et al.*, 1978; Jeffery *et al.*, 1978; Guttmann *et al.*, 1980).

Marquet *et al.*, (1981) found that in the BN to WAG rat donor/host combination modification of immunogenicity of the cardiac graft by treatment of the donor with cyclophosphamide interfered with the enhancing effect of donor-specific blood

transfusions. It was assumed that the reduced immunogenicity of the graft causes an insufficient triggering of the mechanisms of active immunologic enhancement. However, they could not exclude the alternative hypothesis that donor drug-pretreatment leads to transfer of a small amount of drug to the host thus interfering with the response of host lymphocytes (Lafferty *et al.*, 1975; van de Linden *et al.*, 1981a). The effect of the residual drug on the immune capacity of the host would then account for the observed phenomenon.

The aim of the present study was to investigate whether passenger leukocytes play an important role in the donor-specific transfusion phenomenon. The possible role of residual drug after donor pretreatment was excluded by investigating whether modification of the immunogenicity of the graft by irradiation would have the same effect. The passenger leukocyte population was irradiated at a dose (10 Gy) known to abrogate leukocyte proliferation capacities, without interference with their immunogenic properties (Elves, 1969). The functional properties of the passenger leukocytes were modulated at different periods before transplantation to differentiate between the immunogenic properties and proliferative function of these cells.

The results show that reduction of graft immunogenicity (absence of passenger leukocytes) can indeed diminish the favourable effect of DST. We suggest that the dendritic cells in the graft contribute to the induction of specific unresponsiveness as initiated by the donor-specific transfusion to the host.

8.2 Experimental Protocol

Two different groups of experiments can be distinguished as shown in Table 8.1. In the first experimental group the effect of irradiation of the donor with 10 Gy 5 days before grafting on the DST phenomenon was investigated. In the second experimental group the effect of irradiation of the donor with 10 Gy on the day of grafting on the DST phenomenon was investigated. In all experiments one blood transfusion to the host consisted of 1 ml fresh pooled heparinized whole blood. Cardiac function was assessed as evaluated in chapter 3.2.2.2. The results were analyzed using the chi-square test.

8.3 Results

The survival times of the various experimental groups are presented in Table 8.2 and Figures 8.1 and 8.2.

In the first group of experiments it is shown that a single BN blood transfusion of 1 ml given to WAG hosts 7 days before transplantation led to permanent acceptance of BN heart allografts (group 2). Control animals rejected their grafts in 8-9 days (group 1). Pretreatment of the donor with irradiation 5 days before heart transplantation resulted in a slight, but significant prolongation of graft survival in unconditioned hosts. The grafts were rejected in 8-11 days (group 3, $p < 0.05$). If

Table 8.1. Experimental protocol.

Experiment	Group	Donor/Host combination	Donor treatment	Host treatment
I	1	BN/WAG	—	—
	2	BN/WAG	—	day −7, BN 1 ml
	3	BN/WAG	day −5, irradiated	—
	4	BN/WAG	day −5, irradiated	day −7, BN 1 ml
II	1	BN/WAG	—	—
	2	BN/WAG	—	day −7, BN 1 ml
	5	BN/WAG	day 0, irradiated	—
	6	BN/WAG	day 0, irradiated	day −7, BN 1 ml

donor pretreatment with irradiation, 5 days before transplantation and transfusion of the recipient with 1 ml of BN blood were combined, BN heart allografts were rejected in 5, 10, 15, 15 and 29 days. Three grafts were accepted permanently. Thus, in more than 60% of the cases the beneficial effect of DST was abrogated ($p < 0.05$, group 4) (see Table 8.2 and Figure 8.1).

In the second group of experiments it is shown that pretreatment of the donor with irradiation on the day of grafting led to graft survival in the unconditioned hosts similar to the survival in the control animals (group 1 and group 5). If donor pretreatment with irradiation on the day of grafting and DST were combined there was no significant influence on the beneficial transfusion effect. Almost all grafts survived permanently (group 2 and group 6) (see Table 8.2 and Figure 8.2).

8.4 Discussion

The results show that pretreatment of the donor with irradiation (10 Gy) 5 days before transplantation leads to a significant dimunition of the DST effect. However, irradiation of the donor on the day of transplantation did not abrogate the beneficial effect of DST.

Studies in a variety of experimental models have demonstrated that leukocytes carried in transplanted organs (passenger leukocytes or dendritic cells) play an important role in recipient sensitization (Steinmuller, 1967; Elkins and Guttmann, 1968; Guttmann and Lindquist, 1969; Steinmuller and Hart, 1971; Hart and Fabre, 1981c; Lechler and Bachelor, 1982). These cells are Ia positive and according to Hart and Fabre (Hart and Fabre, 1981a; 1981b) are the only site carrying class II antigens in the rat heart. The development of anti-Ia monoclonal antibodies has created the possibility to visualise these cells and they have been demonstrated in the heart (Hart and Fabre, 1981c; Bouwman *et al.*, 1985). It has been demonstrated that 24 hr after treatment with 10 Gy the number of dendritic cells in rat hearts were normal (Hart and Fabre, 1981c; Bouwman *et al.*, 1985). The numbers then declined rapidly, although some dendritic cells still were present at 3 days after irradiation. By day 5 few or no dendritic cells could be seen. The radiation dose employed (10

Table 8.2. The effect of irradiation of the BN donor at different intervals before transplantation on heart allograft survival in the donor-specific transfused WAG recipient.

Group	Donor	Donor treatment	Recipient	Recipient treatment	Number	Survival in days
1	BN	—	WAG	—	10	8,8,8,8,8,8,8,8,9,9
2	BN	—	WAG	day −7, BN 1 ml	25	permanent
3	BN	day −5, irradiated	WAG	—	10	8,9,9,9,10,10,10,11,11,11
4	BN	day −5, irradiated	WAG	day −7, BN 1 ml	8	5,10,15,15,29, permanent 3×
5	BN	day 0, irradiated	WAG	—	5	8,8,9,10,11
6	BN	day 0, irradiated	WAG	day −7, BN 1 ml	8	12, permanent 7×

The donors were irradiated (10 Gy) at day −5 or 0 before transplantation. The recipients were transfused at day −7 with 1 ml of BN blood.

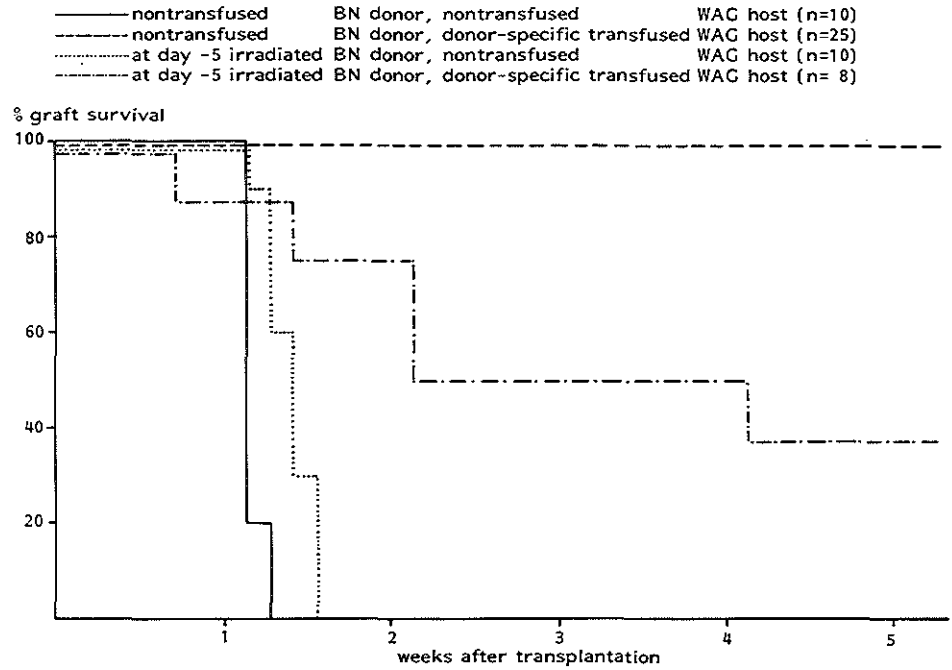


Figure 8.1. The effect of irradiation at day -5 of the BN donor on heart allograft survival in the donor-specific transfused WAG recipient (see Table 8.2).

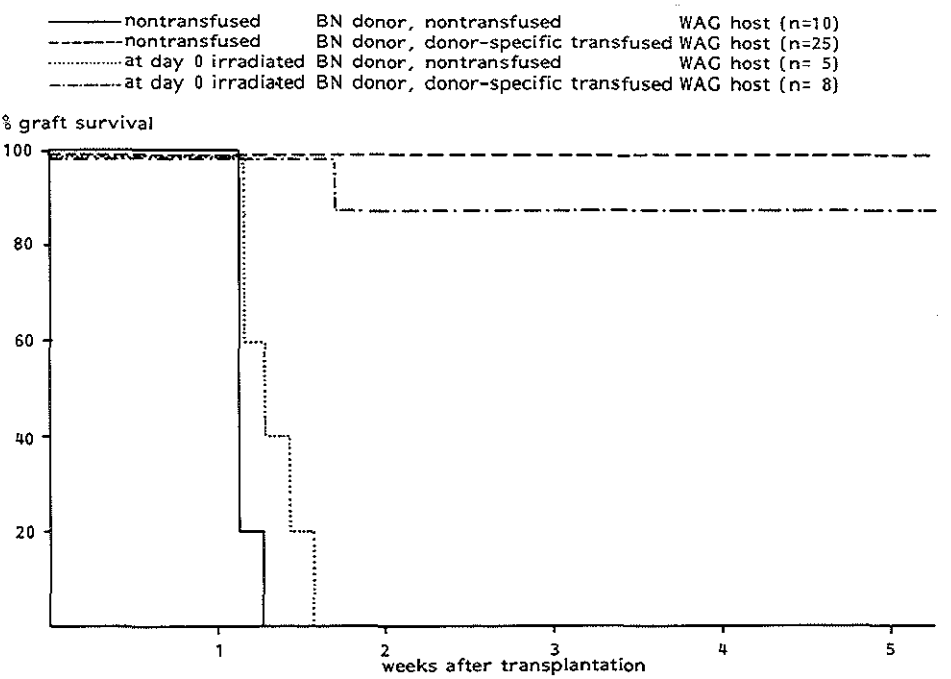


Figure 8.2. The effect of irradiation at day 0 of the donor on heart allograft survival in the donor-specific transfused WAG recipient (see Table 8.2).

Gy), is known to abrogate leukocyte proliferative capacities, without interfering with their immunogenic properties (Elves, 1969). The different timing of irradiation in the current study made it possible to differentiate between the presence of the passenger leukocytes and the role of the proliferative capacity of these cells for the manifestation of the beneficial DST effect.

Irradiation of the donor 5 days before grafting, thus virtually depleting the donor of its dendritic cell population, led to a moderate prolongation of graft survival in the unconditioned host. This reduction of the immunogenicity of the graft did diminish the effect of a donor-specific transfusion in the conditioned host. It can be assumed that the Ia-antigen bearing dendritic cells are necessary for the triggering of the specific unresponsiveness initiated by transfusion of the host with donor blood. This assumption is further corroborated by the finding that irradiation of the donor on the day of grafting, when Ia antigen is still present (Hart and Fabre, 1981c; Bouwman *et al.*, 1985) does not abrogate the effect of a donor specific transfusion. Almost all transfused recipients survived permanently.

Very recently we confirmed these results when we used the OX-6 monoclonal antibody (OX-6 reacts with Ia antigens which are abundantly expressed on dendritic cells). Donor hearts were perfused and incubated (30 minutes) in the OX-6 monoclonal antibody and subsequently washed directly prior to transplantation into DST treated recipients. It was found that all hearts were rejected (Bouwman *et al.*, 1985).

Although it has been demonstrated that enhancement of allografts with antibodies directed against class I associated antigen is possible (Jeekel *et al.*, 1977), there is abundant evidence that passive transfer of alloantibodies directed against donor class II antigens can cause the enhancement of rat heart (Davies and Alkins, 1974), rat kidney (Soulillou *et al.*, 1976; Catto *et al.*, 1977), and mouse skin (Archer *et al.*, 1974; Staines *et al.*, 1974) allografts. Strom *et al.*, (1977) have suggested that active enhancement of organ allografts may be achieved by transferring donor lymphoid cells to the recipient prior to transplantation. They tested the hypothesis that enhancement requires the development of anti-class II immunity. Their experimental data support the concept that enhancement states require active or passive immunity against donor class II MHC antigens at the time of transplantation (Strom *et al.*, 1977). Batchelor *et al.* (1979) and Hart *et al.* (1980) have reported that recipients of long-term surviving allografts behave very similarly in terms of graft survival, antibody response, and histology of rejection to passively enhanced recipients of fresh allografts. These authors raised the possibility that the interstitial dendritic cell might be the major target for passive enhancement. They hypothesized that the immune response to a long-term surviving allograft, being devoid of dendritic cells (Batchelor *et al.*, 1979; Hart *et al.*, 1980), should not be susceptible to further suppression by passive enhancement. Indeed Hart and Fabre (1982) presented data showing evidence for an interaction of enhancing antibody with donor interstitial dendritic cells during the induction phase of passive enhancement. They proposed that passive enhancement, donor pretreatment and Ia matching might operate by the same final mechanism. Thus, it can be assumed

that matching for class II antigens or killing of class II antigen-carrying-cells results in a diminishment of target for the specific unresponsiveness inducing anti-class II antibodies.

Experimental data suggest that this is indeed the case (van Es and Balner, 1979; Bijnen *et al.*, 1982). In a kidney transplantation model in the rhesus monkey van Es and Balner (1979) found that matching for D/DR antigens interfered with the beneficial effect of blood transfusion on kidney graft survival. They speculated that because of sharing of DR antigens, the trigger for suppressor cell induction was absent. Bijnen *et al.* (1982) have confirmed these results in a kidney transplantation model in the dog. They observed that the beneficial effect of blood transfusion is dependent on the degree of matching and suggested that while blood transfusions are on the whole beneficial for unmatched kidneys, they are likely to have no effect, and even to be harmful, for matched kidneys. In the present experiments we too found that class II antigen-carrying cells of the graft, the passenger leukocytes, contribute to the induction of enhancement by DST in the experimental model employed. In clinical transplantation however, most authors have reported that the positive effect of HLA-DR matching on cadaver graft survival is independent of the recipient having received pretransplant blood transfusion or not (Ting and Morris, 1980; Albrechtsen *et al.*, 1981; Goeken *et al.*, 1982; Moen *et al.*, 1983). Only Persijn *et al.* (1981) found that matching for DR antigens gives a less pronounced effect of a blood transfusion to the recipient. They suggest that the effect of pretransplant blood transfusions might be more outspoken in the group of patients who receive a cadaveric graft with two HLA-DR mismatches.

Finally our data confirm those obtained by v.d. Linden *et al.* (1981b) who showed in a kidney transplantation model in the dog that preoperative transfusions cannot successfully be combined with donor pretreatment as commonly practised in man. These findings are a possible explanation for the conflicting data on donor pretreatment in man (Guttmann *et al.*, 1975; Dienst, 1977; Zincke *et al.*, 1978; Jeffery *et al.*, 1978; Guttmann *et al.*, 1980). The clinical data obtained by donor pretreatment should be re-evaluated taking in account the differences in blood transfusion policy between the various centres.

8.5 Summary

It was observed in rats that pretreatment of the donor with irradiation (10 Gy) 5 days before transplantation led to a significant dimunition of the donor specific transfusion (DST) effect, whereas irradiation of the donor on the day of transplantation did not. From these experiments it is concluded that reduction of the immunogenicity of the graft by dendritic cell elimination (irradiation) can diminish the beneficial effect of DST. It is suggested that the dendritic cells in the graft contribute to the induction of specific unresponsiveness as initiated by the DST to the host.

CHAPTER 9

THE ROLE OF PASSENGER LEUKOCYTES IN THE MODIFICATION OF ALLOGRAFT SURVIVAL IN RATS BY BLOOD TRANSFUSION TO THE DONOR

This chapter has been accepted for publication in Transplant. Proc.

9.1 Introduction

It has been reported in rats that blood transfusion(s) given to the donor can markedly influence cardiac and kidney allograft survival if the immune response of the recipient has been modulated (Jeekel *et al.*, 1982; Heineman *et al.*, 1983a, 1983b). When the recipient was pretreated with a donor-specific transfusion, which leads depending on the donor-host combination employed, to a shortened, prolonged or even permanent graft survival, the effect of blood transfusions to the donor on cardiac graft survival was predominantly detrimental. When the cardiac or kidney allograft recipient had been treated with a single injection of respectively 15 mg/kg CsA or 5 mg/kg CsA on the day of operation, the effect of blood transfusions to the donor was beneficial.

We obtained evidence that third party derived leukocytes are responsible for the induction of this donor transfusion phenomenon (Heineman *et al.*, 1983a, 1983b). Previous work from other authors has demonstrated that these transfused allogeneic leukocytes home in small quantities in the graft (van Schilfgaarde *et al.*, 1979). Based on these studies our hypothesis was that the mechanism responsible for the donor transfusion effect might be an interaction between leukocytes from the transfusate and the passenger leukocytes in the graft, leading to altered immunogenicity of these cells.

In the present study attempts were made to substantiate the role of the passenger leukocytes. In the BN to WAG heart transplantation model the functional properties of the passenger leukocytes were modulated by irradiation at a dose of 10 Gy known to abrogate the proliferative capacities of lymphocytes without interfering with their immunogenic properties (Elves, 1969). The functional properties of the passenger leukocytes were modulated at different periods before transplantation to differentiate between the immunogenic properties and proliferative function of these cells.

The results obtained indicate that the beneficial effect of blood transfusions to the donor on graft survival in the model employed, can be abrogated by irradiation of the donor before transplantation. The data suggest a vital role of passenger leukocytes in the manifestation of the donor transfusion phenomenon.

9.2 Experimental protocol

Two different groups of experiments can be distinguished as shown in Table 9.1.

In the first experimental group the effect of irradiation and third party transfusion of the BN donor on heart allograft survival in the unmodified WAG recipient was investigated. The donors were transfused intravenously at different intervals (day –1 and/or –2) with 1 ml of whole Brofo blood and/or irradiated with 10 Gy whole body radiation at day –5.

In the second experimental group the effect of irradiation at different intervals before transplantation and third party transfusion of the BN donor on heart allograft survival in the Cyclosporin A (CsA) treated WAG host was studied. The donors were transfused intravenously at different intervals (day –1 and –2) with 1 ml of whole Brofo blood and/or irradiated with 10 Gy whole body radiation at day –5 or 0. The recipients were injected on the day of grafting intramuscularly in the leg with CsA in a dose of 15 mg/kg body weight. Cardiac function was assessed as evaluated in chapter 3.2.2.2. Statistical analysis of the data was performed by using the Wilcoxon-rank sum test.

9.3 Results

The survival times of the various experimental groups are presented in Tables 9.2 and 9.3 and Figures 9.1, 9.2 and 9.3.

In the first group of experiments the effect of irradiation and third party transfusion of the donor on heart allograft survival in the unmodified recipient was studied. It was found that a single Brofo transfusion to the donor at 2 days before transplantation had no effect on graft survival if the recipient was not immunosuppressed. Similarly as in the control animals, the hearts were rejected in 8-9 days (group 1 and 2).

In the subsequent two experimental groups the donors were irradiated 5 days before grafting. This irradiation led to a moderate, but significant prolongation of graft survival in 60% of the cases. Grafts were rejected in 8-11 days (group 3, $p < 0.05$).

Third party transfusions given to the donor at 2 days and 1 day before grafting in combination with irradiation at 5 days before grafting did lead to graft survival times ranging from 8-12 days (group 4). Graft survival was extended in 60% of the cases as compared to the non-irradiated transfused controls (group 4 vs. group 2: $p < 0.05$), however there was no further prolongation of graft survival as compared to the irradiated non-transfused animals (group 4 vs. group 3: $p > 0.05$) (see Table 9.2 and Figure 9.1).

In the second group of experiments the effect of irradiation before or after donor transfusion on heart allograft survival in rats treated postoperatively with CsA was studied. When using an unmodified donor, a single injection of 15 mg/kg CsA led to graft survival times ranging from 9-15 days (group 5). This group forms the control group.

Table 9.1. Experimental protocol.

Experiment	Group	Donor/Host combination	Donor treatment	Host treatment
I	1	BN/WAG	—	—
	2	BN/WAG	day -2, Brofo 1 ml	—
	3	BN/WAG	day -5, irradiated	—
	4	BN/WAG	day -5, irradiated day -2,-1, Brofo 1 ml	—
II	5	BN/WAG	—	day 0, CsA 15 mg/kg
	6	BN/WAG	day -2,-1, Brofo 1 ml	day 0, CsA 15 mg/kg
	7	BN/WAG	day -5, irradiated	day 0, CsA 15 mg/kg
	8	BN/WAG	day -5, irradiated day -2,-1, Brofo 1 ml	day 0, CsA 15 mg/kg
	9	BN/WAG	day 0, irradiated	day 0, CsA 15 mg/kg
	10	BN/WAG	day -2,-1, Brofo 1 ml day 0, irradiated	day 0, CsA 15 mg/kg

If the donor was transfused with third party blood at 2 days and 1 day before transplantation, the effect of CsA treatment on graft survival was enhanced. Transfusion of the donor led to prolonged graft survival in 75% of the cases (group 6 vs. group 5, $p < 0.05$).

In the subsequent four experimental groups the donors were irradiated. Irradiation of the donor on day -5 or day 0 did not lead to a significant prolongation of graft survival (group 7 and 9). Irradiation on day -5 or 0 combined with transfusions at 2 days and 1 day before transplantation led to abrogation of the donor transfusion phenomenon (group 8 and 10). Graft survival times in these groups were almost similar to the survival times observed in the control group (group 5) (see Table 9.3 and Figures 9.2 and 9.3).

Table 9.2. The effect of irradiation (10 Gy) and third party transfusion of the donor on heart allograft survival in the unmodified recipient in the BN to WAG donor-host combination.

Group	Donor	Donor treatment	Recipient	Recipient treatment	Number	Survival in days
1	BN	—	WAG	—	10	8,8,8,8,8,8,8,8,9,9
2	BN	day –2, Brofo 1 ml	WAG	—	6	8,8,8,9,9,9
3	BN	day –5, irradiated	WAG	—	10	8,9,9,9,10,10,10,11,11,11
4	BN	day –5, irradiated day –2 and –1, Brofo 1 ml	WAG	—	10	8,8,9,9,10,10,11,12,12,12

The donors were transfused intravenously at different intervals (day –1 and/or –2) with 1 ml of whole Brofo blood and/or irradiated with 10 Gy whole body radiation at day –5.

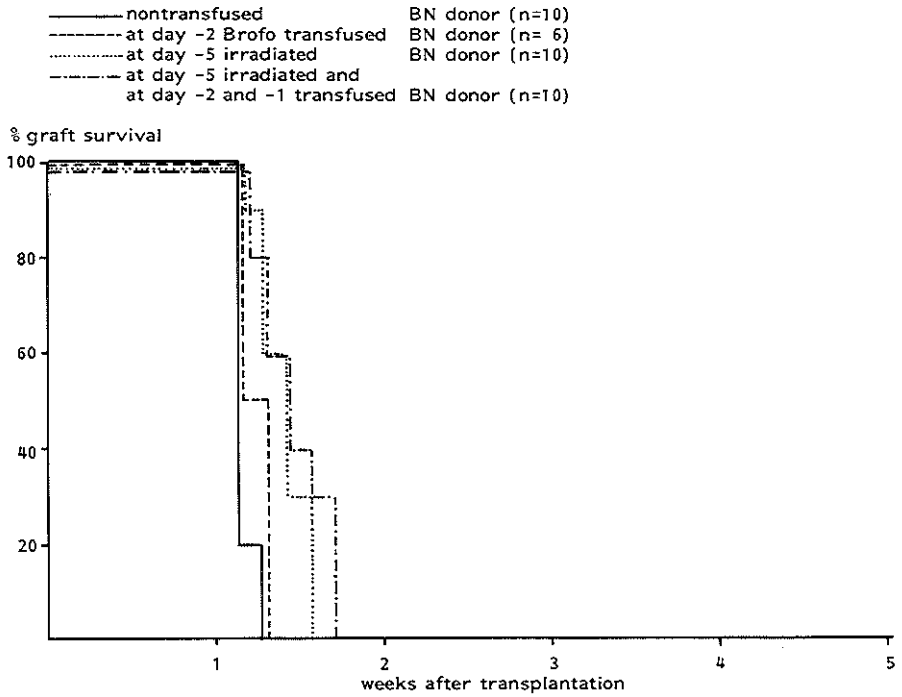


Figure 9.1. The effect of irradiation (10 Gy) and third party transfusion of the donor on heart allograft survival in the unmodified recipient in the BN to WAG donor-host combination (see Table 9.2).

9.4 Discussion

The present study shows that in the employed model the beneficial effect of donor transfusion on graft survival can be abrogated by irradiation either before or after transfusion.

As has already been mentioned in several of the previous chapters it has been demonstrated in a variety of experimental models that leukocytes carried in transplanted organs (passenger leukocytes or dendritic cells) play an important role in recipient sensitization (Steinmuller, 1967; Elkins and Guttmann, 1968; Guttmann and Lindquist, 1969; Steinmuller and Hart, 1971; Hart and Fabre, 1981c; Lechler and Batchelor, 1982). These cells are Ia positive and according to Hart and Fabre (Hart and Fabre, 1981a; 1981b) are the only site carrying class II antigens in the rat heart. The development of anti-Ia monoclonal antibodies has created the possibility to visualize these cells and they have been demonstrated in the heart (Hart and Fabre, 1981c; Bouwman *et al.*, 1985). Hart and Fabre (1981c) and Bouwman *et al.* (1985) investigated in the BN rat the effect of irradiation on the dendritic cell population of the graft. Their results showed that 24 hr after

Table 9.3. The effect of irradiation (10 Gy) and third party transfusion of the donor on heart allograft survival in the BN to WAG donor-host combination after treatment of the recipient with CsA.

Group	Donor	Donor treatment	Recipient	Recipient treatment	Number	Survival in days
5	BN	—	WAG	day 0, CsA 15 mg/kg	12	9,10,11,12,13,13,13,14,14, 14,15,15
6	BN	day –2 and –1, Brofo 1 ml	WAG	day 0, CsA 15 mg/kg	8	11,12,18,19,22,26,28, permanent 1×
7	BN	day –5, irradiated	WAG	day 0, CsA 15 mg/kg	10	12,12,12,12,13,14,22,23, 29,32
8	BN	day –5, irradiated day –2 and –1, Brofo 1 ml	WAG	day 0, CsA 15 mg/kg	7	9,11,11,12,12,13, permanent 1×
9	BN	day 0, irradiated	WAG	day 0, CsA 15 mg/kg	7	9,11,11,11,11,12,13
10	BN	day –2 and –1, Brofo 1 ml day 0, irradiated	WAG	day 0, CsA 15 mg/kg	8	11,11,11,11,12,13,13,14

The donors were transfused intravenously at different intervals (day –1 and –2) with 1 ml of whole Brofo blood and/or irradiated with 10 Gy whole body radiation at day –5 or 0. The recipients were injected on the day of grafting intramuscularly in the hind leg with Cyclosporin A (CsA) in a dose of 15 mg/kg body weight in a volume of 0.1 ml.

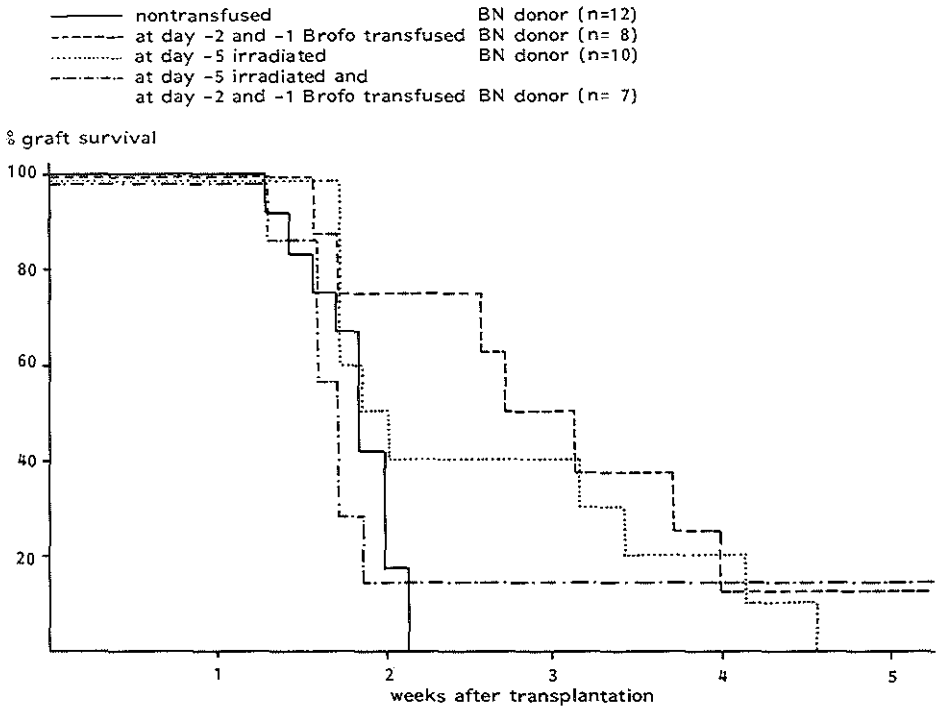


Figure 9.2. The effect of irradiation five days before grafting and third party transfusion of the donor on heart allograft survival in the BN to WAG donor-host combination after treatment of the recipient with CsA (see Table 9.3).

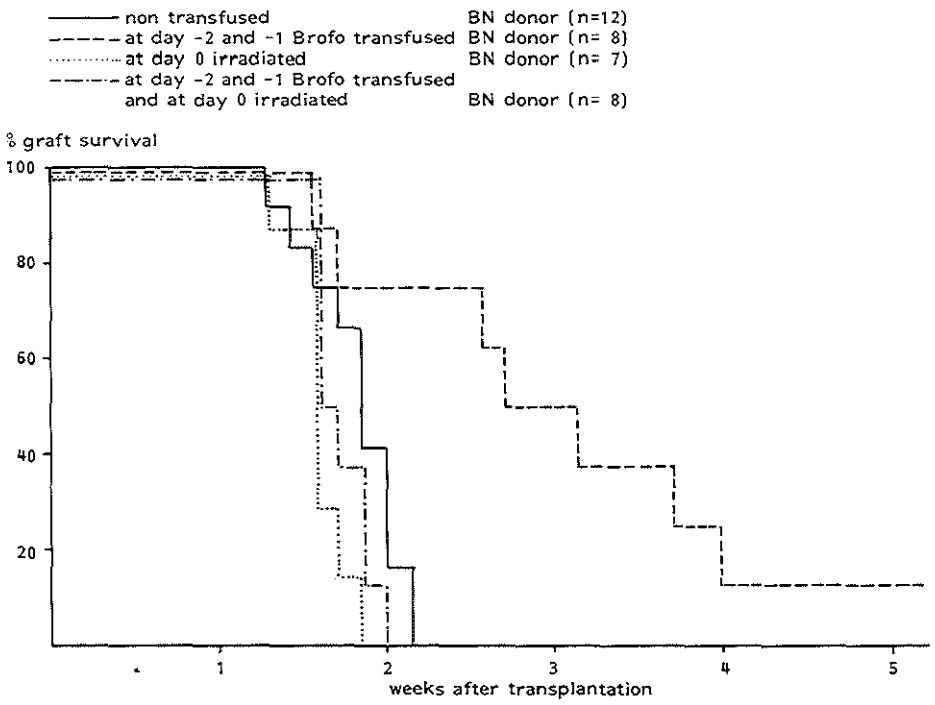


Figure 9.3. The effect of irradiation on the day of grafting and third party transfusion of the donor on heart allograft survival in the BN to WAG donor-host combination after treatment of the recipient with CsA (see Table 9.3).

treatment with 10 Gy the number of dendritic cells in the heart was normal. The numbers then declined rapidly, by the 5th day after irradiation few or no dendritic cells could be seen.

Our hypothesis concerning a mechanism possibly responsible for the donor transfusion phenomenon was that third party transfusion derived cells (leukocytes) interact with the graft dendritic cell population in such a way that these are modified and as a consequence have an altered immunogenicity. Some indirect evidence for this hypothesis could be deduced from our finding that transfusions to the donor did have opposite effects (detrimental or beneficial) depending on the way the recipient had been treated (Heineman *et al.*, 1983b). In the strain combination employed, a donor-specific transfusion to the recipient leads to permanent graft survival (Marquet *et al.*, 1971). It has been demonstrated that this transfusion effect is abolished by treatment of the donor with cyclophosphamide (Marquet *et al.*, 1981), irradiation (Heineman *et al.*, 1985) or anti-Ia monoclonal antibodies (Bouwman *et al.*, 1985). From these experiments it can be concluded that reduction of the immunogenicity of the graft either by dendritic cell elimination (cyclophosphamide, irradiation) or by shielding or alteration of dendritic cell antigens (labeling with anti-Ia monoclonal antibodies) diminishes the beneficial effect of the donor specific transfusion. A similar detrimental influence on the beneficial donor specific transfusion effect has been reported after transfusion of the donor. The third party cells might have interacted with the dendritic cells in such a way that this abrogated the beneficial donor specific transfusion effect. Recipients treated with CsA however, did benefit from donor transfusion. The CsA interacts with the recipient's immune system. These two modifications – transfusion of the donor and CsA treatment of the recipient – did prove to be additive.

The irradiation experiments in the current study were meant to substantiate the role of these passenger leucocytes or dendritic cells in the expression of the donor transfusion phenomenon.

In the first group of experiments (summarized in Table 9.2) recipients were not immunosuppressed. As has been published previously (Heineman *et al.*, 1983b) the donor transfusion phenomenon in the donor-host combination employed is only manifest in conjunction with a minimally immunosuppressed host. The aim of this group of experiments was to determine whether the appearance of the donor transfusion phenomenon could be facilitated by irradiation of the donor. Apparently this was not the case as the combined treatment with irradiation and donor transfusion gave similar results as irradiation alone.

In the second group of experiments (summarized in Table 9.3) the recipients were moderately immunosuppressed with CsA. This immunosuppression of the host allows for the expression of the donor transfusion phenomenon in a significant number of cases (Heineman *et al.*, 1983b).

Irradiation of the donor 5 days before grafting, thus virtually depleting the donor of its dendritic cell population, led to a moderate, though not significant, prolongation of graft survival.

Subsequently, transfusions were given to the donor 3 days after irradiation. This led to graft rejection in the same fashion as in non-irradiated non-transfused control animals. This result indicates that passenger leukocytes need to be present in the donor for the transfusion phenomenon to become manifest.
In two experimental groups donor animals were irradiated shortly before transplantation. The survival time of grafts in the group which received irradiation only was at the control level. This result is in accordance with other reports which show that irradiation of the donor has little or no effect on graft survival if grafts are harvested only a few hours after treatment (Stuart *et al.*, 1971a). In the last experimental group the donor animals were transfused and subsequently irradiated shortly before transplantation. The graft survival times recorded were entirely at the control level. The irradiation again resulted in abrogation of the otherwise beneficial transfusion effect. The timing of irradiation only resulted in abrogation of the proliferative capacities of the passenger leukocytes without interference with their immunogenic properties (Elves, 1969). This leads to the conclusion that not only the presence, but also the intact proliferative capacity of the passenger leukocyte population of the donor is essential for the manifestation of the donor transfusion effect. This suggests that transfusion of the donor provokes a reaction in the passenger leukocytes resulting in a modified activity of these cells towards the responsive cells of the host.

9.5 Summary

It has been shown in rats that third party blood transfusions given to the donor can markedly influence allograft survival if the immune response of the recipient has been modulated by donor-specific transfusion or treatment with Cyclosporin A (CsA). Based on these and other studies our hypothesis was that the mechanism responsible for this effect might be an interaction between leukocytes from the transfusate and the passenger cell population in the graft leading to an altered immunogenicity of these cells. In the present study attempts were made to substantiate the role of the passenger leukocytes. It was observed that irradiation (10 Gy) of the donor either 5 days before transplantation or on the day of transplantation did abrogate the otherwise beneficial donor transfusion effect in the model employed. These results indicate that the presence and intact proliferative capacity of the passenger leukocyte population of the donor is essential for the manifestation of the donor transfusion effect. This suggests that transfusion of the donor provokes a reaction in the passenger leukocytes resulting in a modified activity of these cells towards the responsive cells of the host.

CHAPTER 10

GENERAL DISCUSSION AND CONCLUDING REMARKS

10.1 Introduction

There is now wide acceptance that pretransplant blood transfusions to the recipient produce a strikingly beneficial effect on the post-transplant course and eventual outcome of cadaveric and living related kidneys (Opelz *et al.*, 1981b; Salvatierra *et al.*, 1983). Only recently studies have appeared concerning the effect on graft survival of transfusions given to the donor.

Jeekel *et al.* (1980) were the first authors to describe an effect of blood transfusions given to the donor on kidney graft survival. They described that in the dog prolonged kidney graft survival, which could be induced by one peroperative blood transfusion to the recipient, was reduced to control level by transfusion of the donor on day −1 with 100 ml third party blood. Subsequently 7 retrospective clinical studies on the effect of blood transfusions given to the donor on cadaveric kidney graft survival have been performed. In 4 studies it was reported that blood transfusions to the donor prior to nephrectomy positively influenced graft survival (Jeekel *et al.*, 1980; 1981; Frisk *et al.*, 1981; 1983; Harder *et al.*, 1984). In 3 studies no beneficial effect could be observed (Berg *et al.*, 1982; Bock *et al.*, 1984; Opelz, 1985a).

The many questions raised by these retrospective studies made it necessary to experimentally investigate them in controlled studies. In this thesis experiments in rats are described which had the following objectives: to study the effect of different whole blood transfusions and transfusions of blood components to the donor on graft survival; to determine, in case such an effect was apparent, the underlying mechanism(s); and finally to contemplate possible implications for clinical transplantation.

10.2 Donor transfusion in the rat cardiac and kidney allograft model: summary of the findings

In rats, it was investigated whether third party transfusion(s) to the donor would influence the survival of heterotopically transplanted hearts in nontransfused, transfused and CsA treated recipients. The effect of third party transfusion(s) to the donor on graft survival in the unmodified host was tested in the BN to WAG and the R1 to WAG donor/host combination. It was found that there was no measurable effect of third party (Brofo) transfusion to the donor on heart allograft survival when the WAG hosts did not receive immunosuppression, even if there was

compatibility for class I antigens between donor and host, like in the R1 to WAG combination.

The effect of third party transfusion(s) to the donor on heart allograft survival in DST transfused recipients was tested in three different donor/host combinations, the BN to WAG, the reverse WAG to BN, and the BN to LEW combination.

From the results obtained it was concluded that third party (Brofo or LEW) transfusion(s) of the donor had an effect on cardiac allograft survival in hosts pretreated with DST. It was clearly shown that this effect was not confined to one single donor-host combination, but that it was manifest when different donors and different hosts were employed. Furthermore, in all these combinations it was found that, in DST transfused recipients, grafts from transfused donors fared worse than grafts from nontransfused donors. Thus, a detrimental influence of third party donor transfusion on graft survival was observed, whereas in the clinical setting, a beneficial effect of blood transfusions to the donor had been reported (Jeekel *et al.*, 1980; 1981; Frisk *et al.*, 1981; 1983; Harder *et al.*, 1984). However, the results in one experiment showed that third party blood transfusion to the donor had a beneficial effect on graft survival when the recipient had been treated with CsA on the day of operation. Brofo transfusions to the BN donor led to prolongation of allograft survival in 75% of the cases; LEW transfusions to the BN donor had this effect in even 100% of the CsA treated WAG animals.

The influence of third party transfusions to the donor on the function and survival of heterotopically transplanted kidneys was also investigated. It was observed that a beneficial effect of third party transfusions to the donor on graft function and survival could be elicited under the condition that the recipient was moderately immunosuppressed with CsA. When the host was not treated no effect on graft function or survival could be recorded. Furthermore, it was noticed that there was a dose dependancy of the effect in this model. This was in contrast with the results in the heart transplantation model where we did not notice a difference in graft survival after different volumes of transfused third party blood.

Investigating which characteristics of the transfusate are required to elicit a donor transfusion effect, it was found that the donor transfusion phenomenon was only manifest if viable third party leukocytes were part of the transfusate. Third party erythrocytes or irradiated third party blood did not have an effect on graft survival. For the induction of the phenomenon, mere third party antigen presentation was not sufficient; viable leukocytes appeared to be mandatory. Moreover, it was observed that histoincompatibility for class I antigens between blood donor and graft recipient seemed to be of importance.

10.3 **Possible mechanism responsible for the donor transfusion effect**

Different mechanisms responsible for the donor transfusion phenomenon have been proposed.

It has been speculated that, if there is an effect of peroperative recipient blood transfusion, transplantation of a kidney from a transfused donor might represent

an additional peroperative transfusion of random third party donor leukocytes (Berg *et al.*, 1982).
Furthermore, it has been suggested that the presence of passenger leukocytes of two origins in the kidney at the time of transplantation, i.e. donor leukocytes and third party cells from the blood donor, may be responsible for an altered immunologic reactivity either in the graft or in the host (Frisk *et al.*, 1983; Jeekel *et al.*, 1983). Finally, Bachelor has offered the hypothesis that dendritic cells in the donor kidney might be mobilized in response to a transfusion, thereby rendering the graft depleted of dendritic cells and less immunogenic (Opelz and van Rood, 1983). The first two of these hypothesis have been tested by the author, the third one is under investigation and a few preliminary data are at our disposal.

The data on the influence of transfusion during transplantation surgery are somewhat conflicting. Several authors have reported that patients without pre-transplant transfusions who have been transfused during operation did as well as those transfused prior to transplantation (Stiller *et al.*, 1978; Williams *et al.*, 1980). Others however, have reported transfusions to be effective only when given prior to transplantation (Hourmant *et al.*, 1979; Opelz and Terasaki, 1981; Glass *et al.*, 1982; Opelz, 1985a). In the monkey, dog and rat, it has been found that a single pertransplant third party transfusion had a beneficial effect on allograft survival (van Es *et al.*, 1977; Obertop *et al.*, 1981; Soulillou *et al.*, 1984).

We studied whether a peroperative transfusion with third party blood did influence heart allograft survival in a model in which donor transfusion clearly showed to be effective. The results obtained show that peroperative third party transfusion has no enhancing effect on graft survival when the recipient has been treated with CsA, whereas donor transfusion clearly resulted in a significant beneficial effect in the same model. Thus, the suggestion of Berg *et al.* (1982) that transplantation of a graft from a transfused donor might represent an additional peroperative transfusion of random donor leukocytes, resulting in a beneficial effect on graft survival, does not seem to be the explanation for the donor transfusion phenomenon.

In a next experiment concerning the role of in the graft "stown away" third party leukocytes, it was tested whether transfusion of the donor leads to a specific immune response in the recipient which might be held responsible for the observed effect of donor transfusion. The test employed was a local graft versus host (GvH) assay, called the popliteal lymph node weight (PLN) assay, described by Ford *et al.* (1970). In the experiments (WAG × LEW) F_1 hybrids were used to monitor specific cell mediated immunity of WAG rats subjected to various treatments and allografting. Spleen cells of the animals to be tested were injected into the hind footpads of F_1 rats. The results showed that the assay is indeed suitable to monitor WAG-anti-LEW immunity and that intravenous injection of LEW antigen leads to a significant reduced specific immune competence. Furthermore, it was found that transfer of relevant antigen (LEW) via donor to recipient leads to a significantly reduced specific immune competence as compared to the results obtained after transfer of irrelevant antigen (Brofo). These results convincingly show that the

WAG-recipient has a "memory" for the third party transfusion given to the graft donor. However, the transfer of antigen via the graft, resulting in this "memory" in the recipient, can not be hold responsible for the donor transfusion phenomenon, as the recipients employed did not show the donor transfusion effect. In conclusion, it was found that transfusion of the donor leads to a specific immune response in the recipient, which is however not responsible for the donor transfusion phenomenon This finding is corroborated by the results in the cardiac transplantation study where peroperative transfusion did not enhance graft survival in the model employed.

It has been suggested that the change in graft survival after transfusion of the donor might be explained by attributing a crucial role to the so-called passenger leukocyte or, more specifically, to the dendritic cell. Interstitial dendritic cells are found in the connective tissues of kidney, heart, liver and all other tissues investigated except brain (Hart and Fabre, 1981c; Daar *et al.*, 1983). These cells are of hemopoietic origin and stain intensily for class II major histocompatibility complex antigens; they probably arise from a differentiation pathway distinct from known myeloid and lymphoid elements (Tew *et al.*, 1982). Similar cells isolated from rodent lymphoid tissues (Tew *et al.*, 1982) have been shown to stimulate allogeneic T lymphocytes strongly (Steinman and Witmer, 1978) and interstitial dendritic cells have been suggested to be the significant bone-marrow-derived passenger leukocyte contributing to graft antigenicity (Hart and Fabre, 1981c; Lafferty *et al.*, 1983a). Indeed, long-surviving rat renal allografts lack donor strain dendritic cells (Hart and Fabre, 1981d) and these kidneys show substantially reduced immunogenicity (Bachelor *et al.*, 1979; Hart *et al.*, 1980) on retransplantation into fresh recipient strain animals. Hart and Fabre (1981c) and Bouwman *et al.* (1985) investigated in the BN rat the effect of irradiation on the dendritic cell population of the graft. Their results showed that 24 hr after treatment with 10 Gy the number of dendritic cells in the heart was normal. The numbers then declined rapidly, by the 5th day after irradiation few or no dendritic cells could be seen.

It has also been demonstrated that transfused allogeneic lymphocytes home in small quantities in the graft (van Schilfgaarde *et al.*, 1979). One hypothesis concerning a mechanism possibly responsible for the donor transfusion phenomenon is that third party transfusion derived cells (leukocytes) interact with the graft dendritic cell population in such a way that these are modified (Frisk *et al.*, 1983; Jeekel *et al.*, 1983).

Indirect evidence for this hypothesis can be deduced from the following observations. First, we observed that the donor transfusion effect needs three times more whole third party blood to be elicited in the kidney transplantation model as compared to the cardiac allograft model. Several authors have demonstrated that the BN kidney contains at least four times as many passenger leukocytes as the BN heart (van Schilfgaarde *et al.*, 1979; Bouwman, personal communication). Thus, assuming that transfused third party blood does in equal quantities reach the different organs and that there is indeed an interaction between third party blood derived cells and the passenger leukocytes in the graft, then it can be understood

that the kidney graft needs more third party blood than the cardiac graft. This is indeed what we observed and this finding lends support to the role of passenger leukocytes in the manifestation of the donor transfusion phenomenon. Secondly, it was found that transfusions to the donor did have opposite effects (detrimental or beneficial) depending on the way the recipient had been treated. In the strain combination employed, a donor-specific transfusion to the recipient leads to permanent graft survival (Marquet *et al.*, 1971). We demonstrated that this transfusion effect is abolished by treatment of the donor with irradiation. This is a confirmation of an earlier report of Marquet *et al.* (1981) who treated the donor with cyclophosphamide, and of a recent report of Bouwman *et al.* (1985), who used anti-Ia monoclonal antibodies. From these experiments it can be concluded that reduction of the immunogenicity of the graft either by dendritic cell elimination (cyclophosphamide, irradiation) or by shielding or alteration of dendritic cell antigens (labeling with anti-Ia monoclonal antibodies) diminishes the beneficial effect of the donor specific transfusion. A similar detrimental influence on the beneficial donor specific transfusion effect has been reported after transfusion of the donor. The third party cells might have interacted with the dendritic cells in such a way that this abrogated the beneficial donor specific transfusion effect. Recipients treated with CsA however, did benefit from donor transfusion. The CsA interacts with the immune system of the recipient. These two modifications – transfusion of the donor and CsA treatment of the recipient – did prove to be additive.

The irradiation experiments in the current study were meant to substantiate more directly the role of passenger leukocytes in the expression of the donor transfusion phenomenon. The radiation dose employed (10 Gy) is known to abrogate leukocyte proliferative capacities, without interfering with their immunogenic properties (Elves, 1969). Different timing of irradiation in this study made it possible to differentiate between the presence of the passenger leukocytes and the role of the proliferative capacity of these cells for the manifestation of the donor transfusion effect. It was observed that irradiation of the donor either 5 days before transplantation or on the day of transplantation did abrogate the otherwise beneficial donor transfusion effect in the model employed. These results indicate that the presence and intact proliferative capacity of the passenger leukocyte population of the donor is essential for the manifestation of the donor transfusion effect. This suggests that transfusion of the donor provokes a reaction in the passenger leukocytes resulting in a modified activity of these cells towards the responsive cells of the host. In the light of this result it is worthwhile to contemplate the report from Berg *et al.* (1982) about the effect of transfusion of the donor on allograft survival. In the clinical situation they failed to show any beneficial effect of donor transfusion. From their data it can be deduced that the donors studied were previously included in a prospective trial on donor pretreatment with cyclophosphamide and methyl prednisolone (Corry *et al.*, 1980). Such a donor pretreatment regimen aims at the elimination of the passenger leukocytes. From our data it will be understood that this way of donor pretreatment does interfere with the reported beneficial effect on allograft survival of donor transfusion (Jeekel *et al.*, 1980; 1981;

1982; 1983; Frisk *et al.*, 1981; 1983; Harder *et al.*, 1984). We suggest that their retrospective study would have shown quite different results if this factor would have been included in the analysis.

A third hypothesis concerning the mechanism responsible for the donor transfusion phenomenon has been offered by Batchelor. He suggested that dendritic cells in the graft might be mobilized in response to a transfusion, thereby rendering the graft depleted of dendritic cells and less immunogenic (Opelz and van Rood, 1983). Preliminary data (Bouwman and Marquet, personal communication) show that this is very unlikely. The effect of transfusion on the number of dendritic cells in cardiac grafts has been investigated with anti-Ia monoclonal antibodies. No significant increase or decrease in the number of Ia staining dendritic cells after syngeneic or allogeneic transfusions could be demonstrated.

In conclusion, the studies concerning the mechanism of the donor transfusion effect provide evidence that radiosensitive cells, within grafts, being almost certainly the significant passenger leukocytes, are essential for the manifestation of the donor transfusion phenomenon. Furthermore, it was demonstrated that these cells need to be present in the graft and need to have an intact proliferative capacity. It was also observed that viable leukocytes that differ from the host for at least class I antigens are responsible for the induction of the donor transfusion effect. To understand the interactions that might take place in response to donor transfusion, we accept the stimulator cell model of lymphocyte activation, as proposed by Lafferty (1980), as the relevant model for allograft rejection. Lafferty (1980) makes two postulates concerning the process of immunocyte activation. First, it is proposed that two signals are required for lymphocyte activation. Signal 1 is provided by antigen binding to the potentially responsive lymphocyte. Signal 2 is provided by an inductive molecule (probably interleukin 1), which is said to possess costimulator activity. A corollary of the postulate is the notion that a stimulator cell is required for lymphocyte activation. The stimulator cell is the cell that provides the source of costimulator activity. According to this model, antigen-presenting cells are stimulator cells. The second postulate is "the control postulate": a control molecule on the surface of the stimulator cell regulates production and/or release of costimulator activity. Costimulator activity is released only when the control structure is engaged by the potentially responsive lymphocyte. We hypothesize that third party transfusion of the donor leads to an interaction between third party derived cells, presumably leukocytes as shown by our experiments, and the passenger leukocyte population of the graft. This interaction causes an activation of the passenger leukocyte. This activation bears the characteristics of the transfusate given to the donor and is specific. After transplantation of the transfused graft signal 1 is provided by passenger leukocyte antigen binding to the T-cell receptor. This view is supported by the finding that transfusion does not cause a significant change in Ia staining of dendritic cells (Bouwman and Marquet, personal communication). The provision of the essential signal 2 however, is influenced by the specific metabolic engagement of the passenger leukocyte. We

propose that the T-cell response involving the recognition of the control structure on the surface of the passenger leukocyte and thus, the release of costimulator activity (signal 2), is blocked by this specific metabolical engagement of the passenger leukocyte (Figure 10.1). As a result the route of alloimmunization is changed. The recipient's own antigen presenting cells are handling the alloantigen. This alloantigen is finally presented to the recipient's T helper cells. This indirect antigen presentation to the recipient's immune system is much less efficient than the process of direct antigen handling (Batchelor *et al.*, 1978), and can lead to enhancement of graft survival (Lafferty and Prowse, 1984).

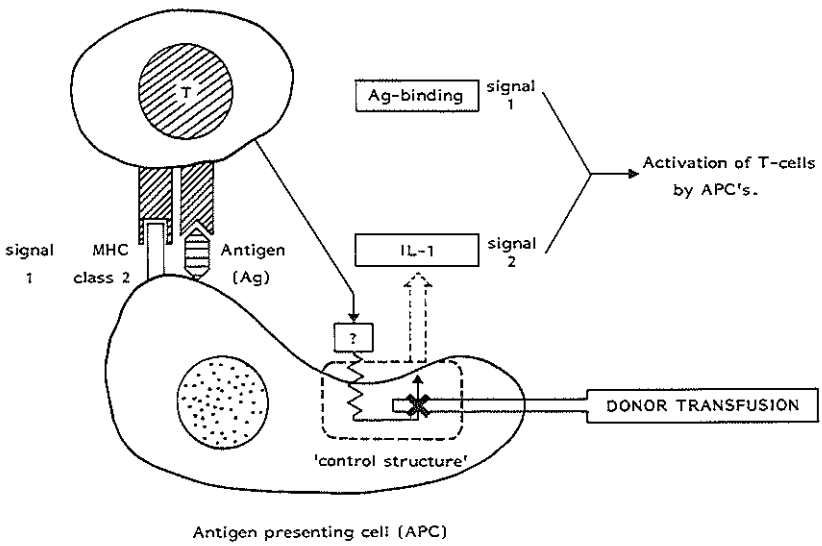


Figure 10.1. Activation of T-cells by antigen-presenting cells influenced by donor transfusion.

Finally, it is of special interest to mention that when the outlined hypothesis holds true, it can be understood why donor transfusion and CsA treatment of the recipient showed an additive, enhancing effect on graft survival, for it has recently been reported that CsA has an inhibitory effect on the production of interleukin 2 (Hess, 1985). These two modifications, transfusion of the donor, leading to interleukin 1 blockade and CsA treatment of the recipient, leading to inhibition of interleukin 2 production, were obviously synergistic in frustrating the rejection reaction. It remains puzzling however, what exactly happens when a transfused donor graft is transplanted into a DST-treated recipient which leads to a detrimental effect on graft survival.

10.4 Concluding remarks

In this thesis retrospective clinical observations, showing a significant effect on graft survival of transfusion of the donor, are confirmed by data obtained in controlled experimental studies. The hypothesis concerning the responsible mechanism points clearly towards the elimination of one of the signals required for lymphocyte activation, in particular the switch off of the second signal which is provided by an inductive molecule, probably interleukin 1. It is proposed that as a result of donor transfusion the route of alloimmunization is changed and that the much less efficient indirect antigen presentation to the recipient's immune system takes place. Further experimental work is necessary to confirm this hypothesis. Of special interest seems to be the effect on graft survival of "anti-interleukins" (agents blocking the production and/or action of interleukins) either given to the donor, to the graft, or to the recipient. If the hypothesis holds true this might have interesting consequences for clinical transplantation as clearly at present donor pretreatment in any form several days prior to transplantation is not clinically applicable.

SUMMARY

It is widely accepted that pretransplant blood transfusions to the recipient produce a significant beneficial effect on the post-transplant course and eventual outcome of cadaveric and living related kidneys. Only recently studies have appeared concerning the effect on graft survival of transfusions given to the donor. Seven retrospective clinical studies on the effect of blood transfusions given to the donor on cadaveric kidney graft survival have been performed. In 4 studies it was reported that blood transfusions to the donor prior to nephrectomy positively influenced graft survival, in 3 studies no beneficial effect could be observed. The many questions raised by these retrospective studies made it necessary to experimentally investigate them in rigidly controlled studies. The effects of donor blood transfusion in the rat cardiac and kidney allograft model and efforts to unravel the mechanism are described in this thesis.

In chapter 1, a review is given of the history and present state of transplantation. Special emphasis is made to describe the several clinical approaches to immune modulation and ample attention is paid to the background of donor pretreatment.

The details of the available seven retrospective clinical studies on the effect of blood transfusion given to the donor on cadaveric kidney graft survival are described in chapter 2. Furthermore, the objectives for the experimental studies and the design of the experiments are formulated.

The various materials and methods used are described in chapter 3.

Chapter 4 deals with the effect of third party whole blood transfusion(s) of the donor on heterotopic heart allograft survival in rats. The following variables were studied: the effect of donor transfusion in different donor-host combinations; the effect of donor transfusion in relation to different types of immune modulation of the host; and finally, the effect of varying number and timing of donor transfusion. It was found that blood transfusion(s) given to the donor did markedly influence graft survival in transfused or immunosuppressed recipients. When the recipient was pretreated with a donor-specific transfusion, which leads, depending on the donor-host combination employed, to a shortened, prolonged or even permanent graft survival, the effect of blood transfusions to the donor on graft survival was predominantly detrimental. When the recipient had been treated with a single injection of 15 mg/kg CsA on the day of operation, the effect of blood transfusions to the donor was beneficial. The donor transfusion phenomenon was not linked to one particular donor-host combination. Furthermore, the donor transfusion phenomenon was not linked to one single type of blood. Different sources of third party derived blood did have the same influence on graft survival. Finally, there was no difference in graft survival after a single or multiple transfusions of the donor, nor was there an effect of timing.

In chapter 5 the donor transfusion phenomenon was studied in a rat kidney allograft model. It was observed that a beneficial effect of third party blood transfusions to the donor on graft function and survival could be elicited under the

condition that the recipient was moderately immunosuppressed with CsA. When the host was not treated no effect on graft function or survival could be recorded. Furthermore, it was shown that the appearance of the phenomenon was dose dependant. It was discussed that this finding lends support to the role of passenger leukocytes in the manifestation of the donor transfusion phenomenon, as the rat kidney contains at least four times as many passenger leukocytes as the rat heart.

Experiments studying the characteristics of the transfusate, required to elicit a donor transfusion effect, were described in chapter 6. More in particular the importance of the presence of leukocytes in the transfusate and of the degree of compatibility for MHC antigens between transfusate and host were studied. It was found that the donor transfusion phenomenon seems to be mediated by viable leukocytes and that the phenomenon is preferentially induced by blood donors that differ from the graft recipient for class I antigens.

One of the hypothesis concerning the mechanism responsible for the donor transfusion phenomenon is that the effect can be ascribed to a peroperative recipient blood transfusion and that transplantation of a graft from a transfused donor might represent an additional peroperative transfusion of random donor leukocytes. This hypothesis is tested in chapter 7. In the first part of the experiments it was studied whether a peroperative transfusion with third party blood did influence heart allograft survival in rats in a model in which donor transfusion clearly showed to be effective. It was found that this was not the case. In the second part it was tested in a popliteal lymph node assay whether transfusion of the donor led to a specific immune response in the recipient. It was observed that transfer of third party antigen via the donor to the recipient led to a reduced specific immune competence, however, it was also noticed that this transfer of antigen via the graft was not responsible for the donor transfusion phenomenon.

In chapter 8 it was investigated whether passenger leukocytes play an important role in the donor-specific transfusion (DST) phenomenon. The passenger leukocyte population was irradiated at a dose (10 Gy) known to abrogate leukocyte proliferative capacities, without interference with their immunogenic properties. The functional properties of the passenger leukocytes were modulated at different periods before transplantation. It was observed that pretreatment of the donor with irradiation (10 Gy) 5 days before transplantation led to a significant dimunition of the DST effect, whereas irradiation of the donor on the day of transplantation did not. From these experiments it was concluded that reduction of the immunogenicity of the graft by passenger leukocyte elimination (irradiation) diminished the beneficial effect of DST. It was suggested that the passenger leukocytes in the graft contributed to the induction of specific unresponsiveness as initiated by the DST to the host.

It has been suggested that the presence of leukocytes of two origins in the graft at the time of transplantation, i.e. donor passenger leukocytes and third party cells from the blood donor, may be responsible for an altered immunologic reactivity in the graft and/or the host. The role of leukocytes in the third party transfusate has been outlined in chapter 6. In chapter 9 attempts were made to substantiate the role

of these passenger leukocytes. Indirect evidence for the importance of passenger leukocytes was deduced from the following observations. First, it was observed in chapter 5 that the donor transfusion effect needs three times more whole third party blood to be elicited in the kidney transplantation model as compared to the cardiac allograft model. Several studies have demonstrated that the BN kidney contains at least four times as many passenger leukocytes as the BN heart. Thus, assuming that transfused third party blood does in equal quantities reach the different organs and that there is indeed an interaction between third party derived cells and the passenger leukocytes in the graft, then it can be understood that the kidney graft needs more third party blood than the cardiac graft. This is indeed what we observed in chapter 5 and this finding lends support to the role of passenger leukocytes in the manifestation of the donor transfusion phenomenon. Secondly, it was found in chapter 4 that transfusions to the donor did have opposite effects (detrimental or beneficial) depending on the way the recipient had been treated. In the strain combination employed, a DST to the recipient led to permanent graft survival. It was demonstrated that this transfusion effect was abolished by treatment of the donor with irradiation (chapter 8). From this experiment it was concluded that reduction of the immunogenicity of the graft diminished the beneficial effect of a DST. A similar detrimental influence on the beneficial DST effect was reported after transfusion of the donor. It was suggested that the third party cells interacted with the passenger leukocytes in such a way that this abrogated the beneficial DST effect. Recipients treated with CsA however, did benefit from donor transfusion. The CsA interacts with the recipient's immune system. Here these two modifications, transfusion of the donor and CsA treatment of the recipient, did prove to be additive (chapter 4). The irradiation experiments in the current study were meant to substantiate the role of these passenger leukocytes in the expression of the donor transfusion phenomenon. It was observed that irradiation (10 Gy) of the donor either 5 days before transplantation or on the day of transplantation did abrogate the otherwise beneficial donor transfusion effect in the model employed. These results indicated that the presence and intact proliferative capacity of the passenger leukocyte population of the donor was essential for the manifestation of the donor transfusion effect. These data suggest that transfusion of the donor provoked a reaction in the passenger leukocytes which resulted in a modified activity of these cells towards the responsive cells of the host.

In the final chapter 10 the results as obtained in the previous chapters are discussed. It is concluded that, taken the stimulator cell concept as the relevant model for allograft rejection, the results point towards the elimination of one of the signals required for lymphocyte activation as the most likely explanation of the mechanism of the donor transfusion phenomenon. It is especially suggested that the switch off of the second signal which is provided by an inductive molecule, probably interleukin 1, is responsible for the described phenomenon. It is suggested that as a result the route of alloimmunization is changed and that the much less efficient indirect antigen presentation to the recipient's immune system takes place.

Finally, it is concluded that further experimental work is necessary to confirm this hypothesis. Of special interest seems to be research on the effect on graft survival of "anti-interleukins" either given to the donor, to the graft, or to the recipient.

SAMENVATTING

Het is algemeen bekend dat bloedtransfusies, welke vóór transplantatie aan de ontvanger worden gegeven, een gunstig effect hebben op de overleving van zowel cadaver- als familie-nieren. Recent zijn een 7-tal retrospectieve klinische studies verschenen betreffende het effect op de transplantaat-overleving van transfusies gegeven aan de donor. In vier van deze onderzoekingen werd een positief effect van donor-transfusie gevonden; in drie onderzoekingen werd geen effect waargenomen. Deze studies wierpen een aantal vragen op, die het beste beantwoord konden worden door gebruik te maken van een preklinisch proefdiermodel.
Het onderzoek dat in dit proefschrift wordt beschreven, omvat experimenten bij ratten en had tot doel het effect te onderzoeken van bloedtransfusies aan de donor op de overleving van hart- en nier-transplantaten. Bovendien werd gepoogd het mechanisme dat ten grondslag ligt aan zo'n effect te ontrafelen.

In hoofdstuk 1 worden de geschiedenis en huidige stand van zaken betreffende weefsel en orgaan transplantatie besproken. Bijzondere aandacht is geschonken aan de verschillende manieren waarop in de kliniek wordt gepoogd de afstotingsreactie te beïnvloeden; verder wordt de achtergrond van donor voorbehandeling aan de orde gesteld.

In hoofdstuk 2 worden de gegevens van de retrospectieve klinische studies naar het effect van donor-transfusie besproken. De doelstellingen van het experimentele onderzoek en het ontwerp van de experimenten worden geformuleerd.

De gebruikte materialen en methoden zijn beschreven in hoofdstuk 3.

In hoofdstuk 4 wordt het effect van derde partij bloed (bloed afkomstig van een stam niet verwant aan donor of ontvanger) aan de donor op de overleving van een heterotoop getransplanteerd hart beschreven. De volgende variabelen werden bestudeerd: het effect van donor-transfusie in verschillende donor-gastheer combinaties; het effect van donor-transfusie in relatie tot immunosuppressieve behandeling van de ontvanger; en tot slot het effect van het aantal en het tijdstip van transfusies. Het bleek dat bloedtransfusies een duidelijk effect hadden op de overleving in ontvangers welke waren getransfundeerd of behandeld met een immunosuppressivum. Afhankelijk van de donor-gastheer combinatie leidt een donor-specifieke transfusie van de ontvanger tot een verkorte, verlengde of zelfs permanente overleving van het transplantaat. In deze donor-specifiek getransfundeerde ontvangers had transplantatie van een hart afkomstig van een getransfundeerde donor, vrijwel altijd een negatief effect op de overleving. Wanneer daarentegen de ontvanger was behandeld met het immunosuppressieve geneesmiddel Cyclosporine A in een dosis van 15 mg/kg lichaamsgewicht, dan bleek dat transfusie van de donor een gunstig effect had. Het bleek dat het vóórkomen van het donor-transfusie effect niet beperkt was tot één enkele donor-gastheer combinatie, maar in tegendeel, in verschillende combinaties kon worden geïnduceerd. Verder

werd vastgesteld, dat bloed van verschillende rattestammen het effect in vergelijkbare mate kon bewerkstelligen. Tot slot werd vastgesteld, dat noch het aantal transfusies aan de donor, noch het tijdstip van transfusie, in het gebruikte model van belang waren.

In hoofdstuk 5 is het donor-transfusie effect bestudeerd in een niertransplantatie model in de rat. Er werd gevonden, dat een derde partij bloed transfusie van de donor een gunstig effect kan hebben op zowel de functie als de overleving van het niertransplantaat. Hierbij was het noodzakelijk, dat de ontvanger een beperkte hoeveelheid Cyclosporine A ontving. Indien dit niet werd gegeven, werd geen effect van transfusie van de donor op de transplantaat-overleving waargenomen. In het niertransplantatie model werd verder gevonden, dat het effect afhankelijk was van de hoeveelheid getransfundeerd bloed. Mede op basis van de bevinding dat, om een vergelijkbaar effect te verkrijgen, er 3 maal zoveel bloed moest worden toegediend in het niertransplantatie-model dan in het harttransplantatie-model, werd de hypothese geponeerd, dat de zgn. "passenger" leukocyten een rol zouden spelen in de manifestatie van dit fenomeen. Achtergrond voor deze suggestie vormde het gegeven, dat de ratte-nier tenminste 4 maal zoveel "passenger" leukocyten bevat dan het ratte-hart.

In hoofdstuk 6 zijn experimenten beschreven waarin is onderzocht aan welke karakteristieken het transfusaat moet voldoen, om een donor-transfusie effect te sorteren. In het bijzonder werd het belang van leukocyten in het transfusaat en van de graad van compatibiliteit voor MHC antigenen tussen transfusie donor en transplantaat ontvanger bestudeerd. Er werd gevonden, dat het donor-transfusie effect tot stand lijkt te komen via levende leukocyten in het transfusaat. Bovendien leek het fenomeen bij voorkeur te worden geïnduceerd door bloed afkomstig van donoren welke tenminste van de ontvanger voor klasse I antigenen verschillen.

Er zijn diverse hypothesen opgesteld over het mechanisme dat aan het optreden van het donor-transfusie effect ten grondslag zou kunnen liggen. Eén zo'n hypothese luidt, dat het effect van donor-transfusie terug te brengen is tot een peroperatieve transfusie aan de ontvanger. Transplantatie van een "getransfundeerd transplantaat" zou een peroperatieve transfusie betekenen van gemigreerde leukocyten uit het transfusaat. Deze hypothese is in hoofdstuk 7 getoetst. In het eerste deel is onderzocht of een peroperatieve transfusie met derde partij bloed, een effect heeft op de overleving van harten in een model waarvan bekend is, dat transfusie van de donor een duidelijk resultaat geeft. Er werd geen effect gevonden. In het tweede deel is, gebruik makend van de "popliteal lymph node assay" bestudeerd, of transfusie van de donor leidt tot een specifieke immuun reaktie in de ontvanger. Er werd gevonden, dat transport van derde partij antigenen via de donor naar de ontvanger plaatsvindt en daar aanleiding geeft tot een verminderende specifieke immunologische reaktiviteit. Echter, tegelijkertijd werd geconstateerd, dat dit transport van antigeen en de verandering in immunologische reaktiviteit niet verantwoordelijk kunnen zijn voor het donor-transfusie effect. Concluderend kan gesteld worden, dat geen aanknopingspunten zijn gevonden voor de juistheid van de hypothese, dat het donor-transfusie effect terug te brengen zou zijn tot een peroperatief transfusie fenomeen.

In hoofdstuk 8 is onderzocht of "passenger" leukocyten een rol spelen bij het tot stand komen van een donor-specifiek transfusie (DST) fenomeen. De "passenger" leukocyten populatie in het transplantaat werd beïnvloed door de donor te bestralen met 10 Gy. Dit heeft tot gevolg dat de proliferatie van deze cellen wordt geremd maar de immunogene eigenschappen ongemoeid blijven. Vijf dagen na bestraling zijn de meeste "passenger" leukocyten uit het transplantaat verdwenen. Waargenomen werd, dat bestraling van de donor 5 dagen vóór transplantatie, aanleiding gaf tot een negatieve beïnvloeding van het gunstige DST effect. Echter, bestraling van de donor op de dag van transplantatie had geen effect. Deze resultaten leidden tot de conclusie, dat vermindering van de immunogeniciteit van het transplantaat door de "passenger" leukocyten uit te schakelen, het gunstige effect van DST te niet doet. Dit gegeven is aanleiding tot de veronderstelling, dat de "passenger" leukocyten een belangrijke rol spelen in de inductie van donor-specifieke tolerantie, zoals wordt bereikt door het geven van DSTs.

Een tweede hypothese omtrent het mechanisme van het donor-transfusie effect is, dat het wordt veroorzaakt door de aanwezigheid ten tijde van transplantatie van leukocyten van twee verschillende origines, nl. donor "passenger" leukocyten en de cellen afkomstig uit het transfusaat. Deze twee populaties zouden zodanig met elkaar interveniëren, dat als gevolg hiervan de immunologische reaktiviteit in het transplantaat of in de ontvanger zou worden veranderd. Het belang van leukocyten uit het transfusaat voor het optreden van een donor-transfusie effect is reeds aan de orde gesteld in hoofdstuk 6. In hoofdstuk 9 is gepoogd de rol van de "passenger" leukocyten nader te definiëren. Indirekt werd het belang van deze "passenger" leukocyten gedestilleerd uit de volgende bevindingen. Ten eerste werd, zoals beschreven in hoofdstuk 5, gevonden, dat, om een donor-transfusie effect te kunnen induceren, in het nier-model drie maal zoveel bloed nodig is dan in het hart-model. In diverse studies is aangetoond, dat de gebruikte BN nier ten minste vier maal zoveel "passenger" leukocyten bevat dan het BN hart. Ervan uitgaande, dat het getransfundeerde bloed de verschillende organen in gelijke mate bereikt, en dat er inderdaad een interactie is tussen leukocyten uit het transfusaat en de "passenger" leukocyten in het transplantaat, kan derhalve worden verondersteld, dat de nier meer derde partij bloed nodig heeft dan het hart om een donor-transfusie effect te laten zien. Dit is inderdaad wat in hoofdstuk 5 werd gezien, en dit gegeven verleent steun aan de hypothese, dat "passenger" leukocyten een belangrijke rol spelen bij het tot stand komen van het donor-transfusie effect. Ten tweede werd in hoofdstuk 4 beschreven hoe transfusies aan de donor tegengestelde effecten (ongunstig versus gunstig) op de transplantaat-overleving hadden, afhankelijk van de wijze waarop de ontvanger was behandeld. In de gebruikte donor-gastheer combinatie geeft een DST aanleiding tot een permanente overleving. In hoofdstuk 8 werd beschreven hoe dit DST effect te niet wordt gedaan door bestraling van de donor. Hieruit werd geconcludeerd, dat vermindering van de immunogeniciteit van het transplantaat een negatief effect heeft op het gunstige gevolg van een DST. Eenzelfde ongunstige invloed werd gevonden na transfusie van de donor. Er is gesuggereerd, dat de derde partij afkomstige cellen zodanig reageerden met de

"passenger" leukocyten, dat het gunstige effect van DST werd verstoord. Ontvangers daarentegen welke met Cyclosporine A werden behandeld, vertoonden een gunstig effect van donor-transfusie. Het is bekend, dat Cyclosporine A met het immuun systeem van de ontvanger intervenieert. Hier hebben deze twee modificaties, nl. transfusie van de donor en Cyclosporine A behandeling van de ontvanger, additief gewerkt (hoofdstuk 4). De bestralingsproeven in dit hoofdstuk zijn bedoeld om de rol van de "passenger" leukocyten bij het tot stand komen van het donor-transfusie effect verder te onderzoeken. Gevonden is, dat bestraling (10 Gy) van de donor danwel 5 dagen vóór transplantatie, danwel op de dag van transplantatie, het gunstige effect van donor-transfusie in het gebruikte model, verloren doet gaan. Deze resultaten geven aanleiding te veronderstellen, dat de aanwezigheid, dus immunogene eigenschappen, en de intacte proliferatieve, dus immunocompetente eigenschappen van de "passenger" leukocyten populatie van de donor essentieel is voor de manifestatie van het donor-transfusie effect. Deze gegevens leidden tot de veronderstelling, dat transfusie van de donor een reactie heeft veroorzaakt in/bij de "passenger" leukocyten, welke heeft geresulteerd in een veranderde activiteit van deze cellen ten opzichte van de responderende cellen in de gastheer.

In het laatste hoofstuk 10 worden de resultaten, uit de voorafgaande hoofdstukken, besproken. Het zogenaamde "stimulator cell" concept wordt geaccepteerd als een goed model om transplantaat afstoting te beschrijven. Met dit model voor ogen wordt geconcludeerd, dat transfusie van de donor mogelijk leidt tot uitschakeling van één van de signalen van de antigeen presenterende "passenger" leukocyt, hetgeen aanleiding geeft tot een vertraging in de activering van de T lymphocyten. Er wordt met name verondersteld, dat het "uitzetten" van het tweede signaal, dat wordt gegeven door een "inductive" molecuul, mogelijk interleukine 1, verantwoordelijk is voor het beschreven fenomeen. Ten gevolge hiervan zou de weg waarlangs immunisatie plaats vindt en dus de afstotingsreactie op gang komt, worden gewijzigd in de veel minder effectieve, indirecte weg van antigeen presentatie. Ten slotte wordt gesteld, dat toetsing van deze hypothese verder experimenteel werk behoeft. Van bijzonder belang lijkt hierbij onderzoek naar het effect op transplantaat-overleving van interleukines en anti-interleukines.

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