"THE HUMORAL IMMUNOSUPPRESSIVE EFFECTS OF BLOOD TRANSFUSION IN RENAL TRANSPLANTATION"

Two Volumes: Volume One

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DECLARATION

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SUMMARY

Renal transplantation offers the optimal quality of life for the patient requiring renal replacement therapy. The main reason for renal allograft failure remains immunological rejection. The discovery that pretransplant blood transfusion is associated with decreased graft loss through rejection led to the introduction of elective transfusion protocols for renal dialysis patients. Despite extensive research, the mechanism of the blood transfusion effect on transplant outcome remains unclear. Of the many suggested explanations, one proposed by MacLeod and her colleagues, claimed that Fcγ-receptor blocking antibodies were produced after blood transfusion and correlated with renal allograft survival. The initial aims of the work described in this volume were to confirm and extend these findings (MacLeod et al, 1982b).

Using IgG prepared from whole serum by standard chromatography, the association between Fcγ-receptor blocking activity and blood transfusion was confirmed for both uraemic and non-uraemic subjects. The development of Fcγ-receptor blocking activity was shown over the course of elective transfusion of previously untransfused renal dialysis patients.

IgG preparations from transfused subjects were shown to interact *in vitro* not only with B lymphocytes as previously described, but also with normal, allogeneic peripheral blood and T lymphocytes, thymocytes, polymorphs, platelets and spermatozoa. The pattern of reactivity did not correspond to the Fcγ-receptor bearing cell populations. The significance *in vivo* of inhibition by IgG preparations from transfused subjects of polymorph phagocytosis and platelet aggregation remains

uncertain. Perhaps relevant to attenuation of allograft rejection was inhibition of the mitogen response which was associated with IgG Fcγ-receptor blocking activity.

Confirmation of the presence of Fcγ-receptor blocking antibodies in patients potentially immunosuppressed by blood transfused was followed by similar experiments using IgG preparations from patients exposed to a wide range of alloantigens, although not necessarily in blood. Patients with haemophilia were chosen, as a group who received clotting factor concentrates derived from multiple donors, and in whom there were recent reports of impaired indices of immune function. Like the recipients of blood transfusion, patients with haemophilia yielded IgG which blocked Fcγ-receptors and inhibited the mitogen response of lymphocytes from normal subjects.

Only a small proportion of the haemophiliac subjects were later found to have antibody against Human T cell Leukaemia Virus Type III, thought now to mark potential development of the Acquired Immunodeficiency Syndrome (AIDS). The epidemiology of AIDS, while consistent with unifactorial viral aetiology, led to suggestions that pre-existing immunosuppression may predispose to the expression of the virus.

Reports of anti-lymphocyte antibodies in the serum of practising homosexual men, the risk group with the highest incidence of AIDS, stimulated experiments to assess the prevalence of Fcγ-receptor blocking antibodies in AIDS patients and in homosexual men with no evidence of the syndrome. IgG preparations from both groups blocked Fcγ-receptors and inhibited the mitogen response of normal lymphocytes. Cross-reactivity with spermatozoa may indicate that anti-lymphocyte antibodies

are induced by rectal insemination. Although these experiments were performed before identification of the virus, and anti-viral antibody status was not checked, the prevalence of infection in comparable populations at the time of testing (1983) has been regarded as too low to account for these findings.

Despite the demonstration of IgG Fcγ-receptor blocking activity in subjects with acquired immune abnormalities, no association was found between renal allograft survival and pretransplant IgG receptor blocking activity, using a variety of donor and third party Fcγ-receptor bearing cells. No conclusive explanation was found for the discrepancy between this and previous work (MacLeod et al, 1982a and b).

Lest the method of preparation of IgG eliminate the suggested association with transplant outcome, pretransplant sera were instead separated into fractions of graded molecular weight. There was a highly significant correlation between renal allograft survival and Fcγ-receptor blocking activity in the highest molecular weight fraction (>19S). Although the serum factor(s) responsible were not identified, their presence appeared to be associated with previous transfusion of blood or blood products, and with diminished response to skin testing with Dinitrochlorobenzene. This may indicate that high molecular weight serum Fcγ-receptor blocking factors are involved in non-specific depression of cell-mediated immunity following blood transfusion, and may be regarded as predictive of successful renal transplantation.

CHAPTER 1

THE NEED FOR RENAL TRANSPLANTATION

The need for renal transplantation in the management of end-stage renal failure is unquestionable. In a field where "the idea that prevention is simple, available or immediately possible, is a fantasy", Robson (1979), the major task has become the maintenance of life by dialysis and transplantation.

Assessment of the relative merits of these treatments in terms of morbidity and mortality has been bedevilled by difficulties in interpretation where patients have been treated with a mixture of modalities and where selection bias in therapy may influence the results (Brunner et al, in press). Vollmer, Wahl and Blagg (1983) attempted to compare patient survival following dialysis and transplantation. Transplantation of a kidney from a live related donor conferred a benefit which was lost when a cadaveric allograft was used; the authors considered that recent improvements in immunosuppressive therapy could eliminate this difference.

More important than survival alone is the quality of life. A number of workers have sought to assess the relative qualities of life for patients on different types of renal replacement therapy, with variable success in controlling the case-mix for the different therapies. The most recent in a series of reports from two American groups, those of Simmons et al in Minneapolis (1985) and Evans et al in Seattle (1985 a and b), consist of detailed cross-sectional multicentre assessment using several recognised indices of physical, emotional and social wellbeing. So far as possible in this type of study the results for the 859 patients studied by Evans et al (1985 a) were adjusted for the case-mix. Both subjective and objective

assessment of quality of life were consistently better for patients with functioning transplants. Indeed, transplant recipients had a subjective quality of life that did not differ significantly from the general population.

It might be naive to assume that improved quality of life were the sole reason for the relatively high proportion of patients in the United Kingdom treated by transplantation. Figures compiled by the Registry of the European Dialysis and Transplant Association (EDTA) show that on 31st December 1984 one half of patients on renal replacement therapy in the United Kingdom had functioning transplants. This proportion is similar to that in Scandinavia and Eire, but considerably greater than, for example, France and the Federal Republic of Germany (both less than one fifth) (Brunner et al, in press). Sells, Macpherson and Salaman (1985) have suggested that "in no other country has the presence of deficient dialysis programmes yielded such an energetic response from affiliated transplant units". The number of successful transplants has a direct bearing on the number of new patients who can be accepted onto dialysis. Figures again from the EDTA Registry show that in 1984 the United Kingdom rate of acceptance onto renal replacement therapy was 35.9 patients per million population. This rate is not only lower than almost all of the rest of Western Europe, but reflects particularly rigorous selection against the elderly (Brunner et al, in press).

The absolute number of renal transplants performed continues to be regarded as limited by the availability of organs (Sells et al, 1985). The analysis of this problem by the Medical Services Study Group of the Royal College of Physicians (1981) pointed out that intensive care facilities available to prospective donors are not uniformly adequate for the provision of viable kidneys. Considerable effort and publicity have been expended to achieve public and professional

acceptance of cadaver organ transplantation with general, if not uniform, success. As "The Lancet" commented in 1984, "within two years we have seen transplantation suffer grievously from a false but newsworthy anxiety about the diagnosis of donor death, and then equally abruptly flourish as a result of a single liver transplant in a child". (Anonymous ,1984). With over 3000 patients in the United Kingdom awaiting renal transplantation, the encouragement of kidney donation remains essential (United Kingdom Transplant Service, 1985).

Crucial to the establishment of transplantation as the treatment of choice for renal failure is an increase not only in the numbers of transplants performed but in their success rate. Major transplant registries are now quoting figures approaching 80% for one-year survival of first cadaver allografts (Opelz 1985 b, Brunner et al in press).

The most important cause of graft failure remains immunological rejection. Four-fifths of graft losses reported in 1980-83 to the EDTA Registry were attributed to rejection (Brunner et al, in press). Interest in the clinical management of rejection has been paralleled by attempts to elucidate the immune response to the renal allograft. Current thinking on this topic is reviewed in the next chapter.

CHAPTER 2

MECHANISMS OF REJECTION OF RENAL ALLOGRAFTS

The mechanism of allograft rejection is dependent on recognition of foreign antigen by the immune system, followed by mobilisation of the available modes of destruction.

1. Recognition of antigen: the afferent limb of the immune response.

Genetic differences between individuals are reflected by differences in the glycoproteins expressed on their cell surfaces. Those which affect allograft outcome are known as histocompatibility antigens. Although both so-called "major" and "minor" histocompatibility antigens are recognised, it is the antigens of the Major Histocompatibility Complex which are regarded as of primary importance.

2. The Major Histocompatibility Complex

The structure of the Major Histocompatibility Complex has recently been summarized by de Vries and van Rood (1985) as follows:

i) HLA class-I molecules

Found on the surface of nearly all nucleated cells (for detailed distribution in man, see Daar et al, 1984 a). They consist of two polypeptide chains, one of which has a molecular weight of 44,000 daltons and is coded in the Major Histocompatibility Complex on chromosome 6. The 12,000 dalton light chain is beta-2-microglobulin, which is coded by a gene on chromosome 15. The heavy chain penetrates the cell membrane, with its extracellular part folded into three immunoglobulin-like domains (α_1, α_2, α_3). B_2 microglobulin

does not penetrate the cell membrane but is associated noncovalently with the α_3 domain. The α_3 domain varies little, but the α_1 and α_2 domains express the polymorphism of three heavy chain genes, known as HLA-A, B and C. Class I molecules act as antigen presenters primarily to cytotoxic T lymphocytes.

ii) HLA class-II molecules

Found primarily on cells of the immune system, such as macrophages and other antigen-presenting cells, B lymphocytes and activated T lymphocytes. Other sites have been charted by Daar et al (1984 b) and include renal vascular endothelium; increased expression of class II antigens has been reported during allograft rejection (Hall et al, 1984). Class-II molecules also consist of two polypeptide chains, both of which penetrate the cell membrane. The 34,000-daltaon α chain is non-covalently associated with the 29,000-dalton β chain. Both chains are coded by genes in the HLA system on chromosome 6. At least 5 class-II α and 7 class-II β genes are located in the HLA system, and named DR, DP and DQ. Class-II molecules provide antigen presentation principally to helper T lymphocytes.

3. The effect of tissue matching on renal transplant outcome

i) Antigen matching

Matching of class I and II antigens between donor and recipient may reduce the likelihood of rejection. Although benefit from matching has not always been apparent in small studies, multicentre data points strongly to the benefits of tissue matching. In particular, matching for both B and DR loci is beneficial; Opelz (1985 b) has recently demonstrated a 20% difference in 1-year graft

survival between donor-recipient pairs matched for no and four B/DR loci. Matching techniques are reviewed by Dick (1979).

ii) Lymphocytotoxicity testing

The methods used for lymphocytotoxicity testing are summarised by Kissmeyer-Nielsen and Dick (1979). If renal transplantation is performed when there is a positive complement-dependent cross-match between recipient serum and donor T lymphocytes, hyperacute rejection will almost invariably occur. The practice therefore is that such a cross-match precludes transplantation of the donor-recipient pair. T cell cytotoxicity is the result of anti-class I antibodies produced following previous transplantation, transfusion or pregnancy, a process referred to as "sensitization".

Conversely there have been several reports that a positive B cell cross-match is not a disadvantage (Ettenger et al,(1976);Morris et al, (1977); d'Apice and Tait,(1979); Ting and Morris (1979). This could be because some positive B cell cross-matches are caused by auto-antibodies (Park, Terasaki and Bernoco,1977). Chapman, Ting and Morris (1985) have recently suggested that non-autoreactive B cell antibodies are associated with poor allograft prognosis.

4. Antigen presentation by the renal allograft

Initiation of the host immune response to the allograft requires donor cells presenting class II antigens. The situation in man differs from that in the rodent models generally employed in the investigation of antigen presentation by renal allografts. Although renal vascular endothelium in the rat does not express class II antigens, in man it does (Daar et al,1984 b). Hirschberg, Bergh & Thorsby(1980)

have shown that antigen-bearing endothelial cells can substitute for accessory cells in the lymphoproliferative response of T lymphocytes to soluble protein antigen. Cerilli et al (1985) claimed that a vascular endothelial cell antigen system, linked to the Major Histocompatibility Complex, is implicated in rejection.

In the rat, the absence of class II endothelial antigens points more clearly to passenger donor cells in the allograft as the source of antigen presentation. A series of experiments before class II antigens were identified led Guttmann, Lindquist & Ockner (1969) to suggest that the important immunogens in renal allograft rejection in rats were not cells from the kidney itself but cells of haematopoietic origin. Klinkert, La Badie & Bowers (1982) demonstrated that a cell type distinct from the macrophage population, the dendritic cell, functioned as the accessory cell required for the rat lymphocyte response to periodate. Lechler and Batchelor (1982) provided further evidence for the importance of the dendritic passenger leucocyte in a series of elegant experiments. Long-surviving, enhanced renal allografts were transplanted into a second, unenhanced rat of the same strain as the first recipient. Rejection did not occur unless donor strain dendritic cells were injected into the new host, with neither T nor B lymphocytes in comparable numbers having any effect. These findings have been confirmed by other workers in different animal models. The expression of class II antigens without dendritic cells may not be sufficient to induce the immune response (Austyn et al, 1985).

5. Destroying the renal allograft: the efferent limb of the immune response

The broad categories of the immune response involved in rejecting

the renal allograft are illustrated in Figures 1 to 3 (adapted from Stiller & Keown (1984). Not illustrated is hyperacute rejection resulting from preformed cytotoxic antibody against antigens of the Major Histocompatibility Complex (Kissmeyer-Nielsen et al, 1966). All the mechanisms illustrated require T lymphocytes; experiments in rodents involving T cell depletion then reconstitution with phenotypes defined by monoclonal antibodies have demonstrated the necessity for the T helper subset (reviewed by Hayry 1984).

Histological examination of rejecting kidneys has traditionally divided early allograft failure other than hyperacute rejection into two major pathways; a) vascular: platelet thrombi and fibrin in capillaries, foci of fibrinoid necrosis in vessel walls, and interstitial haemorrhage; b) cellular: interstitial oedema and focal cortical infiltrates of lymphocytes, plasmacells and macrophage (More ,1985).

Cytological analysis of infiltrating cells has been extended by the development of monoclonal antibodies. Using monoclonal analysis of cells infiltrating rat renal allografts, Bradley, Mason & Morris (1985) have suggested that allograft rejection, in the rat at least, is mediated by specific cytotoxic T lymphocytes, and that the major role of the T helper cell may be to assist this process. (Figure 1)

Relative to man, the rat has a weak delayed hypersensitivity response (Mason, et al, 1984) and so data based on rats may underestimate the importance of this response in man. (Figure 2)

The antibody response may be important even in recipients without preformed cytotoxins. Lymphocytotoxins formed during rejection

are deposited in the kidney (McKenzie & Whittingham, 1968). The histological changes of vascular rejection, in particular, would fit with damage to endothelium by complement-fixing antibody, with secondary platelet and fibrin deposition and thrombosis. Coating of target cells by antibody could also lead to antibody-dependent cell cytotoxicity by K cells. (Figure 3)

Reviewing the evidence on mechanisms of allograft destruction, Hayry concluded that an immunological stimulus so great as a transplanted kidney was likely to provoke a number of modes of response. (Hayry, 1984).

CHAPTER 3

THE DEVELOPMENT OF CLINICAL RENAL TRANSPLANTATION

1. 1900 to 1950

i) Human Organ Transplantation

"The results of clinical organ grafts have now surpassed any reasonable expectation based on immunological theory". Developments in transplantation have not always been so haphazard immunologically as this comment from Calne (1984) might suggest. However, attempts at tissue transplantation in the early part of the century were based as often on folklore and an appreciation of the commercial possibilities as on developments in surgical technique and immunological knowledge.

New techniques of vascular anastamosis, for which Carrel took lasting credit and a Nobel Prize, provided scope for the enterprising surgeon who was not constrained by considerations of species, far less tissue type. Between the wars, an American move to treat sexual dysfunction in men by transplanting monkey testes was transiently lucrative, but gave no lasting benefit either social or scientific (Hamilton, in press and 1984).

It would be surprising if the association with charlatanry deterred the medical profession from exploring the potential of transplantation. Between the wars, however, there was a dearth of clinical study. The Russian surgeon, Voronoy, has been given the credit for the first human cadaveric renal transplant, performed in 1936 and an achievement marred only by the death of the patient two days later (Joekes, Porter and Dempster, 1957).

Unlike the contemporary anecdotes of human organ transplantation, some reports of skin grafting did accept the relevance to graft success of genetic differences between individuals, as expressed at the still relatively unsophisticated level of blood grouping (Masson, 1918; Shawan 1919).

ii) Tissue Antigens in Experimental Transplantation

Scientific interest before 1950 concentrated more on tumour than organ transplantation, not least because microvascular techniques for organ transplantation in laboratory rodents had not yet been devised. It was in this field that Gorer (1937) produced his classic article on "The genetic and antigenic basis of tumour transplantation". By meticulous attention to the purity of his strains of albino mice he was able to demonstrate clearly that sarcomas grown in one strain could be transplanted successfully to other mice of the same strain and to certain F_1 hybrid combinations, but not to outbred mice.

The point that more than simply blood grouping was necessary in skin grafting was summarised by Sir Peter Medawar in 1946: "The idea that there exists an intimate relationship between the forms of incompatibility revealed by blood transfusion and tissue transplantation has been more widely accepted than any concrete evidence for it allows" (Medawar 1946).

In a series of experiments during the 1940s, Medawar and Gibson described the process of skin graft rejection in rabbits (Gibson and Medawar,1943; Medawar, 1945, 1946). Interestingly, although at the time the results may have been regarded by surgeons as a source of

inspiration rather than practical guidance (Calne, 1984), reassessment of the data with hindsight (Medawar, 1946) shows the beneficial effect of blood transfusion on graft survival long before its acceptance in renal transplantation (Opelz et al, 1973).

2. 1950s : Human Renal Transplantation

Attempts at human renal transplantation began in earnest in the 1950s in the absence of maintenance dialysis, tissue matching and sub-lethal immunosuppression. The absence of maintenance dialysis not only reduced the population awaiting transplantation but justified the use of transplantation as a last-ditch manoeuvre to prevent death from uraemia (Hamilton 1984).

Appreciation of the importance of attempting genetic matching led to many of the early renal transplants being between identical twins, although there were notable efforts at transplantation from unrelated donors (Hume et al, 1955; Murray, 1982). Excellent results with the twin pairs were not paralleled for unrelated donors; sadly these transplants only confirmed the importance and histological findings of rejection.

3. 1960s

i) Immunosuppressive Drug Therapy

The limitations of a transplant programme based on the availability of an identical twin highlighted the need for therapy to suppress the immune response to the allograft. During the late 1950s, the staple immunosuppressive therapy was irradiation, which seldom prevented

allograft rejection even in near-lethal doses (Calne, 1984).

The observation that certain organic chemicals could induce immune suppression in laboratory animals had been made in Germany in the early years of the century (Hamilton,1984). However, it was not until the 1950s that 6-mercaptopurine, an antipurine being developed for projected use as an antimetabolite of neoplastic cells, was used to induce selective immune tolerance to foreign protein in rabbits (Schwartz and Damashek, 1959). Calne (1960) showed some inhibition by 6-mercaptopurine of renal allograft rejection in dogs, and followed this up with better results using another purine analogue, Azathioprine (Calne et al, 1962).

Tentative treatment in man began; the first drug-treated recipient of a cadaveric kidney to survive for over a year was reported in 1963 and, in the reminiscences of Murray (1982), "it was immediately evident that drug therapy was more efficient and less dangerous than total body irradiation". The addition of Prednisolone to Azathioprine as standard immunosuppression led to what Murray (1982) has called "the era of boundless optimism" of the mid nineteen-sixties, when the advances of the previous five years were assumed to presage those of the next five.

ii) Leucocyte Antibodies and Antigens

The availability of less crude immunosuppressive therapy was paralleled by the development of maintenance haemodialysis and with it an increasing number of patients for whom transplantation was a possibility (Quinton, Dillard and Scribner, 1960). Transfusion policies for anaemic patients on haemodialysis were possibly, by

today's standards, liberal. It was inevitable that a proportion of patients on regular haemodialysis would acquire antibodies to the newly-described leucocyte antigens (van Rood, van Leeuwen and Bruning, 1967).

In 1966, Kissmeyer-Nielsen et al reported the first two cases of hyperacute rejection of renal allografts in man. Both patients had been heavily sensitized by transfusion and pregnancy, with pretransplant serum containing leucocyte isoagglutinins. Concern about sensitization contributed to a more sparing approach to the use of blood, in retrospect a mistake (Opelz et al, 1973).

The widespread introduction, to try to pre-empt hyperacute rejection, of the lymphocytotoxicity technique described by Terasaki and McLelland (1964) contributed to further elucidation of the HLA system (van Rood et al, 1967). Lymphocytotoxicity proved more sensitive and more reproducible than the previously used leucoagglutination techniques (Dick, 1979). Growing appreciation of the complexity of leucocyte antigens and a modicum of standardization of antigen typing facilitated the establishment of multicentre organ sharing schemes.

Murray's era of boundless optimism (Murray, 1982) faded with the realisation that neither the tissue matching nor the immunosuppressive therapy which was available could guarantee successful transplantation from unrelated donors. Far from it; one-year cadaveric graft survival rates quoted in 1974 were still of the order of 50 %, no better than several years previously (Opelz et al, 1974b). Use of historical controls may have obscured any beneficial effect on graft survival of the introduction of matching for HLA-A and B loci, concurrent as this was with minimization of blood transfusion.

4. 1970s

i) The Blood Transfusion Effect

If the collection of multicentre data needed any justification, it was amply provided by the demonstration of the beneficial effect of pretransplant blood transfusion on renal allograft survival (Opelz et al, 1973). This was not only the most important advance in clinical transplantation over a decade (see Chapter 4) but stimulated considerable scientific interest and controversy (see Chapter 5).

ii) HLA-DR Typing

The advent of HLA-DR typing (Ting and Morris, 1978a), while promising, did not yield widespread immediate benefit. In 1979, Sachs commented that, of the "restricted" number of laboratories in a position to type for DR, "only a few can claim to do so competently". Problems in standardization may have detracted from the perceived impact of DR typing, although the bulk of evidence now is that DR is the most important known locus, with matching for both B and DR improving graft outcome further (Opelz, 1985 b).

5. 1980s

i) Cyclosporin A

After almost twenty years when the staples of convential immuno-suppressive therapy were Prednisolone and Azathioprine, the position changed dramatically. The development of Cyclosporin A has become a legendary piece of pharmaceutical serendipity. Borel's discovery of

the immunosuppressive properties of a cyclic peptide under scrutiny as an antifungal agent, led to its clinical development in Cambridge (Borel, 1976; Calne et al, 1978).

Cyclosporin affects the initiation of the immune response by inhibiting interleukin release (Bunjes et al, 1981). While preventing the activation of precursor cytotoxic T lymphocytes (Hess and Tutschka, 1980), it may spare mature cytotoxic cells and permits the development of both antigen-specific and antigen-non-specific suppressor T lymphocytes (Kupiec-Weglinkski et al, 1984). In man, Cyclosporin seems to affect the primary humoral response to both T-dependent stimuli such as pokeweed mitogen and T-independent stimuli such as Staphylococcus aureus (Paavonen and Hayry, 1980). The primed T helper cell in the secondary humoral response may, however, have lost its susceptibility to Cyclosporin (Kunkl and Klaus, 1980).

The capacity of Cyclosporin to affect primarily the inception of the immune response makes it ideal for organ transplantation, where new antigens are being introduced, but may detract from its application to established immune processes. Reports of successful extension of its use to autoimmune disease are as yet distinctly preliminary (Stiller et al, 1984; Assan et al, 1985).

There is no doubt that the introduction of Cyclosporin has led to significant improvement in renal allograft survival, while not obviating the benefits of HLA-matching and pretransplant blood transfusion (Cats et al, 1984; Opelz, 1985 b). The initial report from the European Multicentre Trial Group (1983) of a one-year first cadaveric allograft survival rate of 72% with Cyclosporin alone against 52% with Prednisolone and Azathioprine has now been followed through to three years with graft

survival of 66% and 42% respectively (Calne and Wood, 1985).

Still poorly established is the need for concomitant steroid therapy. Unlike the European trial, the Canadian Multicentre Study Group trial(1983) included Prednisolone in its Cyclosporin protocol and reported a one-year allograft survival rate of 77%, against 66% with conventional immunosuppression. The concurrent administration of low doses of Prednisolone and Cyclosporin may yield good results with minimum toxicity (Thiel et al, 1984), and the addition of Azathioprine has also been suggested (Sutherland et al, 1985).

Enthusiasm for Cyclosporin has been only partially dimmed by realisation of its side-effects. Lymphomas have been regarded as reversible on reduction or cessation of therapy, and a consequence of removal of T-cell-mediated suppression of B cells previously infected by Epstein-Barr virus (Starzl et al, 1984). Tremor, paraesthesiae, hirstutism and gum hypertrophy have caused less anxiety - to the physician - than has hepatotoxicity, another drug-related problem.

Particularly worrying in the context of renal transplantation, is nephrotoxicity. Hypertension and a raised serum creatinine have been consistent findings from the initial trials onwards. Klintmalm et al (1984) have suggested that the progressive glomrular sclerosis described by Myers et al (1984) may be dose-related. Attempts to avoid long-term nephrotoxicity by weaning patients off Cyclosporin onto conventional therapy at least three months after transplantation have been marred by a significant incidence of rejection (Wood et al, 1983; Adu Michael and McMaster, 1985).

The striking effectiveness of Cyclosporin is underlined by its

widespread adoption despite these problems (Brunner et al, in press).

ii) Monoclonal Anti-lymphocyte Antibodies

Polyclonal anti-lymphocyte globulin preparations have been in use since the 1960s (Calne, 1984). Human lymphocytes or thymocytes are injected into another species, generally horses or rabbits. The resulting immune serum is then absorbed against non-lymphoid cells to try to remove other potentially toxic antibodies, and infused into the transplant recipient. While reported by some centres as effective in the treatment of rejection, the use of anti-lymphocyte globulin has been limited by batch variation, adverse reactions on administration and fears of over-immunosuppression (Najarian and Simmons, 1971; Monaco, Campion and Kapnick, 1977; Stiller and Keown, 1984).

Monoclonal anti-lymphocyte antibodies are a recent refinement of the same principle, (McKearn et al, 1979). Now available against a variety of human lymphocyte subsets, monoclonal antibodies are generally cloned from mice. Their use in clinical renal transplantation remains experimental, although successes (and toxicity) have been reported using antibodies against mature lymphocytes and lymphoblasts (Cosimi et al, 1981; Kirkman et al, 1983; Takahashi et al, 1983).

6. Other Developments

Over the period discussed in this chapter, there have, of course, been considerable advances in areas not mentioned, but relevant to the outcome of transplantation. Notable amongst these are improvements in access to and preservation of donor kidneys, and increased

experience in the maintenance of reasonable general health in patients on regular dialysis. Of the advances mentioned above, more detailed consideration will now be given to the blood transfusion effect on renal allograft survival.

CHAPTER 4

THE BLOOD TRANSFUSION EFFECT IN CLINICAL RENAL TRANSPLANTATION

Concern in the late 1960s about sensitization following blood transfusion divorced from clinical consideration the considerable evidence on blood transfusion and graft enhancement which had accrued over the previous twenty years. Although much of this came from species less prone than man to develop anti-lymphocyte antibodies (Morris, 1980) there were a few pieces of corroborative evidence in man. The claim by Opelz et al in 1973 that pretransplant blood transfusion correlated with renal allograft survival reversed the prevailing clinical dictum and forced a reappraisal of previous work.

1. Early evidence for the transfusion effect

Medawar concluded in 1946 from experiments in rabbits that "immunity to skin homografts following massive intravenous transfusions of homologous whole blood is either trivial or absent". Shortly afterwards this was neatly, if inadvertently, demonstrated in a single case in man (Kearns and Reid, 1949). The success in this case of parent-to-son skin grafting was attributed to prior parent-to-son therapeutic transfusion, and was likened by Snell (1952) to the existing literature on tumour transplantation.

The doyen of studies of tumour transplantation was reported by Flexner and Jobling in 1907. They found that preheated rat sarcoma cells injected intraperitoneally promoted growth of that tumour. Interestingly, neither unheated sarcoma cells nor heated whole blood affected tumour growth.

The work of Kaliss led him to coin the term "immunological enhancement" for "the successful establishment of a tumor homograft ... as a consequence of the tumor's contact with specific antiserum in the host". "Conceivably," he added, "the term could be applied to the experimentally prolonged survival of homografts of normal tissues" (Kaliss and Molomut, 1952; Kaliss, 1958).

Billingham, Brent and Medawar in 1953 described "actively acquired tolerance" in mice and chickens. If exposed to donor cells as neonates they would fail to reject skin grafts as adults. This effect was donor-strain specific.

The finding that survival of skin grafts in even adult mice was prolonged with prior transfusion of donor blood led Marino and Berain (1958) to draw parallels with the description by Felton (1949) of "specific immunoparalysis" to pneumococcal polysaccharide. The conclusion that paralysis rather than saturation of the immune response was responsible for their findings was also reached by Stark and Dwyer (1959), who showed that survival of first and second skin grafts in rabbits was prolonged by pretreatment with donor blood. Peer (1958) reported successful parental skin grafting to human infants after donor blood, although maternal grafting appeared more successful. Stone et al (1965) found that freemartin cattle twins with erythrocyte chimerism showed delayed rejection of exchanged skin grafts.

With the development of the canine renal allograft model, Egdahl and Hume (1956) tried pretransplant cross-circulation between donor and recipient. Conflicting results in different animal pairs may have reflected both sensitization leading to rapid graft loss and active enhancement of graft survival. Less inconclusive results came from

Halasz, Orloff and Hirose (1964), who claimed that prolongation of renal allograft survival was achieved in every dog whom they pretreated with donor blood. A human case of donor-specific transfusion before renal transplantation was reported in 1965 by Eschbach et al. The authors' pragmatic response to renal failure in one patient and bone marrow failure due to acute leukaemia in another was to employ cross-circulation between the two. Both weathered this manoeuvre daily for 4½ months, during which period exchange of skin grafts proved unsuccessful. After the leukaemic girl succumbed to a Pseudomonas septicaemia, one of her kidneys was transplanted into the uraemic youth. On the credit side, he acquired no evidence of Pseudomonas sepsis or leukaemia; unfortunately, the kidney infarcted.

The first case in man where successful renal transplantation was preceded by donor-recipient cross-circulation was reported in 1967 by Dossetor et al. These cases of premeditated donor-specific transfusion were clearly experimental. Moreover, although the animal work on which they were based was sound, extension of it to clinical transplantation was impractical for the majority of patients. Donor-specific transfusion might be feasible for the small group with live prospective kidney donors, but the important issue clinically was the preparation for cadaveric transplantation of patients now being offered maintenance haemodialysis.

Although Dossetor et al (1967) claimed that profusely transfused patients did better, no direct relationship between non-donor blood and allograft outcome was found in other studies (Morris, Ting and Stocker, 1968). Rather, the deleterious effects of sensitization by transfusion were emphasised (Kissmeyer-Nielsen et al, 1966).

At the same time, animal work continued to point to an association between previous exposure to donor cells and allograft survival (Zimmerman et al, 1968; Ockner, Guttmann and Lindquist, 1970). Experiments in rats using donor-strain pretreatment led Fabre and Morris (1972) to underline that "pregraft exposure to blood transfusion might not necessarily be harmful".

2. Clinical confirmation of the transfusion effect

It was against this background of laboratory experience marred by clinical misfortune that Opelz and Terasaki presented their evidence that third party (random donor) blood transfusion was associated with improved renal allograft survival. In a series of papers over several years (1973, 1974, 1978), their multicentre retrospective analyses suggested that patients given whole blood or packed red cells, but not frozen cells, had improved graft survival to a degree dependent on the amount of blood received. For example, in a study of over 1300 cadaveric transplants, graft survival at one year was 42% for recipients who had never been transfused and 71% for those who had received more than 20 units of blood (Opelz and Terasaki, 1978).

The association between transfusion and graft survival was widely confirmed and deliberate policies of pretransplant transfusion implemented. Analysis of results in our own centre (Briggs et al, 1978) showed a striking difference in 1-year graft survival between untransfused recipients (28%) and patients given whole blood (77%).

Less clear, however, was how much and what type of transfusion would produce most benefit with least sensitization. Opelz, Graver and Terasaki (1981 a) took a relaxed view of the risks of sensitization

observing that only 3 of 331 patients studied prospectively formed cytotoxic antibodies to more than 90% of a random donor panel after up to 20 units of blood. In a third of patients the titre of antibody fell after further transfusion (Opelz et al, 1981 b).

On the grounds that matching of histocompatibility antigens of blood donor and recipient might reduce sensitization, Nube et al (1983) gave transfusions matched at the A and B loci, and suggested this manoeuvre elicited less sensitization yet full advantage. If substantiated this finding would suggest that the mechanism of the transfusion effect is not dependent on antigen discordance at the A or B loci. The practical difficulties of adopting a policy of HLA-matched transfusion for all patients are likely to deter other centres from following suit unless more evidence in its favour emerges. Not surprisingly, the main aims in the formation of elective transfusion protocols remain to maximise graft survival and minimize sensitization.

3. Amount of blood required for the transfusion effect

Although Opelz and Terasaki (1978) and Fehrman (1982) suggested a direct dose-response relationship, Persijn claimed, initially retrospectively (Persijn et al, 1979) but later prospectively (Persijn, D'Amaro and van Rood, 1984), that as little as one unit of blood was enough to provide the benefit of transfusion, although not if blood entirely depleted of lymphocytes were used.

This finding was attributed by Terasaki (1984) to crossreactivity among HLA antigens sufficient to allow blood from one donor to immunise to many. Terasaki's own group (Horimi et al, 1983) assessed the

benefit from transfusion of only one unit in terms of 10% 1-year graft survival, a figure estimated by Terasaki to be compatible with the effect of crossreactivity.

A number of different studies looked for the transfusion effect with intermediate amounts of blood. Frisk, Brynger and Sandberg (1982) (2 units), Feduska et al (1982) (1 to 5 units) and Horimi et al (1983) (up to 15 units) all found improved graft survival in transfused subjects. In numbers of patients too small to draw definitive conclusions, Sirchia et al (1982) described a trend towards lower graft survival in patients given, instead of three standard sized transfusions, three small aliquots of packed red cells (mean volume 31 ml).

The enthusiasm of a particular centre for minimising elective transfusion may reflect its experience of sensitization. Also, proponents of a donor-specific mechanism for the transfusion effect might advocate exposure by transfusion to a wide range of alloantigens prior to transplantation of a kidney bearing antigen hopefully rendered familiar. Advocates of a non-specific mechanism for the transfusion effect might argue that there was no necessity to expose the recipient to more than a minimal range of antigens.

4. Timing of transfusion

i) Time interval between transfusion and transplantation

Both Werner-Favre et al (1979) and Hourmant, Soulillou and Bui-Quang (1979) noted that transfusion within the three months prior to transplantation conferred an advantage in cadaveric allograft survival.

This also appeared to be the case in the 402 patients reported by Opelz (1985 a) who had been transfused only one unit of blood previously. No advantage in recent transfusion was seen in nearly 6000 patients in the same study who had had two or more transfusions.

ii) Influence of perioperative transfusion

Despite some suggestions that perioperative transfusion ameliorates transplant survival (Stiller et al, 1978; Williams et al, 1980) there are several reports to the contrary (Feduska et al, 1982; Glass et al, 1982; Opelz 1985 a). However, Terasaki et al (1982) and Opelz (1985 a) did hint at benefit in perioperative blood for patients never previously transfused.

5. Blood components required

i) Experimental models

In the absence of a clearcut mechanism for the transfusion effect (see Chapter 5), trials of various blood components have been conducted in various species and strain combinations. Martin et al (1982) and, more recently, Wood and Morris (1985), gave purified blood components to rats one week before renal transplantation. Whole blood, erythrocytes, peripheral blood lymphocytes and platelets all prolonged graft survival; Martin et al found that macrophages and marrow mononuclear cells, but not thymocytes or plasma, did so too.

ii) Platelets

For obvious reasons, the sort of study described above (i), is

limited to experimental animals. However, there have been clinical trials of platelet transfusions in man. These have been based on observations in Rhesus monkeys. Oh and McClure (1982) suggested that platelets induced a low sensitization rate, possibly due to their lack of class II antigens. Findings in monkeys that platelets improved renal allograft survival without inducing cytotoxic antibodies (Borleffs et al, 1982) were not confirmed by Marquet et al (1983) in beagle dogs, nor by Chapman in man. Chapman et al (1985 a) detected no sensitization following platelet transfusion but the incidence of rejection during the first week following transplantation was higher than would have been expected in unsensitized patients. Only 3 out of 8 patients transfused only with leucocyte-free platelets were alive with a functioning graft at the time of writing, from which Chapman et al concluded that the platelet protocol had not stimulated the transfusion effect.

Few other centres have sought to assess the value of platelet transfusion in renal transplantation. The Collaborative Transplant Study collected information on 36 patients transfused with platelets alone, and concluded that the six-month transplant survival rate of only 55% did not support Borleffs' claim (Opelz 1985 a).

iii) Leucocytes

There is clinical evidence that leucocytes are needed for the transfusion effect in man. This is based on attempts to reduce leucocytotoxin production by administering frozen, filtered or stored blood. Freeze-thawing of blood has been shown to reduce the mitogen response of potentially responsive cells, possibly by reducing their numbers rather than their efficiency (Knight and Farrant, 1978).

One-year graft survival rates given in 1974 by Opelz and Terasaki were 53% for patients transfused with packed red cells or whole blood, 32% for patients who had never been transfused, and only 20% for patients who had received frozen cells only. Persijn et al (1984) compared washed (classed as leucocyte-poor) and filtered (classed as leucocyte-free) red cells and found that while as few as one unit of leucocyte-poor blood enhanced graft survival, there was no evidence of any benefit from leucocyte-free transfusion.

Using a protocol involving storage for at least one week, Light advocated blood storage. This idea centred on in vitro observations that storage led to loss of T but not B lymphocytes. There would be fewer class I antigens on T lymphocytes to induce cytotoxins but plenty of class II antigens on B lymphocytes to induce transplant protection (Light et al, 1982).

Animal work has not contradicted these clinical observations. Strom, Soulillou and Carpenter (1977) have claimed that only cells capable of stimulating in mixed lymphocyte cultures can induce active enhancement of allograft survival in rats. Using a rat cardiac allograft model, Lauchart, Alkins and Davies (1980) showed allograft survival correlated with the percentage of B lymphocytes in the immunizing cell population. Recipients immunized with erythrocytes or T-enriched lymphocytes showed no significant graft prolongation.

6. Donor-specific transfusion

i) Live related donors

With blood transfusion no longer out of bounds, the earlier animal

work on donor-specific transfusion began to be extended to man. Although donor-specific transfusion might have been considered an unwarranted exercise in HLA-identical donor-recipient pairs, and an unjustified risk in one-haplotype-matched pairs with low reactivity in mixed lymphocyte culture, the position was different for pairs with high reactivity. This group had been shown to have a poorer renal allograft prognosis by Cochrum, Salvatierra and Belzer (1974). The same researchers commenced a trial of donor-specific transfusion in this poor-risk group of donor-recipient pairs. The protocol used was that subsequently adopted in most later trials, and indeed in our own patients reported later in this volume; 200 ml of whole blood or packed cell equivalent was administered at 2-weekly intervals on a total of 3 occasions. The results, compared to the historical control group, were excellent. 95% of the patients transplanted had functioning grafts at one year (Salvatierra et al, 1980). Other centres reported similar findings (for example, Takahashi et al, 1982; Leivestad et al, 1982; Mendez et al, 1982).

ii) Unrelated donors

As the practice tends to be to use only close, well matched, well motivated relatives as live kidney donors, experience of donor-specific transfusion between unrelated pairs is relatively limited. Newton and Anderson successfully transplanted two patients after transfusion of peripheral blood lymphocytes from their unrelated kidney donors (Newton and Anderson, 1973 and 1979). Both of two recipients reported by Ruzany et al (1983) suffered early graft loss although they had no evidence of pretransplant cytotoxins. The few cases reported by Sollinger et al (1984) and Bowen et al (1984) were less disastrous. Sollinger gave donor-specific transfusion accompanied by Azathioprine

to 20 recipients. Unrelated donors consisted of 5 wives, 1 husband and 1 friend; there were also 9 two-haplotype-mismatch siblings and 4 non-first-degree relatives. The only graft lost through rejection at the time of reporting was one from a mismatched sibling. Of 8 patients transplanted by Bowen, one graft (from a husband) had been lost at the time of reporting. The total number of recorded cases of donor-specific transfusion from unrelated donors is still too small for definitive comment.

iii) Sensitization following donor-specific transfusion

The major problem encountered in Salvatierra's initial trial (Salvatierra et al, 1980) was the familiar one of sensitization. With the donor-specific transfusion protocol 30% of the 145 transfused subjects developed anti-donor lymphocytotoxins and were excluded from transplantation. This sensitization rate, much higher than that expected from third party blood (Opelz et al, 1981 a) was duplicated (Ettenger et al, 1983) in subsequent reports of donor-specific transfusion, such as those mentioned above (i).

Moreover, continuing transfusion from the same donor may lead to even higher anti-donor sensitization, as shown previously by Ferrara et al (1974). Ferrara's study involved matching normal subjects into 62 donor-recipient pairs. Each recipient then received small aliquots of blood from the same donor at weekly intervals. Fifty recipients developed anti-donor cytotoxins although 5 lost them again after further transfusion. Comparison with standard donor-specific transfusion before renal transplantation must be tempered by the differences that blood was administered more often, and to non-uraemic subjects.

The development of anti-donor lymphocytotoxins has been regarded as the major immunological contraindication to transplantation. Several workers have suggested that this oversimplifies the problem. Some anti-donor cytotoxins produced after donor-specific transfusion may be compatible with good graft survival (Glass et al, 1985). It is also possible that donor-specific transfusion could induce deleterious antibodies other than conventional lymphocytotoxins.

Although it is known that antibodies to blood group ABO antigens are important in renal transplantation, antibodies to the Rhesus and several other blood group systems appear unrelated to graft survival, (Gleason and Murray, 1967). It has been suggested that rejection may be associated with antibodies to Lewis antigens, which are found not only on red blood cells but also on lymphocytes and in plasma (Oriol et al, 1980; Spitalnik et al, 1984).

Gluckman et al (1982) reported the development of antibodies to granulocytes and monocytes following donor-specific platelet transfusion, and suggested that the latter may be relevant to Cerilli's work linking antigens on monocytes to those on renal vascular endothelium. Indeed, Cerilli has suggested that monocyte cross-matches should be performed routinely, since the monocyte expresses the "vascular endothelial cell antigen system", and T and B lymphocytes do not. (Cerilli et al, 1985).

iv) Avoidance of sensitization using immunosuppression

An interesting approach to the avoidance of sensitization during both donor-specific and third party transfusion has been the use of Azathioprine or Cyclosporin. Studies advocating this idea include those by Anderson et al,(1983); Glass et al (1983) and Raftery et al (1985).

Indeed it was in 1973 that Newton and Anderson first reported the use of Azathioprine in this context. Most studies have been successful but small. Garovoy et al (1985), however, have now amassed 60 patients given Azathioprine and 101 given donor-specific transfusion alone. Although Azathioprine appeared helpful in reducing the incidence of T warm antibodies in patients with minimal pre-existing sensitization, those with pre-existing sensitization or previous transplants had a sensitization rate around 30% as before (Salvatierra et al, 1980).

v) Future of donor-specific transfusion

While the use of donor-specific transfusion is scientifically interesting, its apparent advantage over third party transfusion may be obsolete with increasing use of Cyclosporin rather than Azathioprine as post-transplant immunosuppression. Recent data from the Collaborative Transplant Study show that in one-haplotype-matched live related donor transplantation the one-year graft survival rate is around 90%. This figure is irrespective of whether the recipient had received donor-specific or at least three units of third party blood (Opelz, 1985c). From this data Opelz concluded that there was no advantage in donor-specific transfusion for haploidentical recipients.

7. Relevance of the transfusion effect outwith transplantation

"Although it is now generally accepted that blood transfusion may promote the survival of renal allografts in patients treated with immunosuppressive drugs, the possibility that it may also modify the response of other hosts to other immunological stimuli has been litte considered". So wrote Woodruff and van Rood in 1983, introducing

a series of parallels between the transfusion effect and clinical situations outwith transplantation.

Woodruff and van Rood posed the question whether reports of acquired immune deficiency in haemophiliacs (Menitove et al, 1983) could be explained by repeated administration of blood products in the absence of a primary infective agent. The impact on the immune system of clotting factor preparations is discussed further in Chapter 13.

They questioned whether the purported benefits of plasma exchange in autoimmune disease (reviewed by Shumak and Rock, 1984) were due to replacement with plasma rather than removal of immune complexes or autoantibody, and they touched on the apparent effectiveness of intravenous gammaglobulin preparations in the management of idiopathic thrombocytopenic purpura (Imbach et al, 1981, Schmidt et al, 1981).

Another controversial area discussed was the relation between blood transfusion and tumour growth. Evidence that patients on renal replacement therapy have an increased incidence of neoplasm has not been accompanied by evidence on the relation with blood transfusion (Halpert et al, 1985; Hanto et al, 1985; Sheil et al, 1985).

There have been persistent, but poorly controlled, claims that blood transfusion promotes tumour growth. Francis and Shenton (1981) found that transplanted experimental sarcoma in rats grew more rapidly in animals previously given allogeneic blood that in animals transfused with no or syngeneic blood. It has been claimed that the prognosis of colonic cancer is worsened if the patient is given blood (Burrows and Tartter, 1982; Blumberg, Agarwal and Chuang, 1985). Despite claims that transfused and untransfused subjects were well matched, it would

still seem likely on clinical principles that patients with more advanced disease at the time of presentation are more likely to require transfusion.

Moffat and Sunderland (1985) came up with a similar trend on retrospective analysis of patients with renal adeno-carcinoma. However, none of these reports does more than suggest that the association between blood transfusion and tumour growth is worth pursuing.

Woodruff and van Rood (1983) stressed the need for collection of more data to explain the relationship between these various phenomena. The experiments reported later in this volume deal not solely with blood transfusion in transplantation, but also with the impact on the immune system of exposure to other alloantigenic material, including blood products.

Not discussed by Woodruff and van Rood (1983) were attempts to adapt the transfusion effect to the management of pregnancy in women with recurrent spontaneous abortions. Immunization with paternal (Mowbray et al, 1985) or third party leucocytes (Taylor and Faulk, 1981) has been associated with a few successful pregnancies.

Beer et al (1981) suggested that couples with recurrent abortions had an increased frequency of sharing of HLA antigens. Although this finding was disputed by Jeannet et al (1985), such antigen sharing might reduce the likelihood of production of maternal serum factors reducing her immune response against the fetus (Rocklin et al, 1976; Stimson, Strachan and Shepherd, 1979; Power et al, 1983 a and b).

In contrast, in renal transplantation HLA antigen sharing with neither blood (Nube et al, 1983) nor kidney (Opelz, 1985b) appears to be detrimental. It remains to be seen whether the transfusion effect can be extrapolated to pregnancy, and to the other situations mentioned in this section.

CHAPTER 5

THE MECHANISM OF THE TRANSFUSION EFFECT ON RENAL ALLOGRAFT SURVIVAL

The purpose of this chapter is to review some of the mechanisms which have been suggested. Irrespective of the passion with which these may initially have been proposed, none is unequivocally established and few are mutually exclusive.

1. Prolongation of time on dialysis

This indirect mechanism for the blood transfusion effect was based on the observations that:

a) the number of units of blood given for therapeutic reasons increases with time spent on dialysis (Guttman, 1978)

b) elective blood transfusion leads to the chain of sensitization, exclusion from rapid transplantation, hence inadvertent prolongation of uraemia (Cheigh et al, 1981)

c) uraemia itself is immunosuppressive; long periods of it may be more so (Keane and Raij, 1983).

Watson et al (1981) observed that graft survival increased and cell-mediated immunity decreased with time on dialysis but did not separate fully the effects of dialysis time and transfusion. However, it has been argued that the effect of transfusion is independent of that of uraemia, on the grounds that the transfusion effect is seen most clearly in the first three months post-transplant and the effect of dialysis time is spread over years (Terasaki et al, 1982; Bucin et al, 1984; Gilks et al, 1985).

2. Selection of "good risk" patients

i) Immunological selection

Challenging potential recipients with donor antigens in blood could lead to the development of cytotoxins to preclude transplantation of unsuitable donor-recipient pairs. This selection effect could apply to either donor-specific or third party transfusion (Salvatierra et al, 1980; Terasaki et al, 1982). The evidence for or against this hypothesis is confusing.

In the view of Opelz et al (1981 a) patient selection was not the mechanism of the transfusion effect, on the arithmetic ground that the degree of improvement in graft survival could not be explained by the small number of recipients sensitized by transfusion. Fewer than 30% of the patients in this study developed any cytotoxins at all. At the other extreme, of course, is the 30% sensitization rate quoted for donor-specific transfusion (Salvatierra et al, 1980).

Also against this hypothesis is the suggestion that removal of cytotoxic antibodies may enable successful transplantation across a previously positive cross-match. Taube et al (1984) reported that plasma exchange and immunosuppression removed and prevented the resysnthesis of HLA antibodies enabling successful transplantation in 4 out of 5 highly sensitized patients. Using a comparable manoeuvre, Hillebrand et al (1985) noted only a transient fall in titres of cytotoxic antibody.

There is no doubt that cytotoxic antibodies, whether induced by transfusion, transplantation or pregnancy lead to an increased risk of

graft loss (Opelz (1985 b) and accumulation of highly sensitized individuals on transplant waiting lists (Cheigh et al, 1981). The idea that these patients are in some way intrinsically untransplantable has been challenged recently by the United Kingdom Transplant Service (Bradley, Klouda and Ray, 1985). Patients with cytotoxic antibody reactive against more than 85% of a panel have been transplanted against a negative cross-match with regard for HLA matching subsidiary, and so far with moderate success.

Overall, it seems unlikely that selection is wholly responsible for the transfusion effect, although may well contribute to it.

ii) Selection for recipients' original renal disease

Before elective transfusion became widespread, a plausible explanation for the transfusion effect could have been the effect of the recipient's type of disease on his therapeutic blood requirement. Considering the mechanism of the transfusion effect, Opelz et al (1981 a) excluded a role for the original renal disease. Although the supporting data came from 33 centres, there were relatively few patients (174) in broad diagnostic categories. There is no evidence that the recipient's original disease affects graft function, except in the small number of patients who develop recurrent glomular disease or have multisystem disease (Brunner et al, in press).

3. Iron overload

Raised serum ferritin levels, as indicators of iron overload, have been correlated with previous transfusion (Keown, Stiller and Descamps-Latscha, 1984) and with various indices of depressed immune function.

In haemodialysis patients Waterlot et al (1985) found that raised serum ferritin levels were associated with an increased incidence of clinical infection and with diminished neutrophil function (measured in vitro by phagocytosis). Haemoglobin, ferritin and ferric ions can inhibit the lymphocyte response to mitogens and alloantigens (Matzner et al, 1979; Keown and Descamps-Latscha, 1983), possibly through inhibition of interleukin production (Keown and Descamps-Latscha, 1983). Lustbader, Hann and Blumberg (1983) claimed that response to hepatitis B vaccine correlated inversely with serum ferritin concentration.

The relevance of these findings to clinical transplantation was first suggested by results of Stark and Dwyer (1959), that injections of haemoglobin prior to skin grafting in rabbits prolonged graft survival. The possibility that the immunosuppressive effect of blood transfusion might be erythrocyte-mediated was raised by Keown and Descamps (1979), who suggested that phagocytosis of altered erythrocytes impaired subsequent macrophage function. In the later view of Keown et al (1984), this idea was overstated by de Sousa (1983), who attributed the transfusion effect to iron. While iron overload may contribute to non-specific immunosuppression after blood transfusion, its importance is undermined by the evidence for the presence of immunosuppression after small quantities of blood and for the absence of it after leucocyte-free red cells (Persijn et al, 1984).

4. Viruses transmitted by blood

Kirchner and Braun (1982) suggested that pretransplant transfusion might initiate or boost cell-mediated immunity against transmitted cytomegalovirus. This could be beneficial following transplantation,

when cytomegalovirus infection may be associated with serious super-infection and deterioration in renal function (Rubin and Tolkoff-Rubin, 1984). Against this hypothesis is the failure of Takasugi et al (1983) to demonstrate increased titres of anti-viral antibody after transfusion to an extent sufficient to explain the transfusion effect on graft survival.

The role of other viruses has come into prominence with the rise of the Acquired Immunodeficiency Syndrome(AIDS). Transmission of the newly categorized T cell leukaemia viruses by transfusion has been shown (Feorino et al, 1984), but there is no seroepidemiological evidence to link these with the transfusion effect on allograft survival. Similarly, there has been considerable work on Epstein-Barr virus and its relationship to B cell lymphoproliferation, accentuated in renal transplant recipients (Hanto et al, 1985). Reinherz et al, 1980 b) showed that T lymphocyte subsets could be deranged in infectious mononucleosis. This area is discussed further in Chapter 14. The contents of the rest of this volume reflect the author's view that the immunosuppressive effect of transfusion predisposes to rather than results from viral infection.

5. Alteration of T lymphocyte subsets

The availability of monoclonal antibodies to a variety of T cell surface antigens has led to proliferation of studies on T cell subsets in disease (Krensky and Clayberger, 1985). T helper cells, necessary to the initiation of the immune response to the allograft (see Chapter 2), are broadly defined by the OKT4 antibody, and T suppressor cells by the OKT8 antibody. The ratio of OKT4 positive to OKT8 positive lymphocytes has been used as an estimate of the

relative numbers of helper and suppressor T lymphocytes. Changes in lymphocyte subpopulations in blood and kidney after renal transplantation, recently reviewed by Wood, Thompson and Carter (1984), have not borne out the use of the T4:T8 ratio for immunological monitoring of renal allograft recipients.

The relation between blood transfusion and T cell subsets has been investigated by several workers. Mohanakumar et al (1983) showed that in 8 patients awaiting renal transplantation, the T4:T8 ratio fell from 1.9 to 0.9 (mean values) after blood pooled from 5 donors. Kaplan et al (1984) noted reduction in the T4:T8 ratio in non-uraemic subjects with sickle cell anaemia who had received multiple blood transfusions.

Although these findings are interesting, the lack of specificity of the T4:T8 ratio detracts from its contribution to elucidating the transfusion effect (Krensky and Clayberger, 1985). Moreover, in a study reported by Kerman et al (1982) patients who had received more than 5 units of blood showed greater suppressor cell function in mixed lymphocyte culture than those who had received less blood, but had equivalent proportions of OKT8 positive cells.

In the initial enthusiasm for its use, the importance of the T4:T8 ratio has been overstated in the literature.

6. Clonal deletion

Terasaki (1984) proposed a hypothesis which, he claimed, "accounts for most of the known facts about the transfusion effect". The evidence marshalled was attractive if not uniformly consistent with

findings from other groups (Burlingham et al, 1985 a; Streilein, 1985). Using polyclonal T cell activators in mice, Moller (1985) could demonstrate no cytotoxic anti-self T cells, and gave qualified support to the idea that self-tolerance, and possibly transplant tolerance, could be mediated by clonal deletion.

Terasaki's hypothesis is that transfusions immunize patients against a wide range of histocompatibility antigens. On second exposure, this time to antigens with the allograft, a vigorous anamnestic response occurs and clones of reactive cells proliferate. If immunosuppressive therapy is introduced at this point, the proliferating clones are preferentially destroyed, and the host cannot reject the graft.

If this theory is correct, the practical consequence in patient management is that immunosuppression should be introduced when reactive clones are proliferating, that is delayed until several days after transplantation. Terasaki suggested that the most appropriate therapy at that point would be cytotoxic monoclonal anti-blast antibodies.

7. Non-specific suppression of cell-mediated immunity

Evidence for non-specific suppression of cell-mediated immunity following transfusion has been presented by a number of workers in uraemic and, to a lesser extent, non-uraemic subjects. This topic is discussed in more detail in Chapter 10.

Dinitrochlorobenzene skin testing has been used as an *in vivo* correlate of cell-mediated immunity, diminished response being associated with previous blood transfusion and improved allograft survival (Watson

et al, 1979 and 1981; Russ et al, 1984). There have been several studies incorporating the effect of transfusion on the mitogen response in vitro (for example, Fehrman and Ringden, 1981; Jeannet et al, 1982; Klatzmann et al, 1983 a; Smith et al, 1983; Tait et al, 1984). Previous work is discussed more fully in relation to the author's results with mitogen stimulation in Chapter 12.

8. Induction of non-specific serum suppressor factors

i) Alpha-2-macroglobulin

The biochemical and immunological properties of alpha-2-macroglobulin have been reviewed by James (1980) and are discussed further in Chapter 16. The first demonstration of the immunosuppressive properties of alpha-2-macroglobulin was by Kamrin (1959), who found that its administration led to prolonged survival of skin grafts in mature, non-littermate rats. Subsequent reports both confirmed and contradicted these findings (Mowbray, 1963; Davis and Boxer, 1965).

In 1979, Proud, Shenton and Smith claimed an association between the presence in pretransplant plasma of alpha-2-macroglobulin and renal allograft survival, and demonstrated prolongation of cardiac allograft survival in rats given alpha-2-macroglobulin. They speculated that alpha-2-macroglobulin production was increased by transfusion. These findings have not yet been confirmed.

ii) Prostaglandins

Lenhard, Gemsa and Opelz (1985 a) have suggested that blood transfusion induces the release of prostaglandin E_2, which causes

immunosuppression through activation of T suppressor cells.

iii) Unidentified soluble suppressor substances

Non-specific soluble suppressor factors have been described following transfusion, primarily in connection with the specific suppressor cell systems discussed later in this chapter (Sasportes et al, 1980), and therefore mentioned here for completeness only.

9. Suppressor cell induction by transfusion

i) Non-specific suppressor cells

An increase in non-specific suppressor cell activity following transfusion of 2 units of packed red cells in 15 dialysis patients was recorded by Smith et al (1983). Suppressor cell activity, in this case assumed by a method involving suppression of concanavalin A-induced T cell blastogenesis, reached a peak three weeks after transfusion and then tailed off towards 20 weeks. Using similar methods, Lenhard et al (1982) and Tait et al (1984) also showed increased suppressor cell activity after transfusion, although Tait emphasised, appropriately, the heterogeneity of the response in his patients.

Using cell surface markers, Sasportes et al (1980) showed the presence of suppressor T lymphocytes in mixed lymphocyte culture, when the cells had been previously hyperimmunized against the DR incompatibilities depicted in the culture. These cells were independent of cytotoxic effectors and produced soluble suppressor factors. The suppressor supernates inhibited autologous cells in

mixed lymphocyte culture. They also to a lesser extent inhibited allogeneic cells, leading Sasportes to suggest that both specific and non-specific suppressor substances were present (Sasportes et al, 1981).

Data from Lenhard et al (1982) led them to propose that immunosuppression after transfusion occurred in two phases. In the first fortnight immunosuppression was non-specific and mediated by monocytes. Afterwards there was an increase in T suppressor cell activity.

Smith et al (1983) failed to correlate increased suppressor cell activity with renal allograft survival, although by the time of transplantation suppressor cell activity would, from their data, have reverted towards normal. Agostino, Kahan and Kerman (1982) did find that graft outcome correlated with the level of non-specific inhibition of mixed lymphocyte culture by suppressor lymphocytes produced after transfusion. Goeken et al (1982), while demonstrating depression of mixed lymphocyte reactivity following transfusion in 7 of 11 patients, could not show concomitant suppressor cell production in co-culture with pre-transfusion cells.

Most recently, Klatzmann et al (1983 a and b; 1984 a) in a series of papers claimed the production of non-specific suppressor cells after transfusion. Eight out of 21 patients in the first study quoted showed a decrease in mixed lymphocyte reactivity after one transfusion, and another 5 after up to 3 transfusions. Several other patients showed fleeting decreases. Most reactivities were returning towards normal after three months.

The suppressor cells found by Klatzmann were T lymphocytes and acted through soluble mediators which he found to be specific for the

stimulating cells and restricted to the responder lymphocytes. The nature of the suppressor factors was not established and Klatzmann did not speculate on the mechanism of suppressor cell production following transfusion. No correlation was found between suppression of mixed lymphocyte reactivity and suppression of cell-mediated lympholysis, which was also detected following transfusion, in a few subjects. (It should be added that suppression of cell-mediated lympholysis following blood transfusion has not been a universal finding (Goeken et al, 1982; Leivestad et al, 1982)).

ii) Donor-specific suppressor cells

Donor-specific T suppressor cells inhibiting the mixed lymphocyte reaction in man were demonstrated by McMichael and Sasazuki (1977). The patient was a woman who had had 10 children by the same man. Her lymphocytes were unresponsive to lymphocytes from the father, and could suppress the response of HLA matched control lymphocytes to paternal cells. These effects were accentuated when her lymphocytes were enriched for T cells and diminished when enriched for B cells. The same group later suggested that identity at the HLA-D locus between suppressor and responder was required (Engleman et al, 1978).

The presence of donor-specific suppressor cells has also been claimed in patients with long-term renal allograft tolerance (Liburd et al, 1978; Charpentier et al, 1981; Goulmy et al, 1981). There is a considerable body of evidence to support this from animals (for example, Dorsch and Roser, 1977; Hendry et al, 1979; Marquet et al, 1982; Batchelor, Philips and Grennan, 1984; Hall, 1984; Barber, Hutchinson and Morris, 1984).

There is less evidence to link the production of these suppressor cells with blood transfusion, an explanation for the transfusion effect proposed by Dausset and Contu (1981). An in vitro model was discussed by Sheehy, Mawas and Charmot (1979). Specific inhibition of the human mixed lymphocyte response was achieved with autologous lymphocytes already primed with cells from the other donor.

In vivo stimulation for this type of experiment has been provided by donor-specific transfusion of prospective recipients of kidneys from live related donors. Only a handful of the patients described by Cochrum et al (1981) developed reduced responsiveness towards donor cells in mixed lymphocyte culture following donor-specific transfusion. Seven out of 10 patients studied by Leivestad et al (1982) did show donor-specific decreased responsiveness, but this bore no relation to the clinical course following transplantation from the same donor. Half of this group also showed increased donor-specific cell-mediated lysis but again with no association with transplant outcome.

More animal work is available to show the induction of specific suppressor lymphocytes after transfusion. Maki et al (1982) used a mouse skin allograft model and showed antigen-specific suppressor cells following transfusion. In rats studied by Marquet et al (1982) a single transfusion was followed by the production of donor-specific suppressor cells. Transferred to a secondary host, spleen cells with suppressor activity could delay rejection of cardiac allografts culled from the same strain as the blood donor. There was also the suggestion of a weaker, non-specific component to the suppressor cell response to transfusion. Again in a rat cardiac allograft model, Hall (1984) found specific splenic suppressor cells after transfusion.

In a rat skin graft model, Lenhard et al (1985 b) found that a planned transfusion protocol induced strong specific and weak non-specific suppressor cell activity. Anti-donor cytotoxins appeared in increasing titres after up to three transfusions. With numbers of transfusions increasing up to a total of fifteen the antibody titres then fell. This humoral hyporeactivity persisted in spite of rechallenge with antigen and could be transferred by spleen cells to syngeneic untransfused animals. Marquet et al (1982) made the noteworthy comment that, although in inbred rats specific suppressor cells might be prominent in the blood transfusion effect, non-specific suppression might be more important in an outbred species like man.

10. Production of immune complexes

Antigen-antibody complexes have been linked to the induction of immunosuppression. They may bind to Fcγ-receptors to induce T lymphocytes to induce soluble suppressor substances (Greene, Fleisher and Waldmann, 1981.) In the context of experimental enhancement, Hutchinson and Zola (1977) have suggested that antigen-reactive cells are specifically depleted by interaction with antigen-antibody complexes and subsequent opsonization. Previous evidence in man to link complexes with either allograft survival or blood transfusion has been thin, and there is certainly no substantial evidence to link complexes to the transfusion effect. Carpentier found a correlation in 50 patients between allograft survival and the detection of complexes before transplantation (Carpentier et al, 1982). No association with transfusion was shown. Ettenger (1982) showed complexes detected by a similar method in the serum of transfusion recipients. In contrast, Keusch et al (1984) found no correlation between complexes and either prior transfusion or subsequent allograft survival.

11. The production of beneficial antibodies

Numerous explanations for the blood transfusion effect have implicated the production of some sort of beneficial antibodies. The concept that antibodies could be other than harmful owes much to the studies of passive graft enhancement by administration of alloantibody, as reviewed by Morris (1980). Morris used the term passive enhancement to denote suppression of the immune response by passively administered donor-specific alloantibody (in contrast to active enhancement where immunosuppression is achieved by prior administration of antigen, and possibly mediated by antibody). Attempts to achieve passive enhancement of renal allograft survival in higher animals and man have not met with the conspicuous success of rodent models (Morris, 1980).

i) Lymphocytotoxins directed against B lymphocytes

Iwaki et al (1978) attributed the transfusion effect to panel-reactive B lymphocytotoxins of IgM class and detected at 4°C only. A weakness of this paper was that transfusion details were not supplied. More solid results were provided by d'Apice and Tait (1979). Thirty out of 78 recipients of cadaver renal transplants had a positive B cell cross-match, a finding associated with improved graft outcome. All patients had been transfused but the group with a positive B cell cross-match had received significantly more blood.

Other centres did not concur (Ting and Morris, 1979) and in a follow-up report d'Apice and Tait (1980) concluded that a positive B cell cross-match was more likely to be a consequence of transfusion independent of graft enhancement. Only 14 out of 34 cross-match-positive

sera had anti-DR specificity and there was marked heterogeneity of response to temperature variation and platelet absorption. On these grounds the authors discounted the parallel drawn in the previous study with enhancement of rodent allografts by antibodies with anti-class II specificity (Davies and Alkins 1974).

Ettenger et al (1982) confirmed the production after transfusion of cold B lymphocytotoxins, but in too small a proportion of patients to account for the transfusion effect. Mohanakumar et al (1985) found that pretransplant sera from patients with a positive B cell cross-match and a successful transplant appeared to define membrane-bound immunoglobulins rather than DR antigens, although the relation to previous transfusion was not established. The recent suggestion by Chapman et al (1985 b) that only autoreactive B lymphocytotoxins are associated with satisfactory graft outcome emphasise the failure of this type of antibody to explain the transfusion effect.

ii) Anti-idiotype antibody

All antibody molecules share "constant" regions (the Fc portion) which do not contribute to antigenic specificity. Parts of immunoglobulin molecules associated with the antigen-combining region (the $F(ab')_2$ portion) can serve as antigenic determinants and are known as idiotypes. Antibody can be produced against such idiotypes and is known as anti-idiotype antibody.

Anti-idiotype antibody reacts not just with antibody but with T and B lymphocytes with the same idiotype expressed on the cell membrane (Roitt et al, 1981). Jerne (1974) postulated that idiotypic interactions unified the cellular and humoral components of the immune system.

This "network" regulated the immune system and was disturbed only when antigen was introduced. The concept of anti-idiotypic tuning of the immune system has been examined in the context of autoimmune disease (Plotz, 1983; Cooke, Lydyard and Roitt, 1984). Manipulation of the idiotypic network has been advocated as a clinical tool (Olson, Wagner and Leslie, 1982).

The relevance of the idiotypic network to transplantation was suggested initially by studies of enhancement. Hart et al (1972) suggested the use of anti-idiotype antibody for specific immunosuppression on the basis of experiments in mice. Although unable to achieve enhancement of rat renal allografts by anti-idiotype antibody alone, Stuart et al (1976) claimed that treatment with donor spleen cells and alloantibody yielded prolonged graft survival in conjunction with the appearance of anti-idiotype antibody and reduction in cell-mediated immunity. The mechanism suggested was specific interaction of anti-idiotype antibody with T cell surface receptors preventing the initiation of the immune response. Binz and Wigzell (1976) claimed the induction of specific tolerance to skin grafts when rats were autoimmunized against their own idiotypes. Stuart, Fitch and McKearn (1982) later showed some prolongation of rat renal allograft survival by passive administration of recipient strain anti-idiotypic antibody raised against an anti-class I hybridoma monoclonal antibody.

A single report of anti-idiotype antibody in a patient with a long-surviving renal allograft came from Miyajima et al (1980). The patient's serum suppressed the mixed lymphocyte reaction between cells from the patient and from the kidney donor. The factor causing suppression was removed from serum by rabbit anti-human IgG, and found to have a molecular weight of 7S, as expected for IgG.

Singal, Joseph and Szewczuk (1982) proposed that blood transfusion led to the production of anti-T cell idiotype antibodies which enhanced allograft survival. The same group have accumulated a number of pieces of evidence in favour of this hypothesis. They duplicated Miyajima's report using third-party cells with the same HLA-B type as the donor (Singal et al, 1982). They associated serum anti-idiotype activity with previous transfusion in 17 uraemic and 2 non-uraemic subjects (Fagnilli and Singal, 1982; Singal, Fagnilli and Joseph, 1983), and again used the molecular weight of the suppressor factor to claim that it was IgG.

In human renal transplantation, Burlingham et al (1985 a and b) have shown the development after donor-specific transfusion of serum factors which specifically inhibit the donor-recipient mixed lymphocyte reaction. Their conclusion that IgG is responsible is based on absorption studies using Protein A. Reed et al (1985) have also claimed that specific anti-idiotype antibody develops after donor-specific transfusion; the same group claimed that pregnancy sera contained specific anti-idiotypic autoantibody that reacted with T cell receptors for alloantigens (Suciu-Foca et al, 1985). In contrast, after transfusing rats, Lenhard et al (1985 c) found suppressor cells and failed to find anti-idiotype antibody. After transfusing renal patients, Takeuchi et al (1985) found both, and claimed on the strength of 4 patients that they were related. Further, in a total of 12 patients they claimed an association between post-transplant rejection episodes and failure to produce anti-idiotype antibody after pre-transplant transfusion.

The weaknesses of all these studies are small numbers, heterogeneity of recipient response to transfusion and failure to show a definite

relationship to graft survival.

iii) Anti-F(ab')$_2$ antibody

One group has looked at renal allograft survival in relation to pretransplant anti-F(ab')$_2$ antibodies (Chia et al, 1982; Horimi et al, 1982). Using the same method, Nasu et al (1980) had found 20% of normal subjects to be antibody positive. Although the initial report on 253 patients looked as though antibody might be associated with improved graft survival (Horimi et al, 1982), the association only just reached statistical significance in the follow-up with 429 patients. Moreover, stratification of the patients for previous transfusions did not support an association between antibody and the transfusion effect (Chia et al, 1982). The authors considered also that lack of specificity precluded an anti-idiotype phenomenon.

iv) Fcγ-receptor blocking antibody

In a series of studies, MacLeod and her colleagues have provided evidence that non-cytotoxic antibodies, detected by their capacity to block the Fcγ-receptor on B lymphocytes, develop following blood transfusion (MacLeod et al, 1982b, 1983), are HLA-linked (MacLeod et al, 1985 a) are allo- rather than autoantibodies (MacLeod et al, 1985b) and are associated with human renal allograft survival (MacLeod et al, 1982, a and b). The background to these studies is discussed more fully in the next chapter.

12. Conclusions

The uniformity of the association between blood transfusion and

renal allograft survival is not matched by the theories to explain the phenomenon. Marrying two of the major current schools of thought, van Rood (1983) has suggested that transfusion initially evokes non-specific suppressor cells which suppress production of antibody against class I antigens. Then anti-idiotype antibodies form and prevent activation of T lymphocytes by HLA-specific antigen. Despite intense research effort, no single convincing explanation for the transfusion effect has been found. A combination of factors may prove responsible. The last word again goes to Calne, who commented in 1984 that "numerous speculations have been advanced to explain the phenomenon, indicating the degree of our ignorance".

CHAPTER 6

FcγR-RECEPTORS AND RENAL TRANSPLANTATION

1. Distribution of FcγR-receptors in peripheral blood

Surface receptors for the reacted Fc portions of IgG, or FcγR-receptors, are present on a variety of cell types (Unkeless, Fleit and Mellman, 1981). Around 25% of peripheral blood lymphocytes carry FcγR-receptors (Clements and Levy, 1978), including almost all B lymphocytes and K cells (Sandilands et al, 1978 a and b) and the so-called TγR subfraction of the T lymphocyte population (Moretta et al, 1979). Most, but not all, FcγR-receptor bearing T lymphocytes belong to the suppressor fraction as now defined by functional assays and monoclonal antibodies (Reinherz et al, 1980 a ; Callard et al, 1981). Normal human polymorphs, monocytes and platelets express FcγR-receptors (Janossy, 1981).

There appears to be marked variation in cell-bound FcγR-receptors. Not only may there be genetic polymorphism (Kaneoka et al, 1983; Tax et al, 1985), but receptors on different cell types may vary in molecular weight (Cohen, Sharp and Kulczycki, 1983), avidity for IgG (Sandilands et al, 1978 a) and antigenicity (Winfield, Lobo and Hamilton, 1977; Cunningham-Rundles et al, 1980).

There have also been reports of the receptor on neoplastic lymphoid cells. Ferrarini et al (1975) reported its presence on B cells from around half of patients with chronic lymphatic leukaemia and Habeshaw et al (1979) reported it in 10% of patients with non-Hodgkins lymphoma.

Not all FcγR-receptors are restricted to the cell surface. Some

may elute spontaneously and be found in cell-free culture supernatants (Lethibichthuy et al, 1980) and normal human serum (Reid et al, 1983).

2. Fcγ-receptors and the immune response

Fcγ-receptors, either alone or in combination with macromolecular antigen:antibody complexes, have been attributed various roles in immune modulation (Fridman et al, 1981; MacLean et al, 1984). Fcγ-receptor bearing T lymphocytes may suppress T-dependent B cell differentiation (Moretta et al, 1979). Soluble Fcγ-receptors, derived possibly from suppressor T lymphocytes, may impair antibody synthesis *in vitro*, (Lethibichthuy et al, 1980), and may restore receptor function on T cells *in vivo* following modulation of previous receptors by immune complexes (Samarut and Revillard, 1980). Recent evidence suggests that lymphokines released by activated T cells lead to increased Fcγ-receptor expression on antigen-presenting cells, and an augmentation control loop on T cell activation by the interaction between existing antibody and the monocyte Fcγ-receptor (Chang, 1985).

In the field of renal transplantation, Charpentier et al, (1983), showed the existence, in patients with long-surviving allografts, of T cells which specifically suppressed the proliferative response of recipient to donor cells, and expressed both the OKT8 antigen and Fcγ-receptor.

Fcγ-receptors on macrophages are responsible for clearance of immune complexes by the reticuloendothelial system (Chang, 1985).

3. Fcγ-receptor blocking antibody and the immune response

Considering the evidence for an immunomodulatory function of the receptor population (s), it is perhaps not surprising that antibodies blocking the Fcγ-receptor have been implicated in the immune response. Antibody to fluid-phase receptor could lead to formation of circulating immune complexes with consequent activation of suppressor mechanisms (Moretta et al, 1979) and/or saturation of receptor-mediated reticuloendothelial system clearance (Imbach et al, 1981). Antibody blocking cell-bound Fcγ-receptors has been shown to trigger a variety of suppressor mechanisms beyond simply the elution of antibody-receptor complexes into the serum (Daeron and Fridman, 1985).

Aggregated IgG may act on the Fcγ-receptor similarly to immune complexes (Dickler and Kunkel, 1972). It is thought that only the Fc portion of the antibody molecule can block the receptor directly. Reports of blocking by $F(ab')_2$ fragments (Soulillou et al, 1978; MacLeod et al, 1982 b) may be explained by co-capping and stripping of the receptor (Sarmay et al, 1979).

The finding by Ritz and Schlossman (1982), confirmed by Roberton et al (1984), that human complement cannot lyse effectively cells coated with mouse monoclonal antibody, suggests that Fcγ-receptor mediated-reticuloendothelial clearance may be the major mechanism for removal of cell-antibody complexes in the therapeutic use of monoclonal antibodies. Saturation of Fcγ-receptor-mediated clearance mechanisms has been mooted as the mode of action of pooled immunoglobulin in suppressing autoimmune disease (Imbach et al, 1985), although alternative mechanisms are anti-idiotypic suppression of autoantibodies (Sultan et al, 1984) or enhanced suppressor T cell function (Delfraissy et al, 1985).

4. Fcγ-receptor blocking antibody in renal transplantation: the background

The relevance to transplantation of Fcγ-receptor blocking antibody was first raised in the early 1970s in connection with studies of passive enhancement of rodent renal allografts. Subsequent exploration of this topic has been based on work linking:

i) passive enhancement with the Fc portion of IgG

ii) Fcγ-receptors with class II antigens

iii) passive enhancement with antibodies against class II antigens

i) The Fc portion in passive enhancement

There is considerable evidence that the mechanism of passive enhancement is Fc-dependent, and, by implication, dependent on the interaction between this portion of the antibody molecule and the recipient's Fcγ-receptor population(s) (Hoffman & Kappler, 1978).

French and Batchelor (1972) were unable to show enhancement using relatively pure $F(ab')_2$ preparations. Studying anti-lymphocyte serum, Lance, Medawar and Taub (1973) found that $F(ab')_2$ fragments did not enhance, although all the serum enhancing activity was in the 7S (or IgG peak) serum fraction. Kaliss, Sinclair and Cantrell (1976) showed only slight suppression of cell-mediated immunity to murine tumour allografts using $F(ab')_2$ fragments. One group showed that $F(ab')_2$ fragments alone enhanced neither mouse skin nor sarcoma allografts (Capel et al, 1979; de Waal et al, 1979). Convincing evidence from a rat renal allograft model that enhancement required the Fc portion was provided by Winearls et al (1979).

The proposal by Hutchinson and Zola (1977) that antigen-reactive cell opsonization could explain passive enhancement hinged on the necessity for the Fc portion to bind to Fcγ-receptors on monocytes and macrophages (Hutchinson 1980). Hutchinson and Brent (1982) went on to exclude the possibility that the Fc portion was needed not to bind to the receptor but to fix complement. They found that decomplementation with cobra venom had no effect on passive enhancement of mouse skin allografts. Lems et al (1984) found that complement-fixing IgG2 and non-complement-fixing IgGI antibodies were equally effective in enhancement, but IgM was not (Lems et al,1981).

ii) The relation between Fcγ-receptors and Class II antigens

Dickler and Sachs (1974) speculated that the Fc -receptor on mouse B lymphocytes was either identical to or closely associated with the Ia alloantigens determined by the Ir region of the H-2 complex (equivalent to class II antigens). In 1975, Soulillou et al, carried this a step further. They found that rat alloantiserum absorbed with donor erythrocytes or platelets was as effective as the unabsorbed antiserum in inducing passive enhancement of rat renal allografts. Absorbed antibodies inhibited donor-recipient mixed lymphocyte culture and specifically blocked donor splenic lymphocyte Fcγ-receptors. This activity was maintained through to $F(ab')_2$ fragments. Soulillou et al (1975) proposed that anti-Fcγ-receptor were actually anti-Ia antibodies, and that they produced enhancement by blocking class II antigen presentation. Dickler (1976) claimed that anti-Ia $F(ab')_2$ fragments blocked Fcγ-receptors on both B and T cells. Using an erythrocyte-antibody (EA) rosette inhibition assay to define Fcγ-receptor blocking activity, Suthanthiran et al (1977b) showed that cytotoxic anti-Ia serum inhibited the Fcγ-receptor whereas the receptor was unaffected by serum

against class I antigens. In contrast, Dobloug et al (1981) reported that antibody against beta-2-microglobulin, but not antibody against HLA-DR antigens, would block EA rosette formation in man. Other workers also concluded that the Fcγ-receptor and Ia antigen were not one and the same structure (Rask et al, 1975; Solheim Thorsby and Moller, 1976; Wofsy McDevitt and Henry, 1977; Soulillou and Peyrat, 1979).

In a series of experiments using anti-Ia and anti-beta-2-microglobulin, Sarmay, Sanderson and Ivanyi (1979) claimed that Fcγ-receptors could be blocked by antisera not just to Ia antigens but to any cell surface receptor. If, however, the relation found previously between anti-Ia and anti-Fcγ-receptor activity were entirely by chance, one might expect anti-Ia serum to block other cell surface receptors too. But Soulillou, Peyrat and Guenel (1978) found no evidence of blocking of the C3 receptor as detected by an EAC rosette inhibition assay. Klinkert et al (1982) failed to find Fcγ-receptors on rat dendritic cells, while noting that the cells expressed Ia antigens and may be crucial to antigen presentation of the renal allograft (Austyn et al, 1985). The body of evidence, however, appears to be in favour of some unspecified association between Fcγ-receptors and class II antigens.

iii) Anti-class II antibody in passive enhancement

Davies and Alkins (1974) suggested that enhancement was mediated by class II antigen:antibody complexes. Catto et al (1977) found passive enhancement in a rat renal allograft model to be dependent on anti-Ia activity. The necessity for anti-Ia activity in enhancing serum was challenged by Jeekel et al (1977) who reported enhancement of rat renal

allografts with antibodies against antigens associated with erythrocytes.

The findings of Suthanthiran et al (1978; 1979) of production of anti-Ia antibody during allograft rejection, and apparent abrogation of anti-Ia antibody production in passively enhanced recipients, led to their suggestion (Suthanthiran et al, 1978) that passive transfer of anti-Ia antibody could mediate enhancement by interfering with the recipient's own response to donor class II antigen. Otsubo et al (1983) claimed that anti-mouse Ia monoclonal antibody prolonged canine renal allograft survival. However, Soulillou et al (1980) had previously tried to enhance canine renal allografts with specific anti-class II serum, and commented that "it would seem that the rodent enhancement model works too smoothly".

5. Fcγ-receptor blocking antibody and renal transplant outcome

The blurred distinction between Fcγ-receptors and class II antigens led to the use of EA rosette inhibition assays, indicating Fcγ-receptor blocking activity, in attempts to extrapolate to man rodent data concerning enhancement by anti-class II antibody. Early findings were not encouraging. Suthanthiran et al performed an EA rosette inhibition assay on both pre-(1977) and post-transplant (1978) serum. Inhibition of rosette formation was associated with graft loss. Using unseparated normal human peripheral blood lymphocytes, Bakkaloglu et al (1980) correlated rosette inhibition by post-transplant serum with rejection episodes. Indeed, Suthanthiran et al (1977) proposed that further larger studies should establish whether the EA rosette inhibition assay should be recommended as a "routine cross-match procedure to preclude renal transplantation".

The work of Suthanthiran relied on the use of recipient serum ultracentrifuged to remove immune complexes and aggregates which, rather than IgG, could block the Fcγ-receptor and inhibit rosette formation. Soulillou et al (1978) looked at the capacity of pre-transplant recipient serum to inhibit EA rosette formation by donor B-enriched lymph node lymphocytes. Further, in 3 out of 55 cases, IgG and $F(ab')_2$ fragments derived from the serum samples were tested in parallel with serum; EA rosette inhibition was found with serum, IgG and $F(ab')_2$ fragments. No significant difference was seen in allograft outcome between recipients with or without pre-transplant EA rosette inhibitory activity, although if anything those with inhibitory activity fared slightly better.

The most recent work on the relation between Fcγ-receptor blocking antibody and renal allograft survival has come from Aberdeen, as mentioned briefly in discussion of the blood transfusion effect. The presence in pre-transplant serum of non-cytotoxic Fc -receptor blocking activity, as detected by EA rosette inhibition, against donor B lymphocytes correlated well with one-year renal allograft survival. Graft outcome also correlated, although less well, with inhibition of rosette formation of B lymphocytes from panels of normal donors and patients with chronic lymphatic leukaemia. Rosette inhibition was maintained in preparations of IgG and $F(ab')_2$ fragments (MacLeod et al, 1982 a and b).

The same group has also provided impressive evidence for the development of such antibodies against paternal antigens during successful pregnancy (Power et al, 1983 a and b; Stewart et al, 1984). Family studies in the contexts of both pregnancy and transfusion have shown antibody development to be apparently HLA-linked, again raising

the question of the relation between Fcδ-receptors and class II antigens (Power et al, 1983b; MacLeod et al, 1985a). These exciting findings led to the series of experiments described below.

CHAPTER 7

SUBJECTS STUDIED

1. Normal subjects

Forty-five healthy volunteers with no past history of renal disease or blood transfusion. Details of sex and parity are given overleaf. The age range was 10 to 57 years.

Abbreviations are as under:

N : Nulliparous

P : Pregnant

M : Parous - at lease one full-term pregnancy

Normal subjects

Initials	Sex	Parity
RA	M	
CB	F	M
DB	M	
EB	F	M
JB	M	
MC	F	M
WC	M	
CF	F	M
GF	M	
MF	F	N
KG	F	N
EH	F	P
BJ	M	
BK	F	P
AL	M	
CL	M	
GL	M	
JL	M	
NL	F	M
SL	F	M
CMcG	F	P
DMcK	F	P
JMcK	M	
SMcK	F	P
CMcL	F	N
HMcM	M	
AM (1)	M	
AM (2)	F	N
AM (3)	F	M
CR	F	P
FR	M	
MR	F	M
SR	F	N
CW (1)	M	
CS (2)	F	N
CS (3)	F	M
GS	M	
MS	F	P

Normal subjects

Initials	Sex	Parity
AT	G	P
AW	F	N
CW	M	
MW (1)	M	
MW (2)	F	M
MW (3)	F	M
PW	F	N

2. Transfused patients without known renal disease

Fifteen patients attending the Western Infirmary, Glasgow. All had previously received at least 5 units of blood for a variety of indications as listed overleaf. No patients with haemophilia were included in this group. The mean age was 57 years with a range of 22 to 84.

Transfused subjects without known renal disease

Initials	Age	Sex	Diagnosis	No. of units of blood transfused
AC	26	F	Post-partum haemorrhage	6
EC	84	F	Pernicious anaemia	5
HF (1)	79	M	Gastro-intestinal Angiodysplasia	>100
HF (2)	72	M	Pancreatitis	5
JF	57	M	Gastro-intestinal bleeding	5
WF	43	M	Gastro-intestinal bleeding	5
DL	55	M	Diverticulitis	6
EL	22	F	Trauma	>10
MMcC	46	F	Gastro-intestinal bleeding	15
JMcM	55	M	Carcinoma of colon	5
WMcM	57	M	Aplastic anaemia	98
CMcQ	81	F	Sideroblastic anaemia	10
GP	75	F	Gastric ulcer	5
MP	65	F	Gastro-intestinal bleeding	6
MS	40	F	Gastro-intestinal bleeding	8

3. Patients with known renal disease

Patients studied were those attending the Renal Unit of the Western Infirmary, Glasgow, for inpatient or outpatient care, and/or dialysis and transplantation. Overleaf are details for each patient of age, sex, primary renal disease, type of renal replacement therapy where appropriate and transfusion history. Where sequential serum samples were used, the transfusion details in this section refer to the transfusion status at the time of the first sample. Where accurate transfusion details are not available, this is indicated by "greater than" the minimum number of units which the patient is known to have received.

Abbreviations for the patient's renal disease are as follows:

BP : hypertensive nephrosclerosis
DM : diabetes mellitus
GN : glomerulonephritis
O : obstructive nephropathy
PCK: polycystic kidney disease
PN : pyelonephritis
R : reflux nephropathy
U : uric acid nephropathy

Abbreviations for the mode of renal replacement therapy are as follows:

C : continuous ambulatory peritoneal dialysis
H : haemodialysis
T : functioning renal transplant

Patients with known renal disease

Initials	Age	Sex	Renal disease	Renal replacement therapy	No. of units of blood transfused
CA	51	F	U	C	20
DA (1)	23	M	PN	H	27
DA (2)	27	M	O	H	5
JA (1)	43	M	DM	C	5
JA (2)	35	M	Amyloid	H	9
WA	39	M	GN	H	8
AB	31	M	DM	C	5
BB	20	M	O	C	7
CB	20	M	GN	H	-
DB	75	M	GN	-	-
EB (1)	62	M	TB	-	27
GB (2)	65	M	O	C	6
JB (2)	56	M	PCK	H	5
JB (3)	30	F	DM	C	5
JB (4)	27	F	GN	C	5
MB (1)	59	F	PN	C	5
MB (2)	42	F	BP	H	11
MB (3)	68	F	GN	-	-
NB	53	F	BP	C	5
PB	22	M	O	H	>20
SB	42	F	PN	H	7
CC	24	F	Calculi	-	6
IC (1)	38	M	PN	H	5
IC (2)	66	F	Calculi	-	-
JC (1)	55	M	Lupus	-	-
JC (2)	31	M	Alport's	T	>10
JC (3)	49	F	PCK	H	5
LC	64	F	O	H	5
MC (1)	45	F	O	H	>60
MC (2)	39	M	PCK	-	>30
PC	57	M	BP	-	-
TC (1)	36	M	R	H	13
TC (2)	34	M	GN	H	5
TC (3)	52	M	PN	H	5
VC	56	M	PN	H	13

Patients with known renal disease

Initials	Age	Sex	Renal disease	Renal replacement therapy	No. of units of blood transfused
WC (1)	53	M	U	H	14
WC (2)	39	M	DM	-	-
WC (3)	50	M	BP	-	-
AD	44	F	PN	C	7
DD	42	M	GN	H	13
HD	43	M	PN	H	10
JD	53	M	BP	H	12
KD	20	M	GN	H	7
PD	22	M	GN	H	20
RD	57	M	GN	H	10
WD (1)	56	M	PN	H	-
WD (2)	55	M	PN	H	17
WD (3)	48	M	PN	T	6
EF	66	F	Lupus nephritis	-	-
RF	26	M	R	C	5
AG (1)	54	M	PCK	H	5
AG (2)	51	M	GN	C	5
AG (3)	27	M	R	H	35
EG	58	F	GN	-	-
JG	20	M	GN	-	-
TG (1)	22	M	GN	T	7
TG (2)	49	M	BP	H	6
WG (1)	50	M	GN	-	-
WG (2)	22	M	DM	-	-
AH (1)	26	F	PN	H	8
AH (2)	50	F	PCK	-	-
AH (3)	40	F	BP	H	>50
CH (1)	61	F	PN	T	5
CH (2)	29	F	GN	H	33
DH	33	M	GN	H	48
FH	32	M	GN	-	-
HH	19	M	Nephronophthisis	H	39
JH (1)	36	M	GN	H	14
JH (2)	50	M	GN	-	-
JH (3)	41	M	GN	H	>20
JH (4)	49	F	PN	C	6

Patients with known renal disease

Initials	Age	Sex	Renal disease	Renal replacement therapy	No. of units of blood transfused
JH (5)	26	F	GN	H	6
MH (1)	36	F	GN	-	-
MH (2)	35	F	GN	-	-
SH (1)	65	M	GN	-	-
SH (2)	40	M	GN	-	-
WH	42	M	U	H	10
DI	35	M	GN	H	8
DJ	44	M	DM	C	-
NJ	35	M	DM	C	16
AK	27	M	GN	H	-
DK	32	M	PN	-	-
EK	30	F	DM	H	20
TK	48	M	PN	H	7
WK (1)	45	M	GN	-	-
WK (2)	37	M	GN	H	-
DL	18	F	Nephronophthisis	H	>40
EL	21	F	PN	H	12
RL	44	M	GN	-	-
AMcC	40	F	PN	C	5
MMcC	53	F	Analgesic	C	9
AMcD	61	F	PCK	H	8
B McD	25	M	O	H	5
G McD	25	M	O	H	15
MMcD	42	F	GN	T	12
RMcF	35	M	GN	-	-
HMcG	46	M	BP	H	5
CMcG	61	F	Analgesic	-	2
JMcG (1)	50	M	BP	H	7
JMcG (2)	40	F	GN	H	10
MMcG	50	F	BP	H	-
EMcI	49	F	Analgesic	H	10
JMcI (1)	63	M	BP	-	-
JMcI (2)	63	M	BP	-	-
JMcK	62	M	GN	-	-
MMcK	59	F	DM	C	
FMcL	22	M	PN	H	5

Patients with known renal disease

Initials	Age	Sex	Renal disease	Renal replacement therapy	No. of units of blood transfused
JMcL	38	M	GN	H	-
LMcL	54	F	PN	-	-
MMcL	45	M	BP	H	4
PMcL	18	F	R	-	-
DMcM	51	M	DM	-	-
WMcM	45	F	R	-	-
RMcR	30	M	DM	H	
AM (1)	51	F	PN	H	5
AM (2)	36	F	GN	H	7
CM (1)	<15	F	Renal agenesis	C	>10
CM (2)	45	F	GN	H	7
IM	23	M	GN	H	5
JM (1)	17	M	BP	H	66
JM (2)	28	M	GN	H	5
JM (3)	35	M	GN	H	9
PM (1)	29	M	GN	H	6
PM (2)	25	F	GN	H	-
TM (1)	33	M	DM	T	5
TM (2)	53	M	DM	-	-
EN	27	F	PN	H	11
GN	55	M	GN	C	6
IO'D	49	F	PN	C	7
EO'N	58	F	PCK	H	6
SO	58	F	PN	H	12
FP	38	F	PN	T	5
MP (1)	20	F	GN	H	8
MP (2)	24	M	Alport's	H	>40
MP (3)	49	F	GN	-	-
MP (4)	33	F	GN	H	>80
EQ	24	F	DM	C	4
AR	20	M	PN	H	19
GR	29	F	DM	C	5
FR	55	M	Analgesic	C	-
IR	38	M	PN	H	72
JR (1)	57	M	PCK	H	5

Patients with known renal disease

Initials	Age	Sex	Renal disease	Renal replacement therapy	No. of units of blood transfused
JR (2)	57	F	PN	H	9
MR (1)	37	F	GN	H	6
MR (2)	26	F	PN	H	5
AS	46	F	PN	H	18
CS (1)	32	M	GN	H	-
CS (2)	64	M	BP	-	-
ES	54	M	BP	H	12
IS	22	M	PN	H	5
JS (1)	54	F	Nephrocalcinosis	C	-
JS (2)	72	M	GN	-	-
JS (3)	54	F	PCK	-	>10
MS (1)	49	F	GN	H	
MS (2)	50	F	GN	H	9
MS (3)	63	F	PCK	H	>50
MS (4)	54	F	Nephrocalcinosis	-	-
MS (5)	44	F	PN	H	
NS	56	M	Alport's	H	11
SS	16	M	0	H	15
TS (1)	50	M	PCK	H	5
TS (2)	47	M	PCK	H	5
WS	75	M	Past ATN	-	5
JT (1)	32	M	R	C	7
JT (2)	46	M	GN	H	5
PT	52	F	BP	H	>10
AW	26	M	GN	H	1
GW	41	M	PCK	H	6
JW	26	F	R	H	8
MW	54	F	Amyloid	-	>20
SW (1)	23	M	GN	H	10
SW (2)	43	M	PCK	H	5
FY	49	M	GN	H	7

4. Patients with haemophilia

Thirty males with Factor VIII deficiency and 9 with Factor IX deficiency. No patient had serum Factor VIII inhibitory activity.

No patient had clinical evidence of AIDS nor of unexplained lymphadenopathy persisting for more than three months. None was known to be homosexual. None had recently travelled to Africa or the Caribbean.

Six of 24 patients tested since the period of this study have been found to have antibodies against human T-cell leukaemia virus Type III. All have Factor VIII deficiency and had used commercial Factor VIII preparations before 1979. For five years before this study none of the patients had received other than locally produced Factor VIII/IX, that is donated by voluntary donors in the West of Scotland.

The mean age was 28 (range 18 to 67 years). Listed overleaf are details of

a) endogenous clotting factor production (expressed as a percentage of normal)

b) current use of therapeutic clotting factors. The patients have been divided into three categories depending on the number of units of Factor VIII or Factor IX used during the previous year:

low	less than 10,000 units	14 patients
medium	10,000-40,000 units	9 patients
high	more than 40,000 units	16 patients

c) previous blood transfusion: again, three categories:

never transfused	20 patients
transfused less than 5 units of blood	7 patients
transfused 5 or more units of blood	12 patients

Haemophilia A

Initials	Age	Endogenous Factor VIII/IX (expressed as % normal)	Factor VIII/IX usage in past year	No. of units of blood transfused
AB	18	<1	High	<5
JB	33	<1	High	>5
AC	32	<1	High	>5
IC	19	2	High	0
JC	21	<1	Medium	0
AD	21	<1	High	0
PD	22	5	High	0
AF	36	<1	Low	0
DF	34	4	Low	>5
EF	29	<1	Low	0
DG	32	<1	High	<5
GH	41	2	Low	<5
DI	25	<1	High	0
MJ	18	<1	Medium	0
MK	18	7	Low	0
JMcC	67	5	Medium	0
IMcE	35	<1	High	<5
WMcK	26	<1	High	>5
GMcN	26	15	Nil	0
JMcS	29	<1	Medium	>5
BM (1)	38	<1	Nil	<5
BM (2)	28	5	Nil	<5
HM	43	<1	High	>5
NM	30	10	Medium	<5
TM	27	<1	High	>5
RR	33	<1	Medium	>5
NS	23	<1	High	0
RS	28	<1	Low	>5
SU	28	<1	High	0
PW	60	<1	Low	>5

Haemophilia B

Initials	Age	Endogenous Factor VIII/IX(expressed as % normal	Factor VIII/IX usage in past year	No. of units of blood transfused
RB	20	<1	Low	0
IF	20	<1	Medium	0
AH	35	<1	High	0
KMcD	20	5	Low	0
RM	23	5	Low	0
TM	26	5	Medium	0
DS	20	<1	Low	0
MS	34	<1	Medium	>5
RS	38	<1	High	>5

5. Patients attending a Genitourinary Clinic

Thirty-two patients presenting to the Genitourinary Clinic at the Southern General Hospital, Glasgow, with symptoms attributable to sexually transmitted disease. Details of individual patients are not given. In the 20 cases on whom a diagnosis was recorded at the clinic visit, diagnoses were as follows:

non-specific urethritis	8 cases
gonorrhoea	6 cases
genital warts	2 cases
syphilis	1 case
urinary tract infection	1 case
genital Herpes infection	1 case
moniliasis	1 case

Sexual orientation was as follows:

male homosexuals	11 cases
male heterosexuals	10 cases
female heterosexuals	11 cases

The mean age of the patients was 27 (range 19 to 43 years).

6. Patients with AIDS

a) One patient treated in the Western Infirmary. Full details of this case were published by Shiach et al (1984). The patient was a Scottish heterosexual male who had spent some years in Africa. After the development of screening for antibody to

HTLV-III, stored serum was found to be antibody-positive.

b) Six patients with clinically defined AIDS treated in San Francisco. All were male homosexuals. Three had Kaposi's sarcoma, and all presented with a variety of opportunistic infections.

CHAPTER 8

TECHNIQUES USED

1. Preparation for experiments of cells from peripheral blood

Whole blood was taken from the subjects specified and kept until use (generally within one hour) in bottles containing a few drops of preservative-free heparin.

i) Peripheral blood lymphocytes (PBL)

Human mononuclear cells were obtained from heparinised whole blood by centrifugation over a Ficoll-metrizoate (SG = 1.077) column (Boyum, 1968). Cells aspirated from the plasma/Ficoll interface were washed twice in Hepes buffered Earles medium (HEM), pH = 7.3 (Gibco Laboratories, Glasgow) then resuspended in HEM supplemented with 10% fetal calf serum (HEM + S). Approximately 10 x 10^6 mononuclear cells in 2ml HEM + S were added to plastic tissue culture plates and incubated at 37°C for 2 hrs in 5% CO_2 in air. Non-adherent cells were removed by repeated gentle washings with a Pasteur pipette (Ross et al, 1973). The non-adherent cells were found to have lymphoid cell morphology (more than 97%) and were virtually all viable as assessed by the exclusion of trypan blue (greater than 97%). Lymphocyte suspensions were washed twice in (HEM), and adjusted to a final concentration of 4 x 10^6 cells/ml in HEM.

ii) T or B cell enriched lymphocyte suspensions

Equal volumes of PBL and neuraminidase-treated sheep erythrocytes (NSE) were mixed (for preparation of NSE please see 8:4:iii) and

centrifuged at 400g for 10 mins. The cell pellet was incubated at $4^{o}C$ for 1 hour prior to resuspension by end over end mixing and layered onto Percoll(Pharmacia, Uppsala, Sweden) (SG = 1.077) in the proportions of 3 ml cell suspension to 1 ml Percoll. Following centrifugation at 400g for 20 mins, cells were removed from the interface, i.e. 'B' enriched and from the pellet, i.e. 'T' enriched. Contaminating NSE were removed from the T cell suspensions by hypotonic lysis. Both 'B' and 'T' enriched populations were washed three times in HEM and adjusted to a final concentration of 4×10^{6}/ml. 'B' cell enriched populations contained less than 2% NSE rosetting cells while 'T' enriched contained greater than 90% NSE rosetting cells.

iii) Monocytes

After the removal of the non-adherent lymphoid cells from plates as described above (8:1:i), the residual, adherent cells were found to be mainly monocytes (more than 95%) as assessed by morphology, latex bead phagocytosis and non-specific esterase straining. Monocytes were maintained in HEM + S at room temperature in 5% CO_2 in air until required.

iv) Polymorphonuclear leucocytes (polymorphs)

Polymorphs were recovered from the bottom of the Picoll-metrizoate columns as described by Boyum (1968). The erythrocyte/polymorph containing cell pellet was mixed with an equal volume of autologous plasma recovered from the top of the Ficoll-metrizoate columns and incubated at $4^{o}C$ for 30 mins. Pre-cooled 6% Dextran 110 was added (400µl Dextran/ml cells in plasma) and incubated at $4^{o}C$ until the erythrocytes sedimented by 50% of the total volume. The

polymorph rich plasma was then recovered and the cells washed twice in HEM by centrifuging at 200g for 5 mins. Any contaminating erythrocytes were removed by hypotonic lysis and the cell suspensions adjusted to an appropriate concentration in HEM. These cell suspensions were viable (greater than 97%) as assessed by dye exclusion and contained greater than 95% polymorphs as assessed by morphology and latex bead phagocytosis.

v) Erythrocytes

Erythrocytes were obtained by sedimentation with dextran from the cell pellet at the bottom of the Ficoll-metrizoate column, as described in section 8:1:iv.

vi) Platelets

Pooled human platelets were obtained from the Glasgow and West of Scotland Blood Transfusion Service, Law Hospital, Carluke). Contaminating leucocytes were initially removed by centrifugation of platelet-rich plasma at 150g for 30 minutes and pure platelets obtained by further centrifugation at 400g for 10 minutes.

2. Preparation for experiments of cells from lymphoid organs

i) Splenic B lymphocytes

Splenic lymphocytes from cadaveric kidney donors were obtained from the Tissue Typing Laboratory at Glasgow Royal Infirmary, where they had been frozen and stored in liquid nitrogen. Enrichment for B lymphocytes was performed according to the method described above (8:1:ii).

ii) Thymus-derived T lymphocytes

Thymus tissue was collected at open heart surgery from infants with no known immune abnormality. Thymocytes were prepared by mechanical separation and resuspended until use in HEM.

3. Preparation of serum for experiments

Serum samples were taken from subjects as specified below and prepared for use in a variety of ways.

i) Unseparated serum

This was frozen and stored until use at -20°C.

ii) Ultracentrifuged serum

Serum either fresh or thawed was ultracentrifuged at 105,000g (model L2-65B Beckman ultracentrifuge) immediately prior to use; the lower third of the contents of each ultracentrifuged tube was discarded.

iii) Serum fractions of graded molecular weight

These were prepared as described by Reid et al (1983). Five hundred microlitre aliquots of serum were fractionated by ultracentrifugation at 105,000g for 16 hrs at 4°C over 4 ml 20-40% discontinuous sucrose gradients using a model L2-65B Beckman ultracentrifuge. Five fractions (F1-5) each of 720ul were collected in

turn from the bottom of the gradient, plus one larger fraction (F6) of 900ul. Each serum fraction was dialysed into phosphate buffered saline (PBS) at 4°C for 16 hrs. The approximate molecular weight of serum components in each fraction was estimated by screening a small aliquot by radial immunodiffusion using four markers: IgM, IgG, C1q and transferrin. In all experiments, IgM was found to peak in F2, C1q in F3, IgG in F4 and transferrin in F5. Serum fractions were stored at -20°C until use.

iv) IgG

IgG was obtained from serum by DEAE cellulose chromatography (DE52-Whatman). Both serum and DE52 were equilibrated as to pH and conductivity with 10mM phosphate buffer of pH 7.8 prior to chromatography. IgG preparations were dialysed into phosphate buffered saline (PBS) pH 7.2 for 18 hours at 4°C and ultracentrifuged at 105,000g for 1 hour prior to use. Small aliquots (500µl) were stored at -20°C and repeated freeze-thawing was avoided. The IgG preparations obtained were pure as assessed by immunoelectrophoresis and on radial immunodiffusion. The monomeric nature of these IgG preparations was confirmed by polyacrylamide gel electrophoresis and by gel filtration (sephacryl S300-Pharmacia). In all experiments, unless otherwise stated, IgG preparations were used at a concentration of 1mg/ml in PBS.

v) Absorbed IgG preparations

A variety of cells were used, as specified in the relevant chapters, and obtained as recorded above. IgG preparations were absorbed three times at 37° for 1 hour using 200µl of packed cells in HEM per 500µl IgG (1 mg/ml) in PBS).

4. Experimental techniques using lymphocytes

i) Inhibition of EA rosette formation

Full details of this method have been described previously (Sandilands et al, 1980).

i, a) Preparation of antibody-sensitised chicken erythrocytes (EA)

A 5% suspension of washed chicken E was mixed with an equal volume of a 1 in 500 dilution of rabbit anti-chicken E serum and incubated at 37°C for 1 hrs, washed twice, and adjusted to a 1% suspension in HEM. The preparation of this antibody to chicken E, which is predominantly of IgG class, has been described elsewhere (Sandilands et al, 1975).

i,b) Inhibition of EA rosette formation

One million monocyte-depleted human lymphocytes (separated from peripheral blood or lymphoid organs as described above (8.1) were resuspended in 120µl test or control substrate. Whole serum, serum fractions and IgG were prepared as above (8.2) for testing. Controls used were fetal calf serum, phosphate buffered saline, IgG derived from serum from the normal donor of the target lymphocytes, and known inhibitory and non-inhibitory IgG preparations, where appropriate. Following incubation at 4°C or 37°C for 30 minutes, cells were washed twice and resuspended in 120µl HEM, and mixed with an equal volume of chicken EA, prepared as described above. Tubes were then centrifuged at 200g for 5 minutes prior to resuspension of the EA rosette pellet and fixation in 3% glutaraldehyde. Cells were resuspended in 0.75%

trypan blue and inspected under sealed coverslips. At least 200 lymphocytes on each slide were counted and the percentage of lymphocytes forming rosettes, defined as binding two or more chicken EA, was determined. Results were expressed as the percentage inhibition of EA rosette formation from the formula:

$$\% \text{ inhibition} = \frac{\begin{array}{l}\% \text{ rosette-forming lymphocytes in control (mean of 3)} - \% \text{ rosette-forming} \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad \text{lymphocytes in test}\end{array}}{\% \text{ rosette-forming lymphocytes in control (mean of 3)}} \times 100$$

ii) Inhibition of EAC rosette formation

ii,a) Preparation of antibody-sensitised erythrocytes (EAC)

Five millilitres of a 5% suspension of washed sheep erythrocytes was mixed with an equal volume of rabbit anti-sheep erythrocyte IgM, diluted 1 in 100 in HEM (Haemolysin- Flow Laboratories Ltd., Irvine, Aryshire). After incubation for 30 minutes at 37°C the preparation was washed twice in HEM, then adjusted to give a 1% suspension in HEM. An equal volume of fresh normal human serum, diluted 1 in 80, was added and, following a further incubation period of 30 minutes at 37°C, the complexes were washed twice and adjusted to a final concentration of 0.5% in HEM.

ii,b) Inhibition of EAC rosette formation

One million monocyte-depleted human lymphocytes (separated from peripheral blood as described above (8.1)) were resuspended in 120µl test or control substrate (as defined above 8:4,i)). Following incubation at 37°C for 30 minutes cells were washed twice and resuspended in 120µl

HEM, and mixed with an equal volume of EAC. Following centrifugation at 200g for 5 minutes, the pellet was incubated at 37°C for 30 minutes, then resuspended by vortex mixing and fixed in 3% glutaraldehyde. Cells were resuspended in 0.75% trypan blue and inspected under sealed coverslips. At least 200 lymphocytes on each slide were counted, and results were expressed as the percentage inhibition of rosette formation produced by test compared with control preparations, according to the formula detailed above for the EA rosette inhibition assay (8:4,i).

iii) Inhibition of NSE rosette formation

iii,a) Preparation of neuraminidase-treated sheep erythrocytes (NSE)

Three hundred microlitres of washed packed sheep erythrocytes was mixed with 60µl neuraminidase and 1.8ml HEM. This was incubated at 37°C for 1 hour, washed twice and adjusted to a 0.5% suspension in HEM.

iii,b) Inhibition of NSE rosette formation

One million monocyte-depleted human lymphocytes (separated from peripheral blood or lymphoid organs as described above (8:1) were resuspended in 120µl test or control substrate (as defined above (8:4,i). Following incubation at 37°C for 30 minutes cells were washed twice and resuspended in 120ul HEM, then mixed with an equal volume of NSE. Following centrifugation at 200g for 5 minutes the preparations were placed overnight in an ice bath at 4°C. After gentle resuspension by end-over-end mixing the cells were fixed with

3% gluteraldehyde. Cells were resuspended in 0.75% trypan blue and inspected under sealed coverslips. Results were expressed as the percentage inhibition of rosette formation produced by test compared with control preparations, according to the formula detailed above for the EA rosette inhibition assay (8:4,i).

iv) Inhibition of phytohaemagglutinin-induced blastogenesis(PHA)

Normal human peripheral blood mononuclear cells (PBMC) were resuspended in RPMI(Gibco)culture medium supplemented with 10% heat-inactivated autologous serum and antibiotics at a final concentration of 4×10^6/ml. Fifty microlitres of PBMC (2×10^5 cells), 50 µl serum fraction in PBS and 10µl PHA (100ug/ml) were incubated together in the wells of a flat bottomed microtitre plate. Following incubation at 37°C for 66 hours in a humidified atmosphere of 5% CO_2 in air the cells were pulsed with ^{14}C-thymidine (0.02µCi/well) for 6 hours, harvested on to fibreglass filters using a multiple-channel automated cell harvester and washed in turn with distilled water, 5% trichloroacetic acid and methanol. The filters were dried at 37°C, placed in glass vials containing 5mls of scintillation fluid and the radioactivity counted in an automated B counter (Packard). Results were expressed as the percentage inhibition of PHA response produced by the IgG as compared with control wells (Controls >3000 cpm).

v) Inhibition of antibody-dependent cell-mediated cytotoxicity and Natural Killer cell function.

Peripheral blood mononuclear cells, pretreated with IgG obtained from subjects as described above, (8:3:iv), were incubated with ^{51}Cr-labelled target cells a) antibody (IgG) sensitised Chang liver

cells at a lymphocyte:target cell ratio of 33:1 for 20 hrs at 37°C or b) K526 cells at 20:1 for 4 hrs at 37°C. ^{51}Cr release was measured in the supernatant using an automated gamma counter. Minimum and maximum release was determined by incubating labelled target cells in medium alone or in 10% SDS respectively. In the antibody-dependent cell-mediated cytotoxicity assay spontaneous cytotoxicity (in the absence of anti-Chang serum) was also calculated and subtracted from the total cytotoxicity to give a value for net antibody dependent cytotoxicity. Results were expressed as percentage inhibition of cytotoxicity induced by IgG as compared with cell suspensions which were incubated with medium above in place of IgG (Sandilands et al, 1976; Kay, Fagnani & Bonnard, 1979).

vi) Exclusion of complement-dependent cytotoxicity

Samples of serum and IgG were screened for lymphocytotoxicity at 22°C against peripheral blood lymphocytes from the donor panel used routinely in transplantation by the Tissue Typing Laboratory at Glasgow Royal Infirmary. Testing was performed, courtesy of Professor H.M.Dick, by staff of the Tissue Typing Laboratory, using standard techniques (Kissmeyer-Nielsen and Dick, 1979).

5. Experimental technique using monocytes: inhibition of EA rosette formation

Human monocytes adherent to plastic plates (as prepared in sections 8:1,i and 8:1,iii) were incubated at 37°C for 30 minutes in 5% carbon dioxide in air, together with Iml HEM + S (8:1,i) and 500ul test or control substrate. The plates were then rinsed 6 times with HEM prior to rosette formation with chicken EA in situ as described previously (8:4,i; Sandilands et al, 1980).

6. Experimental techniques using polymorphs

i) Inhibition of EA rosette formation

The same method was employed as for lymphocytes (8:4:i) using one million polymorphs suspended in 250µl HEM.

ii) Inhibition of phagocytosis of IgG-coated latex beads

Normal human peripheral blood polymorphs were preincubated with IgG prepared from serum as described above (8:3:iv). Following two washes in HEM, 2 x 10^6 polymorphs were resuspended in 50µl HEM + S and incubated at 37°C for 30 mins together with an equal volume of IgG coated latex beads (Wellcotest 1µm beads - diluted 1:2 HEM + S). Following fixation with 3% glutaraldehyde the cells were resuspended in 1 drop of 0.75% trypan blue and counted under coverslips. Cells containing ten or more beads within the cytoplasm were counted as positive. Results were expressed as the percentage inhibition of phagocytosis of latex-IgG as compared with control suspensions pre-treated with PBS or with normal IgG. Under these conditions approximately 85% of the polymorphs observed appeared to have ingested latex-IgG.

iii) Inhibition of polymorph phagocytosis of radiolabelled Staphylococcus aureus

A modification of the method of Peterson et al (1977) was used. 10 ml of Mueller-Hinton broth was inoculated with Staphylococcus aureus (Cowan strain). To measure bacterial uptake the inoculated broth also contained 100ul of ^{3}H adenine (20uCi mmol specific activity:Radiochemical Centre, Amersham). The broths were incubated overnight at 37°C. The bacteria were washed 3 times in sterile saline. The concentration of

organisms was adjusted to 1 x 10^7 colony forming units/ml using a Cecil spectrophotometer. An optical density of 0.025 at 620nm corresponded to 1 x 10^7 colony forming units/ml. This was checked by pour plate methods.

Bacteria were opsonized with an equal volume of pooled human serum diluted in Gel-Hanks (Gibco). Opsonization of Staphylococcus aureus with 10% serum was found to give optimum phagocytosis. The bacteria were incubated in serum at 37°C in an orbital shaking incubator (150 rpm). After 15 minutes the bacteria were centrifuged at 3000 rpm for 20 minutes and resuspended in the original volume of Gel-Hanks.

Normal polymorphs (obtained using method 8:1:iv) were incubated at 37°C for 1 hour in IgG preparations (8:3:iv) or RPMI medium (Gibco) as control. After incubation samples were removed, washed and resuspended in Gel-Hanks at 1 x 10^7/ml.

To measure phagocytic uptake triplicate scintillation vials containing 0.1 ml of polymorphs and opsonized bacteria were incubated at 37°C in an orbital shaking incubator. A 1:1 ratio of polymorphs: bacteria was found to give optimum phagocytosis. After 15 minutes the vials were removed and 3 ml scintillation fluid added to one (A) of each trio (United Technologies, Packard, Caversham). Three ml of ice-cold phosphate buffered saline was added to the second vial (B). To the third vial (C) was added 1 ml of 500ug/ml lysostaphin (220 U/mg:Sigma) in phosphate buffered saline. This vial was reincubated at 37°C for 20 minutes, washed 3 times with phosphate buffered saline, and 3 ml scintillation fluid was added.

All 3 vials were washed 3 times with phosphate buffered saline at

900 rpm for 5 minutes. After the final wash the pellet was resuspended in scintillation fluid. The amount of radioactivity was determined using a liquid scintillation counter.

All experiments contained control vials (D) in which polymorphs were replaced by Gel-Hanks.

The percentage of bacteria taken up or ingested by the polymorphs was calculated from the number of counts per minute from each vial, using the formulae below:

$$\% \text{ uptake} = \frac{B - D}{A} \times 100$$

$$\% \text{ ingestion} = \frac{C - D}{A} \times 100$$

7. Experimental technique using platelets: inhibition of aggregation

Normal human platelets were obtained fresh from whole blood anticoagulated with 3.8% trisodium citrate by centrifugation to obtain platelet rich plasma. The rate of platelet aggregation was measured by the standard turbidometric technique as described by Born (1962). Briefly, a standard volume (100 µl) of various dilutions of IgG (normal or from transfused patients) in saline (100µl) were added to 200 ul platelet rich plasma and incubated at 37°C for 5 minutes prior to the addition of 1 µg collagen. The rate of platelet aggregation was then estimated using a Malins two channel aggregometer (change in optical density per second) and the results expressed as the percentage inhibition of aggregation induced by IgG from patients or from normal controls.

8. Detection of antibodies to spermatozoa: The Tray Agglutination Test

Antibodies to spermatozoa were detected by the tray agglutination test as described by Dor, Rudak & Aitken (1981). Briefly, 5 ul aliquots of IgG at dilutions ranging from 1 mg/ml to 0.05 ug/ml in PBS were incubated under liquid paraffin for 1 hour at 37°C with 1 ul fresh normal human semen. Agglutination was recorded using an inverted microscope at a magnification x 100.

9. Extraction of IgG-binding proteins from Factor VIII concentrate using affinity chromatography

IgG prepared from serum by DEAE chromatography (8:3:iv) was adjusted to a concentration of 10mg/ml in phosphate buffered saline and coupled with cyanogen bromide activated Sepharose 4B beads (Pharmacia) to give an 8 ml volume of beads. This was added to 6 ml reconstituted lyophilized Factor VIII preparation (Hemofil Batch 059IV048A, obtained from Glasgow Royal Infirmary). After end-over-end mixing at 4°C for 18 hours, the beads were washed in phosphate buffered saline until all non-specifically absorbed protein (assessed by measuring the optical density at 280nm) was removed, and a column was prepared (1 x 15 cm column - Pharmacia).

IgG-binding proteins were eluted using glycine HCl buffer at pH 2.8; 4 x 2 ml fractions were collected once the pH of the eluate began to fall. Each fraction was neutralized with solid Na_2HPO_4, then dialysed into phosphate buffered saline. The fraction containing most protein (assessed as previously by optical density) was designated that containing Hemofil-derived "antigen".

10. Polyacrylamide (SDS-PAGE) slab gel electrophoresis

A 7.5% non-reducing slab gel was prepared after the method of Laemmli (1970). Two glass slab gel plates were clamped and sealed with 30% acrylamide. Between them was poured 7.5% running gel, consisting of 6 ml 30% acrylamide, 6 ml buffer (TRIS 185g/l, SDS 4g/l, pH adjusted to 8.9 with N HCl), 100 ul 10% ammonium persulphate, 10ul M tetramethylenediamine, and 12 ml distilled water. After addition of a layer of isobutanol the gel was left to set for one hour. After excess isobutanol was tipped off, the remainder of the space between the plates was filled with 3% stacking gel, consisting of 2.4ml 30% acrylamide, 6 ml buffer (TRIS 59g/l, SDS 4g/l, pH adjusted to 6.7 with N HCl), 200 ul 10% ammonium persulphate, 10 ul tetramethylenediamine, and 15.6 ml distilled water.

To create wells for substrate, a plastic comb was inserted while the gel set. After the comb was removed, to each well was added 50 ul of the sample substrate to undergo electrophoresis, and 10 ul of sample buffer. This buffer was prepared by mixing 7.5 ml stacking gel buffer, 1g SDS and 2.5 ml distilled water. The mixture was warmed until the SDS dissolved, and then 1 ml glycerol and 4 ml 1% bromophenol blue dye were added. Dalton markers (Sigma) were included in all experiments.

The gel plates were clamped to the electrophoretic apparatus. The upper tank (negative terminal) was filled with buffer consisting of TRIS 6.3g/l, glycine 4g/l and SDS 1g/l. The lower tank (positive terminal) was filled with buffer consisting of TRIS 0.1g/l and SDS 1g/l, with pH adjusted to 8.1 using 5 N HCl. The gel was subjected to a current of 40 mA until the dye front was almost at the foot of the plates.

After the current and plates were disconnected, the gel was incubated at 37°C for 18 hours in 0.1% PAGE Blue stain (BDH), in a solution consisting of methanol, distilled water and glacial acetic acid (volumes in ratio 5:4:1). Then, until the gel pattern appeared clear, the gel was suspended in a solution of distilled water, methanol and glacial acetic acid (volumes in ratio 8:1:1).

11. Absorption of substrate with Protein A

One millilitre of Sepharose 4B beads, alone or with Protein A (Pharmacia), in phosphate buffered saline, was resuspended in 0.5 ml substrate (for example, serum fraction 1, prepared by method 8:3:iii above). After end-over-end mixing at room temperature for one hour, the beads were removed by centrifugation and the supernatant used for subsequent experiments.

12. Statistical analysis

Statistical analysis of results was performed using standard methods detailed in the text and described by von Fraunhofer and Murray (1976).

CHAPTER 9

THE PREVALENCE OF Fcγ-RECEPTOR BLOCKING ANTIBODIES IN NORMAL SUBJECTS

1. Introduction

i) Background

As outlined in Chapters 5 and 6, the production of Fcγ-receptor blocking antibodies was suggested in 1982 as the missing link between blood transfusion and renal allograft survival (MacLeod et al, 1982 b). Despite further promising evidence from the same group, no reports have emerged of similar work from other centres, apart from that described in this volume.

At the start in 1983 of this study the general aims were to confirm and extend MacLeod's findings. It was decided to use the EA rosette inhibition assay already in standard use in this laboratory for assessment of Fcγ-receptor blocking (Sandilands et al, 1980; and 8:4:i). At the outset the intention was to focus on IgG class antibody. Because of the possibility of serum factors other than IgG blocking the Fcγ-receptor and giving a "false positive" result, the decision was taken to use not serum but IgG for experimental work.

Using this assay, Bakkaloglu et al (1980) found in 50 normal subjects that the upper limit of inhibition of EA rosette formation of normal human peripheral blood lymphocytes by whole serum was 30%. No "normal" limits for this assay have been defined using IgG.

ii) Aims of the study

The purpose of the experiments described in this chapter was to define the extent in the author's hands of EA rosette inhibition by IgG from normal subjects, to use for comparison with subjects studied in later chapters.

The reports by Power et al (1983 a and b) and Stewart et al (1984) of Fcγ-receptor blocking antibodies in association with successful pregnancy led to specific consideration of pregnant and multiparous women as subgroups of the normal population.

2. Materials and Methods

i) Subjects studied

Serum samples were obtained from the following groups of normal volunteers, none of whom had previously received blood or blood products:

a) 13 male members of hospital staff (mean age 34, range 17 - 60 yrs)

b) 7 nulliparous female members of hospital staff (mean age 21, range 19 - 26 yrs)

c) 7 pregnant members of hospital staff (mean age 26, range 23 - 35 yrs). All were in the second trimester of their first pregnancy.

d) 11 multiparous members of hospital staff or their families (mean age 46, range 33 - 56 yrs)

ii) Methods used

IgG was obtained from serum samples by DEAE Chromatography as described above (8:3:iv) and adjusted to a concentration of Img/ml

in phosphate buffered saline. IgG preparations were ultracentrifuged at 105,000g for one hour prior to use lest aggregates of IgG had formed during freeze-thawing (Sandilands et al, 1980).

The EA rosette inhibition assay was performed on IgG preparations as described above (8:4:i). Peripheral blood lymphocytes, obtained from unselected healthy members of hospital staff, were used as the source of Fcγ-receptor bearing cells. The assay was performed at both 4°C and 37°C. Controls used at each temperature were phosphate buffered saline alone, and phosphate buffered saline containing IgG obtained from serum from the donor of the target lymphocytes. Control percentages of at least 15% EA rosette-forming cells were deemed acceptable (Sandilands et al, 1980). Cytotoxicity was excluded by 2-stage testing at 22°C against a 23-donor panel of peripheral blood lymphocytes.

3. Results

Control results were comparable for both temperatures and for phosphate buffered saline alone or with IgG from the normal lymphocyte donor. In no IgG preparation was significant cytotoxicity against peripheral blood lymphocytes demonstrated. Results of the EA rosette inhibition assay, taken to reflect degree of Fcγ-receptor blocking activity, for the various groups studied are depicted below (Figures 4 to 6).

Figure 4 shows percentage inhibition of EA rosette formation by IgG preparations from the 13 males and 7 nulliparous females. The maximum percentage inhibition at either temperature was 35%, with no difference detected between males and females. Mean $\pm$ standard

deviation percentage inhibition for all 20 patients was 15 $\pm$ 12 at 4°C, 7 $\pm$ 9 at 37°C, and 11 $\pm$ 11 overall.

Figure 5 shows percentage inhibition of EA rosette formation by IgG preparations from the 7 nulliparous, 7 pregnant and 11 multiparous women. Three subjects in each of the latter two groups showed percentages at both temperatures outwith the ranges depicted in Figure 4. Using non-parametric statistical analysis (Mann-Whitney U test), only the difference between the nulliparous and pregnant women reached statistical significance ($P<0.05$).

Figure 6 shows the result of an experiment to see whether Fcγ-receptor blocking activity was removed by ultracentrifugation at 100,000g for one hour. The failure to do so suggests that IgG, rather than contaminating aggregates, was responsible for blocking activity in the 6 preparations tested.

Table 1 shows the results obtained when simultaneous testing was performed on 10 control lymphocyte preparations rosetted in the presence of phosphate buffered saline alone, and with IgG from a known "positive" and a known "negative" preparation. This indicates the extent of internal and intra-observer variation of the assay in the hands of the author.

4. Discussion

The results presented in this chapter provide a reference range for comparison of results in later chapters using the EA rosette inhibition assay to test preparations of IgG at concentration Img/ml in phosphate buffered saline. The decision as to which percentage of inhibition of EA rosette formation to designate "significant" is

arbitrary, and previous workers have adopted differing criteria (for example, Suthanthiran et al, 1977; Sandilands et al, 1980; MacLeod et al, 1982 a).

In the normal nulliparous subjects studied here, no IgG preparation inhibited rosette formation to a degree greater than 35% of the control value. Overall, the mean percentage inhibition of rosette formation at either temperature was 11%, and the value for the mean + 2 standard deviations of the mean was 33%. On the basis of these results it was decided to regard percentages greater than 35% as outwith the normal range, denoting significant inhibition of rosette formation.

It is necessary also to take into account women's obstetric histories. The results reported here confirm the demonstration by Power et al (1983 a and b) that a proportion of women presently or previously pregnant may have non-cytotoxic Fcγ-receptor blocking IgG class antibody. In these experiments the IgG preparations were not tested against paternal lymphocytes. Although previous work suggests that testing against paternal lymphocytes would yield a higher proportion of "positive" results, the women studied here were being considered for use as a control population for a population of multiparous renal dialysis patients, for whom testing against unrelated donors was more practicable than against their husbands (see Chapter 10).

It is interesting to note that the studies of Power et al (1983 a and b) and Stewart et al (1984) show EA rosette inhibitory activity of maternal sera against paternal B lymphocytes. Although some of the inhibitory activity of the IgG preparations here may be directed against the B cell Fcγ-receptor, inhibitory activity as great as 98% (Figure 5) can only be explained by inhibition of more than just the B cell receptor (Sandilands et al, 1980).

5. Conclusions

i) Normal values for the EA rosette inhibition assay were obtained using IgG preparations in phosphate buffered saline. It was decided to regard percentages of EA rosette inhibition greater than 35% as outwith the normal range for IgG preparations from men and nulliparous women.

ii) Several pregnant and multiparous women had results outwith this range.

CHAPTER 10

THE ASSOCIATION BETWEEN BLOOD TRANSFUSION AND FcγR-RECEPTOR BLOCKING ANTIBODIES

1. Introduction

i) Background

The association between blood transfusion and serum FcγR-receptor blocking activity has been noted in renal dialysis patients by MacLeod et al (1982b, 1983, 1985a), using B lymphocytes from normal subjects and patients with chronic lymphatic leukaemia. Discussed in this chapter are experiments to assess the prevalence of FcγR-receptor blocking antibodies in IgG preparations from transfused subjects with and without renal failure.

ii) Aims of the study

The experiments described in this chapter were designed to answer the following questions:

a) Is previous blood transfusion associated with the presence of non-cytotoxic IgG class FcγR-receptor blocking antibodies in renal dialysis patients?

b) Are similar antibodies found in transfused subjects without known renal disease?

c) Is the presence of antibodies associated with clinical variables other than transfusion?

d) Does antibody activity vary with the lymphocyte donor used? If so, can the variation be attributed to the donor's tissue type?

2. Materials and Methods

i) Subjects studied

Serum samples were taken from the groups of subjects listed below:

a) Normal subjects: the results for these subjects were obtained as described in Chapter 9. Twenty (13 male) had had no transfusions or pregnancies, and 11 were untransfused multiparous women (considered separately below in Figure 8).

b) Untransfused uraemic subjects: Ten (6 male) subjects with renal disease (mean age 45, range 22 - 72 years). All had a serum creatinine greater than 600 μmol/l. None had received blood or blood products. Two had had previous full-term pregnancies.

c) Transfused uraemic subjects: Fifty-four (30 male) uraemic subjects on maintenance dialysis (mean age 39, range 19 - 65 years). All had received at least 5 units of whole blood or packed cells, the Renal Unit protocol for elective transfusion prior to entry to the pool waiting for cadaveric transplantation. Nineteen patients of both sexes were on continuous ambulatory peritoneal dialysis and the rest on haemodialysis. Nine of the women had had previous full-term pregnancies.

d) Transfused non-uraemic subjects: Ten subjects (5 male) with no known renal disease and a serum creatinine less than 130 μmol/l (mean age 60, range 26 - 84). Two had had previous full-term pregnancies. All had received within the past 5 years at least 5 units of whole blood or packed cells, for a variety of indications detailed in Chapter 7:2.

ii) Methods used

IgG prepared from serum samples as described above (8:3:iv) was tested for Fcγ-receptor blocking activity using the EA rosette inhibition assay (8:4:i). Peripheral blood lymphocytes, obtained from unselected healthy members of hospital staff, were used as the source of Fcγ-receptor bearing cells. The assay was performed at both 4°C and 37°C. Phosphate buffered saline alone was used for the controls at each temperature with control values of at least 15% rosette-forming lymphocytes deemed acceptable. Cytotoxicity was excluded by 2-stage testing at 22°C against a 23-donor panel of peripheral blood lymphocytes.

3. Results

Figure 7 shows percentage inhibition of EA rosette formation at 4°C and 37°C by IgG preparations from the subjects detailed above. Previous transfusion of at least 5 units of blood is associated in both uraemic and non-uraemic subjects with the presence of Fcγ-receptor blocking IgG antibody.

That Fcγ-receptor blocking is not a consequence of uraemia is shown by results for the untransfused uraemic and transfused non-uraemic subjects.

IgG preparations from all of the 10 transfused non-uraemic subjects gave Fcγ-receptor blocking at both temperatures.

Of the 54 transfused uraemic subjects, at 4°C 14 and at 37°C 31 had Fcγ-receptor blocking antibody activity. This difference between the prevalences of antibody activity at the two temperatures was highly statistically significant ($P<0.001$ X^2 test). No patient had antibody activity at 4°C alone. Similarly, the differences in the prevalences of antibody activity at both temperatures between IgG preparations from transfused uraemic and non-uraemic subjects were highly significant (X^2 test, 4°C $P<0.001$, 37°C $P<0.01$).

There was no evidence that variables other than transfusion caused the increased prevalence of Fcγ-receptor blocking activity seen in transfused subjects. None of the patients studied was pregnant. Only two of the non-uraemic subjects had had pregnancies, one two and one over fifty years previously. This is insufficient to explain the uniform Fcγ-receptor blocking activity in the non-uraemic transfused group.

Nine of the transfused uraemic group had had full-term pregnancies, all at least five years previously. In this they were similar to the multiparous control group, to whom they are compared in Figure 8. At 4°C there was no difference between the Fcγ-receptor blocking activity of IgG from transfused and untransfused women. However, at 37°C IgG from 6 out of 9 transfused and from only 3 out of 11 untransfused women showed blocking activity outwith the normal range. Using the X^2 test with Yates correction, this difference between the groups reached statistical significance ($P<0.05$).

The transfused subjects as a whole were older than the untransfused subjects. However, within each group there was no evidence of increasing antibody activity with age.

Within the group of transfused dialysis patients there was, as shown in Figure 7, considerable variation in the capacity of IgG preparations to inhibit EA rosette formation. No association was found between inhibition at either temperature and the patient's age, sex, primary renal disease, mode of dialysis, length of time on dialysis or HLA type.

Nor was any association seen between rosette inhibition and biochemical control of renal failure, as reflected by routine measurements at the time of sampling of serum urea, creatinine and phosphate. Serum ferritin was not measured.

There was no obvious association with drug therapy. Unlike the non-uraemic transfused subjects, almost all the dialysis patients were taking multivitamin preparations and aluminium-based phosphate binders; there was no apparent association between rosette inhibition and routine measurements of serum aluminium.

In one of the 54 patients the transfusion history was confused by his having had a number of units of blood in another centre. For the other 53 patients the number of units of blood (of any type) transfused at the time of the sample used was plotted against Fcγ-receptor inhibition at 37^{o}C in Figure 9. No correlation was seen; the same applied when the results at 4^{o}C were used (not illustrated).

The variation was unlikely to be due to differences in concentration of antibody, as all IgG preparations had been standardized by

spectrophotometry to a concentration of Img/ml. Figure 10 shows that, despite variation in degree of rosette inhibition with serial dilutions of IgG preparations, Fcɣ-receptor blocking activity was not completely removed by dilution to 1 in 10^6 of preparations from transfused dialysis patients.

Table 2 shows the variation in results obtained when IgG preparations from 15 transfused uraemic subjects were tested for inhibition of EA rosette formation at 37^oC against peripheral blood lymphocytes from 4 unrelated normal subjects in parallel. No obvious pattern of reactivity against the antigens common to the lymphocyte donors was seen.

Only 3 of the IgG preparations studied had cytotoxic activity against more than 10% of the donor lymphocyte panel used for tissue typing. All 3 subjects were dialysis patients who had received multiple transfusions; one had also had two full-term pregnancies.

4. Discussion

The findings in this chapter confirm the association reported by MacLeod et al (1982 b) between blood transfusion and Fcɣ-receptor blocking antibodies. Previous work has concentrated on B lymphocytes rather than unseparated peripheral blood lymphocytes, because of early evidence linking Ia antigens to Fcɣ-receptors on the surface of B lymphocytes (Chapter 6:4:ii). The results described here show that antibodies associated with transfusion may be detected using peripheral blood lymphocytes. It is unlikely that the high levels of inhibition of EA rosette formation seen in some subjects can be explained by antibodies blocking only B lymphocyte Fcɣ-receptors (Unkeless et al, 1981). Experiments to confirm Fcɣ-receptor blocking activity against

T lymphocytes are described in Chapter 12.

The use of IgG separated by DEAE chromatography, while more time-consuming than the use of serum, confirmed that IgG was responsible for the Fcγ-receptor blocking activity. This does not preclude the existence in serum of other Fcγ-receptor blocking factors.

It is interesting that more IgG preparations were found to have Fcγ-receptor blocking activity when the EA rosette inhibition assay was performed at 37°C than at 4°C. Only at 37°C was a significant difference found between transfused and untransfused multiparous women. This may be compared to the increased sensitivity at 37°C of lymphocytotoxicity testing (Kissmeyer-Nielsen and Dick, 1979), and may reflect temperature-dependent differences in antigen expression on the cell surface. It is unlikely to be a kinetic phenomenon as continuing the period of incubation at 4°C for up to one hour did not increase the sensitivity of the assay (results not shown). In each case, peripheral blood lymphocytes from the same normal donor were used for testing at both temperatures and no IgG preparation had Fcγ-receptor blocking activity at 4°C only.

Previous studies of transfusion-related Fc -receptor blocking antibodies have employed EA rosette inhibition at 4°C only (MacLeod 1982b, 1983, 1985a). In the first report from the Aberdeen group linking the transfusion effect to Fcγ-receptor blocking antibodies (MacLeod et al, 1982b), 10 out of 11 patients with, and 9 out of 20 patients without pretransplant serum Fc -receptor blocking activity, had functioning transplants after one year.

As a test to predict renal allograft survival the assay had, from these figures, much greater specificity (92%) than sensitivity (53%)

(Alderson, 1976). Of the 54 transfused uraemic subjects reported here 14 (26%) at 4°C and 31 (57%) at 37°C had Fcγ-receptor blocking activity against lymphocytes from a normal donor. It is tempting to speculate whether doubling the sensitivity of the assay by performing it at 37°C might also double its sensitivity as a predictive test for renal allograft survival.

In contrast to the results for the uraemic subjects, IgG preparations from all 10 transfused subjects without known renal disease had Fcγ-receptor blocking activity at both temperatures. This homogeneity was not reflected in the patients' clinical histories, the only common factor being that each had in the past received at least 5 units of blood (Chapter 7:2).

The difference in prevalence of antibody between the uraemic and non-uraemic groups in this study could not be explained by an increased prevalence of cytotoxicity of IgG preparations from the non-uraemic group (Morito et al, 1978). There have been suggestions that cytotoxic antibody formation may be greater in non-uraemic patients. Ferrara et al (1974) repeatedly transfused normal recipients with blood from the same donor, and found that all but 20% of recipients eventually developed anti-donor cytotoxins. The majority of patients studied by Varghese et al (1981) and Fehrman and Ringden (1982) had detectable cytotoxins after transfusion for chronic anaemia in the absence of renal failure. There have been no previous reports of non-cytotoxic Fcγ-receptor blocking antibodies in transfused non-uraemic subjects.

Assessment of the "normal" response to transfusion is complicated by the difficulties in dissociating the effects of transfusion from the effects of the underlying disease. For example, it has been reported that activated T lymphocytes may suppress haematopoiesis in patients

with aplastic anaemia (Bacigalupo et al, 1980; Torok-Storb and Hansen, 1982; Nakao et al, 1984). Yet the uncertainty remains that these suppressor lymphocytes could be a consequence of transfusion not disease (Singer et al, 1978; Torok-Storb et al, 1980; Zoumbos et al, 1985).

The effects of transfusion and uraemia have also been difficult to distinguish (Guttman, 1978). Scanty transfusion details hinder interpretation of the bulk of the literature (reviewed by Goldblum and Reed, 1980; Keane and Raij, 1983). There have been conflicting reports on the antibody response in patients with uraemia although serum immunoglobulin levels have been regarded as normal (Goldblum and Reed, 1980)

Balch (1955) found no evidence of impairment of the capacity of patients with post-traumatic acute renal failure to synthesise tetanus antitoxin after booster inoculation with tetanus toxoid. Stoloff et al, (1958) reported normal responses to booster diphtheria toxoid in Schick-negative uraemic patients. Wilson, Kirkpatrick and Talmage (1965) found that uraemic subjects had poor responses to typhoid vaccine despite having encountered it previously.

More recently, Bramwell et al (1985) found that impaired response to hepatitis B vaccine in dialysis patients was predicted by impairment of cell-mediated immunity, as assessed by skin testing with Dinitrochlorobenzene. Although the link with transfusion was not discussed, the same group had shown a relationship among prior transfusion, poor response to Dinitrochlorobenzene and improved renal allograft survival (Watson et al, 1979 and 1981).

The results in this study suggest that uraemia may be associated

with reduction in the prevalence of Fcγ-receptor blocking antibodies in transfused subjects. Multiparous women may have antibody activity, but no further clinical variables associated with the presence of antibodies were identified.

The Fcγ-receptor blocking activity of IgG preparations from transfused dialysis patients varied with the donor of the receptor-bearing lymphocytes. The apparent lack of association with the HLA-A, B or DR type of the lymphocyte donor echoes the findings of MacLeod et al (1982a), although MacLeod et al (1985a) suggested from a family study that Fcγ-receptor blocking antibodies showed HLA linkage. It is possible that the antibodies are directed against an undefined class II antigen system expressed on the cell surface in association with the Fcγ-receptor (MacLeod et al, 1982b).

Genetic polymorphism of the Fcγ-receptor itself could also contribute to the variation. Kaneoka et al (1983) and Tax et al (1985) have proposed that polymorphism of Fcγ-receptors is not HLA-linked and is responsible for the heterogeneity of the human lymphocyte response to mouse monoclonal antibodies.

It is possible that the antibodies are directed against the range of alloantigens expressed by previous blood donors. Access to information about blood donors, for example their HLA types, was not readily available. The situation in which it would be feasible to study the antibody response to a particular donor is that of donor-specific transfusion prior to haploidentical live related donor transplantation. Experiments with donor-specific transfusion are discussed in Chapter 11.

The results of this cross-sectional study show a strong association

between previous blood transfusion and the presence of Fcγ-receptor blocking antibodies. Confirmation of the association would be previded by following untransfused patients over a protocol of elective transfusion. This is discussed further in the next chapter.

5. Conclusions

i) Previous blood transfusion was associated with the presence of non-cytotoxic IgG class antibodies detected by their capacity to block Fcγ-receptors on peripheral blood lymphocytes from normal subjects.

ii) More IgG preparations from transfused renal dialysis patients were found to have Fcγ-receptor blocking activity when tested at 37°C than at 4°C.

iii) IgG preparations from fewer uraemic than non-uraemic subjects had Fcγ-receptor blocking activity.

iv) No clinical variables other than previous transfusion and pregnancy were identified as associated with the presence in uraemic subjects of Fcγ-receptor blocking antibodies.

v) IgG preparations varied in Fcγ-receptor blocking activity with the peripheral blood lymphocyte donor used.

CHAPTER 11

THE PRODUCTION OF FcγR-RECEPTOR BLOCKING ANTIBODIES FOLLOWING ELECTIVE BLOOD TRANSFUSION IN RENAL DIALYSIS PATIENTS

1. Introduction

i) Background:

The last chapter illustrated the association between previous blood transfusion and non-cytotoxic IgG class antibodies detected by their capacity to block the FcγR-receptors on peripheral blood lymphocytes. Demonstration of this association is, however, incomplete in a purely cross-sectional study. The experiments presented in this chapter follow renal dialysis patients over the course of the elective transfusion protocols designed to prepare them for transplantation.

ii) Aims of the study

The experiments described in this chapter were devised to answer the following questions:

a) Do FcγR-receptor blocking antibodies develop over the course of elective third party blood transfusion?

b) Do donor-specific FcγR-receptor blocking antibodies develop after donor-specific transfusion from a haploidentical relative?

2. Materials and Methods

i) Subjects studied

a) Ten untransfused renal dialysis patients (8 male, mean age 37, range 19 - 49 years) undergoing elective third party transfusion before entering the pool for cadaveric renal transplantation. The elective transfusion protocol consisted of five units of packed cells from different donors transfused at fortnightly intervals.

b) Ten renal dialysis patients (5 male, mean age 27, range 15 - 52 years) undergoing donor-specific transfusion from a haploidentical relative. The donor-specific transfusion protocol adopted in Glasgow was that of Salvatierra et al (1980). The prospective renal allograft donor provided one unit of blood, which was given to the recipient in three aliquots at fortnightly intervals. All but 2 patients had received previous third party blood. The donor in 6 cases was a sibling and in the other 4 a parent.

ii) Methods used

Serum samples were taken from each subject before the course of transfusion and two weeks after each transfusion. IgG preparations were made from each sample, and stored and ultracentrifuged as before prior to use (8:3:iv).

IgG preparations from the group undergoing third party transfusion were tested for Fcγ-receptor blocking activity against normal human peripheral blood lymphocytes, using the EA rosette inhibition assay at both $4^{o}C$ and $37^{o}C$. IgG preparations from the group undergoing donor-specific transfusion were tested against peripheral blood lymphocytes from the blood donor, from other first degree relatives

and from unrelated controls. Phosphate buffered saline alone was used for the controls. Cytotoxicity was excluded by 2-stage testing at 22°C against a standard tissue typing panel as previously.

3. Results

Figure 11 shows the percentage inhibition of EA rosette formation at 4°C and 37°C by IgG prepared from serum samples taken from patients before and after the 5 unit course of third party blood. A significant increase in inhibition at both temperatures was noted after transfusion ($P<0.05$, Wilcoxon Matched Pairs Signed-Rank Test). Two patients in this group were found to have cytotoxic antibody following transfusion.

Figure 12 shows more detailed results for 2 patients, testing IgG preparations after each unit of third party blood. Patient B had no antibody response to transfusion in this assay. Although IgG from patient A developed Fcγ-receptor blocking activity at 4°C later in the transfusion protocol than at 37°C, this was not a consistent finding in other patients studied.

The results of donor-specific transfusion are shown in Figure 13. No subject had had a successful pregnancy, and it would seem likely that the 3 patients who had evidence of anti-donor Fcγ-receptor blocking antibody even before donor-specific transfusion had acquired it through third party transfusion. One of only 2 previously untransfused patients developed anti-donor cytotoxic antibody, and the other showed no Fcγ-receptor blocking activity after transfusion against either peripheral blood or B lymphocytes from the donor sibling, their parents or 4 other siblings. Only 2 of the 10 patients studied developed non-

cytotoxic anti-donor Fcγ-receptor blocking antibody. Using the Wilcoxon test again no significant change in anti-donor antibody activity at either temperature was demonstrated after transfusion.

The high incidence of prior third party transfusion and the small number of patients developing non-cytotoxic antibody after donor-specific transfusion unfortunately render non-contributory the family studies, which were undertaken in order to try to demonstrate HLA-linkage of antibody production! Both patients who developed non-cytotoxic anti-donor antibody had received third party blood before donor-specific transfusion from their mothers. Each failed to develop anti-paternal antibody, but was found to have Fcγ-receptor blocking activity after transfusion in each case against one of three siblings. However, in one family pretransfusion IgG also blocked the sibling's peripheral blood lymphocyte EA rosette formation. In the other the relevant sibling was an identical HLA match to the patient. In each case donor-specific transfusion was accompanied by the development of anti-body against unrelated subjects as well as the blood donor.

4. Discussion

The results in this chapter show that IgG class antibodies which block Fcγ-receptors on normal peripheral blood lymphocytes develop over the course of a three-month, five unit, elective blood transfusion protocol in renal dialysis patients. This has already been shown for B lymphocytes by MacLeod et al (1982b, 1983).

The relative clarity of the results for third party transfusion contrasts with those for donor-specific transfusion. Although one family study has been published by MacLeod et al (1985a) there have been

no published reports of Fcγ-receptor antibody production in protocols of donor-specific transfusion prior to haploidentical live related donor renal transplantation.

The reports of anti-idiotypic antibody after donor-specific transfusion are discussed in Chapter 5:11:ii, and share the weaknesses of the results of donor-specific transfusion in this study (Burlingham et al, 1985a and b ; Reed et al, 1985; Takeuchi et al, 1985). Although the number of patients is relatively respectable (10), 8 of these had had previous third party blood. Had the antibody response to donor-specific transfusion been less varied, it might have been easier to comment on the results.

In particular, the effort expended on family studies was not worthwhile. The lack of HLA-linked pattern of Fcγ-receptor blocking activity could in most cases have been ascribed to previous third party blood. Of the 2 patients who had never been transfused, one developed cytotoxic antibody and the other developed no Fcγ-receptor blocking antibody activity against unseparated or B lymphocytes from any family member. The latter patient had excellent graft function 18 months after receiving a kidney from the blood donor.

Transplant outcome bore no relation to the development of Fcγ-receptor blocking antibodies against donor peripheral blood lymphocytes following donor-specific transfusion. Four out of the 10 original donor-recipient pairs did not proceed to transplantation because of the development of cytotoxic antibodies in the recipient or the discovery of medical contraindications in the donor. Of the 6 who did reach transplantation, 5 kidneys were functioning at one year. The sixth was lost through rejection at 9 months. The recipient had developed

non-cytotoxic anti-donor Fcγ-receptor blocking antibodies during the course of donor-specific transfusion, but had also had large amounts of third party blood.

Consideration of these results suggests that any follow-up study should include only patients who have never had third party blood. The number of such patients in any one centre may be small. It is perhaps worth noting that, although donor-specific transfusion has been widely used, the Collaborative Transplant Study has recently failed to report any advantage in transplant survival over third party blood (Opelz, 1985c). Our own centre has now discontinued donor-specific transfusion after hyperacute rejection of a kidney. This case was not one of those studied here and rejection may have been related to the later finding of a positive monocyte crossmatch (Cerilli et al, 1985).

5. Conclusions

i) Elective third party blood transfusion is associated in renal dialysis patients with the development of non-cytotoxic IgG class antibodies detected by their capacity to block Fcγ-receptors on peripheral blood lymphocytes from normal subjects.

ii) No consistent association was seen between donor-specific transfusion and the development of similar antibodies directed against lymphocytes from the blood donor. This may have been related to previous third party transfusion.

CHAPTER 12

THE SPECTRUM OF CELLULAR REACTIVITY OF TRANSFUSION-RELATED Fcγ-RECEPTOR BLOCKING ANTIBODIES

1. Introduction

i) Background

Having confirmed in the previous chapters the association between blood transfusion and the production of Fcγ-receptor blocking antibodies, the next task was to delineate the spectrum of cellular activity of IgG preparations obtained from multiply transfused subjects.

ii) Aims of the study

The experiments described in this chapter were designed to answer the following questions:

a) Can transfusion-related IgG class antibodies, detected by their capacity to block Fcγ-receptors on allogeneic peripheral blood lymphocytes, be shown to block Fcγ-receptors on other cells?

b) Can IgG preparations from transfused subjects inhibit Fcγ-receptor mediated cell functions *in vitro*?

c) Do IgG preparations with Fcγ-receptor blocking activity block other cell surface receptors also?

2. Materials and Methods

i) Subjects studied

Peripheral blood lymphocytes were obtained (8:1:i) from the following subjects:

a) 40 untransfused normal volunteers

b) 11 patients with serum creatinine greater than 600 micromols/l who were not on maintenance dialysis and had never been transfused

c) 5 patients with serum creatinine greater than 600 micromols/l who had received at least 2 units of blood but were not on maintenance dialysis

d) 10 patients with functioning renal transplants (serum creatinine less than 300 micromols/l) who had received at least five units of blood prior to transplantation and were on maintenance immunosuppressive therapy with Prednisolone (range 10 - 15 mg daily) and Azathioprine (dosage range 50 - 150 mg daily)

In 20 subjects, distributed among the study groups, simultaneous serum samples were taken and IgG preparations made (8:3:iv).

Additional IgG preparations used in the experiments described in this chapter were those from transfused and untransfused subjects as described in Chapters 7, 9 and 10.

The number of samples analysed varied between assays; the numbers of subjects studied are given beside the relevant results.

ii) Methods used

The percentage of EA rosette-forming cells in peripheral blood lymphocyte preparations from study subjects was assessed (8:4:i). Concurrent IgG preparations from these subjects were tested for inhibition

of EA rosette formation by allogeneic peripheral blood lymphocytes.

IgG preparations from normal and transfused subjects were tested against cells from normal donors:

a) for inhibition of EA, EAC and NSE rosette formation simultaneously against peripheral blood lymphocytes from the same normal donor. (Methods described: 8:4:i; 8:4:ii; 8:4:iii).
b) for inhibition of EA rosette formation (8:4:i) by B and T cell enriched lymphocyte preparations (8:1:ii).
c) for inhibition of phytohaemagglutinin (PHA)-induced lymphocyte blastogenesis (8:4:iv).
d) for inhibition of antibody-dependent cell-mediated cytotoxicity and Natural Killer cell function (8:4:v)
e) for inhibition of polymorph EA rosette formation (8:6:i)
f) for inhibition of polymorph phagocytosis of IgG-coated latex beads (8:6:ii)
g) for inhibition of uptake and ingestion by polymorphs of 1^{125}-labelled Staphylococcus aureus (8:6:iii)
h) for inhibition of monocyte EA rosette formation (8:5)
i) for inhibition of collagen-induced platelet aggregation (8:7)

IgG preparations from 3 transfused subjects were tested for inhibition of EA rosette formation by peripheral blood lymphocytes from 1 normal donor (8:4:i) before and after absorption with the following types of cells (8:3:v); erythrocytes (8:1:v); platelets (8:1:vi); thymocytes (8:2:ii) and B lymphocytes (8:1:ii) from a patient with chronic lymphatic leukaemia. These last were classified as B cells according to their reactivity with monoclonal antibodies (OKT 11, 8 and 4 (Becton Dickinson) and Pan B (DAKO)); they carried surface immunoglobulin and formed spontaneous rosettes with mouse erythrocytes but

failed to form rosettes with chicken EA.

3. Results

i) Lymphocytes

Figure 14 shows the percentages of EA rosette-forming lymphocytes in peripheral blood from groups of transfused and untransfused subjects. Statistical analysis, performed by two-tailed Student's t test assuming normal sample distribution, gave the levels of significance in Table 3 for the differences between results for the groups. No correlation was found (Figure 15) between the percentage of EA rosette-forming peripheral blood lymphocytes and the capacity of the corresponding IgG preparations to inhibit rosette formation by allogeneic peripheral blood lymphocytes.

Figure 16 shows the results for inhibition at 37°C of EAC and NSE rosette formation of normal human peripheral blood lymphocytes by IgG preparations from 10 untransfused subjects, 6 of whom were healthy and 4 of whom were on regular dialysis. These results are provided for comparison with those in Figure 17, where IgG preparations from 14 transfused dialysis patients were tested simultaneously for inhibition at 37°C of EA, EAC and NSE rosette formation by peripheral blood lymphocytes from the same donor. (Controls $>$ 25% EAC, $>$ 75% NSE rosettes).

Although some inhibition of EAC rosette formation was noted, this occurred to a lesser degree than did inhibition of EA rosette formation. In no patient was NSE rosette formation inhibited.

Interestingly, when IgG preparations from 3 women in the second trimester of pregnancy were tested specifically against paternal

lymphocytes, inhibition of both EA and EAC rosette formation was noted (Figure 18). Percentages for inhibition of EA rosette formation were outwith the range already defined as normal (Chapter 9). Using a Mann-Whitney U test IgG preparations from the 3 pregnant women inhibited EAC rosette inhibition to a significantly greater extent than did IgG preparations from the 6 untransfused normal subjects in Figure 16 ($P<0.05$).

Figure 19 shows the results for inhibition of EA rosette formation at 37°C by IgG preparations from 10 transfused dialysis patients. The Fcγ-receptor bearing cells used were T and B cell enriched lymphocyte preparations from a single normal donor. (Controls >15% T, >35% B).

The upper limit of normal inhibition of EA rosette formation by IgG was again taken to be 35%. Although values for T and B cell enriched preparations were not established as for peripheral blood lymphocytes (Chapter 9), in none of 4 normal IgG preparations tested was inhibition greater than 10% (results not illustrated) and the limit used by MacLeod et al (1982 a and b) for B lymphocytes was 40%. In 7 out of 10 cases illustrated there was concordance between the results for the two cell preparations. One IgG preparation had Fcγ-receptor blocking activity against the T but not the B cell preparation, and 2 inhibited rosette formation of the T but not the B cell preparation.

Table 4 shows values for inhibition of K and NK cell cytotoxicity by IgG preparations from 5 and 9 transfused dialysis patients respectively. In none of 3 normal subjects did inhibition of cytotoxicity exceed 15%, but no significant difference between the normal and transfused subjects was found (Mann-Whitney U test).

IgG preparations from 11 normal subjects and 9 transfused dialysis patients were tested with peripheral blood lymphocytes from a single donor for inhibition of both EA rosette formation (at 37°C) and inhibition of PHA-induced blastogenesis. Figure 20 shows the results of inhibition of PHA-induced blastogenesis (performed in triplicate with mean control value 3967 counts per minute). IgG preparations from the transfused patients inhibited blastogenesis to a significantly greater degree than did IgG preparations from the normal subjects ($P<0.01$, Mann-Whitney U test). Mean ($\pm$ standard deviation) percentage inhibition was 30 ($\pm$ 4) for the normal, and 46 ($\pm$ 10) for the transfused group. Inhibition of EA rosette formation by each IgG preparation is plotted against inhibition of blastogenesis in Figure 21, with a statistically significant correlation noted between the two percentages ($P<0.01$).

ii) Polymorphs

Figure 22 shows the results of the EA rosette inhibition assay (37°C) using polymorphs from a normal donor. The IgG preparations came from 6 normal controls and 27 transfused subjects, all but 2 of whom were uraemic subjects on dialysis. None of the 6 normal subjects had antibody activity inhibiting polymorph EA rosette formation by more than 25%, but samples from 14 of the transfused subjects (including both of those without renal disease) gave higher levels of inhibition (normal versus transfused groups, $P<0.001$, Mann-Whitney U test).

In Figure 23 the results of EA rosette inhibition for polymorphs are plotted against those for peripheral blood lymphocytes, using the same IgG preparations and the same normal cell donor. No significant correlation is seen.

Figure 24 shows the effect of IgG preparations from 6 normal and 17 transfused uraemic subjects on the percentage of IgG-coated latex beads taken up by polymorphs from a normal donor. Control tests showed that no beads were ingested without prior coating with IgG. No significant difference between the normal and transfused subjects was detected (Mann-Whitney U test).

The one normal subject whose IgG preparation gave 70% inhibition of ingestion of latex beads was a healthy multiparous woman. Three women in each of the normal and transfused groups had had previous full-term pregnancies; if they are excluded from consideration the difference between the normal and transfused groups by the same statistical analysis has a p value of 0.05. In the absence of more data the association between previous transfusion and IgG inhibition of polymorph phagocytosis of IgG-coated latex beads remains not proven.

No correlation was found between percentage inhibition of phagocytosis of the beads and percentage inhibition of EA rosette formation of polymorphs from the same normal donor (Figure 25).

Uptake of IgG-coated latex beads is mediated through the Fcγ-receptor; phagocytosis of Staphylococcus aureus is, however, related to the C3b receptor (Ross, 1982). The results of experiments to assess the effect of IgG preparations on uptake and ingestion by normal polymorphs of radiolabelled Staphylococcus aureus are presented in Figure 26. IgG preparations from 6 normal and 6 transfused uraemic subjects were tested. Both uptake and ingestion were impaired in the transfused uraemic, as opposed to the normal, subjects (Mann-Whitney U test, $P<0.05$ for both parameters). There was a close correlation between uptake and ingestion ($r = 0.85$, $P<0.001$)

iii) Monocytes

On parallel testing against cells from the same normal donor, none of 6 IgG preparations found to inhibit EA rosette formation by peripheral blood lymphocytes and polymorphs inhibited monocyte EA rosette formation. The assay was performed at 37°C only. Mean ± standard deviation percentage inhibition of EA rosette formation for each of the cell types tested was as follows:

monocytes	1 ± 2
lymphocytes	67 ± 7
polymorphs	54 ± 12

iv) Platelets

IgG preparations from 6 normal and 6 transfused subjects (all but one uraemic) were tested for their capacity to inhibit collagen-induced aggregation of normal platelets. Figure 28 shows the results, with corresponding values for peripheral blood lymphocyte Fcγ-receptor blocking activity. The difference in degree of inhibition of platelet aggregation between IgG preparations from normal and transfused subjects was found to be statistically significant ($P<0.05$, Mann-Whitney U test).

v) Absorption experiments

IgG preparations from 3 transfused subjects were tested for their capacity to inhibit EA rosette formation by peripheral blood lymphocytes from a single normal donor before and after absorption with a variety of cells. The results (Figure 28) show that Fcγ-receptor blocking activity was removed by absorption with platelets, which carry the receptor, but not by erythrocytes, which do not. Inhibition of EA rosette formation

was also removed after absorption with thymocytes and B cells from a patient with chronic lymphatic leukaemia, neither of which expressed Fcγ-receptors, as assessed by failure to form EA rosettes.

4. Discussion

The results presented in this chapter show the effects of IgG preparations from untransfused and transfused subjects on a variety of cell types and functions. Although the intention was to look primarily at IgG preparations in relation to their Fcγ-receptor blocking activity, it seems likely that the range of antigens presented by blood transfusion leads to other types of antibodies contributing to the results found here.

In some of the experiments, IgG preparations were tested in parallel with antiserum to membrane-labile Fcγ-receptors derived from normal peripheral blood lymphocytes. Results for this antiserum have been published elsewhere (Sandilands et al, 1985) and provide a standard for comparison with the effects of Fcγ-receptor blocking IgG preparations.

If Fcγ-receptor blocking antibodies suppress the immune response to the renal allograft *in vivo*, they may do so by blocking antigen-presenting cells in the graft (Jones, Hans-Hartmut and Feldman, 1972; Hart and Fabre, 1982). The alternative would be to block the patient's own lymphocytes. MacLeod et al (1985b) suggested that Fcγ-receptor blocking antibodies are allo- rather than autoantibodies. Reactivity of B cell antibodies with B cells from patients with chronic lymphatic leukaemia has been taken to indicate that they are not autoantibodies (Ting and Morris, 1978b). Fcγ-receptor blocking activity was removed from IgG preparations in this study by absorption with B cells from a leukaemic patient.

Blood transfusion has been reported as leading to increased expression of OKT8 positive, Ia postive, Fcγ-receptor bearing T lymphocytes (Mangan et al, 1982; Gascon et al, 1984; Nakao et al, 1984). However, if transfusion were associated with the development of antibodies blocking autologous Fcγ-receptors, one might expect to find in transfused subjects a decrease in the percentage of lymphocytes bearing unblocked receptors. Circumstantial evidence for this was provided by the reduced percentage of EA rosette-forming lymphocytes found in the transfused subjects studied here. No conclusion could be drawn without further experiments. It would be interesting to test IgG from transfused subjects for inhibition of EA rosette formation by autologous lymphocytes obtained and frozen prior to transfusion.

IgG preparations from transfused patients blocked EAC and NSE less readily than EA rosette formation. This suggests that the antigen(s) against which transfusion-associated antibodies are produced are in some way linked to the Fcγ-receptor rather than the other receptors on the cell surface. At the same time, removal of Fcγ-receptor blocking activity by absorption with non-Fcγ-receptor bearing thymocytes and leukaemic cells underlines the distinction between the receptor and the putative antigen(s) (Janossy, 1981; Soulillou and Peyrat, 1979).

In contrast, the pregnant women tested had EAC as well as EA rosette inhibitory activity against paternal peripheral blood lymphocytes. If this were confirmed in larger numbers of patients it might indicate a difference between Fcγ-receptor blocking antibodies associated with transfusion and pregnancy.

IgG preparations from transfused subjects had Fcγ-receptor blocking

activity against both T and B cell enriched lymphocyte populations, although not all preparations had activity against both types of cells from the same donor. The discrepancy could be explained by the suggestion that T and B cell Fcγ-receptors are antigenically different (Cunningham-Rundles et al, 1980) but it is interesting that $F(ab')_2$ fragments from the Fcγ-receptor antiserum gave almost identical levels of inhibition of EA rosette formation by both cell preparations (Sandilands et al, 1985).

No IgG preparations affected the Killer or Natural Killer cell functional assays. The assay for Natural Killer cell function is independent of the Fcγ-receptor but antibody-dependent cell-mediated cytotoxicity is mediated through the Fcγ- receptor (Dickler, 1974; Kay et al, 1979).

Like the IgG preparations studied here, the anti-Fcγ-receptor antiserum preparation did not inhibit antibody-dependent cell-mediated cytotoxicity, a finding attributed to the difference between the Fcγ-receptors predominant on Killer cells and the membrane-labile receptors against which the antiserum was raised (Sandilands et al, 1985).

Reduced Natural Killer cell function has been described in transfused non-uraemic subjects (Gascon et al, 1984). Bender et al (1984) reported that sera from uraemic subjects depressed the function of Natural Killer cells from normal subjects, but use of only a handful of patients prevented them from drawing conclusions about the role of transfusion.

There have been consistent reports of depression of cell-mediated

immunity with clinical consequences ranging from the prolongation of allograft survival, described first for skin grafts in 1957 (Dammin, Couch and Murray) to the occurrence of tuberculosis in populations with renal failure at up to 12 times the rates in control populations (Andrews et al, 1980). Although other factors, such as debility and poor nutrition, may contribute to these findings, transfusion may also be important. The association between transfusion and skin testing with Dinitrochlorobenzene has already been discussed (Chapter 5:7).

The T cell mitogen response to phytohaemagglutinin was selected as an index of T cell function for the experiments reported here (Mills, 1966). This has been widely used in previous studies in uraemic patients with emphasis placed on the phytohaemagglutinin response of lymphocytes from uraemic patients. Several groups have now found this to be normal except in patients who had had around 20 units of blood. Failure to consider transfusion histories may have contributed to previous conflicting reports. (Boulton-Jones et al, 1973; Fehrman and Ringden, 1981; Jeannet et al, 1982; Klatzmann et al, 1983a).

There have been a number of suggestions that serum from uraemic subjects can reduce T lymphocyte responses. Newberry and Sanford (1971) showed that serum from patients with renal failure but not from normal subjects was associated with depression of the phytohaemagglutinin response. Using a model of adoptive transfer of tuberculin sensitivity in acutely uraemic guinea pigs, Johnston and Slavin (1976) suggested that lymphocyte unresponsiveness was a consequence of uraemia. Hanicki et al (1976) and Harwick, Kalmanson and Guze (1978) claimed that depression of the mitogen response was a direct chemical effect of renal failure. Sengar et al (1975) found in haemodialysis patients that the

plasma fraction containing IgG could inhibit mixed lymphocyte reactivity, but did not associate this finding with transfusion.

Studies of anti-idiotypic antibody production following blood transfusion in renal dialysis patients have concentrated on the capacity of these antibodies to depress mixed lymphocyte reactivity rather than the mitogen response (for example, Singal et al, 1983; Burlingham et al, 1985b).

The results reported here show that following transfusion, some dialysis patients possess IgG with the capacity to inhibit the mitogen response in vitro. This appears to be associated with Fcγ-receptor blocking activity but it is possible that both effects are unrelated consequences of transfusion. Immune complexes have been implicated in the release of soluble suppressor factors by interaction with T cell Fcγ-receptors (Greene et al, 1981; Moretta et al, 1979); although not described, it is possible that Fcγ-receptor blocking IgG could initiate the same process.

Contamination of the IgG preparations with aggregated immunoglobulin or immune complexes could have given artefactual inhibition of the mitogen response. However, IgG preparations were ultracentrifuged prior to use, and there was no evidence of contamination in preparations tested for purity with gel electrophoresis.

Untransfused uraemic subjects were not studied in this set of experiments so inhibition of the mitogen response due to renal disease alone cannot be excluded. However, a direct effect of uraemia is unlikely with the extensive dialysis entailed by preparation of IgG from serum before testing. The experiments provide the first evidence

for a possible mechanism of action of IgG preparations with Fcγ-receptor blocking activity in the suppression of the immune response.

Because polymorph phagocytosis may be mediated through the Fcγ-receptor, it was decided to assess the effect of Fcγ-receptor blocking antibody preparations on phagocytosis both dependent on and independent of the receptor (Ross, 1982). Polymorph phagocytosis has been reported as normal or depressed in renal failure (reviewed by Goldblum and Reed, 1980). Not only are the reports contradictory but transfusion history has not generally been considered a possible influence on the results.

A recent exception to this was the study by Waterlot et al (1985). They claimed that raised serum ferritin concentrations consequent on transfusion were associated with depressed phagocytosis by polymorphs from dialysis patients. They also linked this finding to the incidence of clinical infection.

As Keane and Raij (1983) commented, "while there is a general clinical impression that uraemic patients have an inherent increased susceptibility to infection .... this has been difficult to document from the literature and in our own experience". Keane and Raij quoted a figure of 20% for the proportion of deaths in dialysis patients attributed to infection.

No attempt was made in the study reported here to draw an association between infective episodes and the effects on normal polymorphs of IgG preparations from transfused subjects. Serum ferritin was not routinely measured.

Although IgG preparations from some transfused subjects blocked

polymorph EA rosette formation, no association was found with inhibition of rosette formation by peripheral blood lymphocytes from the same normal donor. The antiserum raised by Sandilands et al (1985) against lymphocyte-derived Fcδ-receptors did not block polymorph Fcγ-receptors. It may be that Fcδ-receptors on polymorphs and peripheral blood lymphocytes are antigenically distinct. No panel experiments were performed using polymorphs.

Although the difference between untransfused and transfused subjects did not reach statistical significance for Fcγ-receptor mediated phagocytosis (uptake of IgG-coated latex beads) the number of untransfused subjects was small. In contrast, with only 12 subjects studied there was a striking difference in inhibition by IgG preparations from untransfused and transfused subjects of uptake and ingestion of Staphylococcus aureus.

The clearance of complement-coated bacteria is mediated not through the Fcδ-receptor but through the CR_1 receptor (Complement Receptor Type I; Ross, 1982). Although IgG preparations from transfused subjects failed to show substantial inhibition of EAC rosette formation by peripheral blood lymphocytes from a normal donor, they may block the CR_1 receptor on polymorphs. IgG preparations from untransfused subjects with renal disease were not used in this series of experiments but the method of preparation of IgG from serum should have eliminated a direct biochemical effect of uraemia in the transfused group.

Staphylococci have been regarded as the major bacterial pathogen in dialysis patients (Keane and Raij, 1983). If IgG from transfused dialysis patients were shown to inhibit in vitro phagocytosis not only of Staphylococcus aureus but also of strains of Staphylococcus epidermidis

antibody-related depression of phagocytosis could be relevant in vivo to infections related to Tenckhoff catheters and vascular access.

In view of the evidence linking Fcγ-receptors on antigen presenting cells to augmentation of T cell activation (Chang, 1985), and the central role of antigen presenting cells in the initiation of rejection (Austyn et al, 1985), it is perhaps a little surprising if antibodies which block the Fcγ-receptor and are associated with allograft survival have no effect on monocyte Fcγ-receptors. IgG preparations tested in the experiments reported here did not inhibit monocyte EA rosette formation despite marked inhibition of EA rosette formation by lymphocytes and polymorphs from the same normal donor. The antiserum against lymphocyte Fcγ-receptors raised by Sandilands et al (1985) had no effect on monocytes either.

Inhibition of platelet aggregation by IgG preparations from transfused subjects has not been reported previously, although there has been considerable interest in abnormalities of platelet function in uraemia (reviewed by Eschbach, 1983). Curry, Messner and Johnson (1984) claimed that platelet aggregation was inhibited by a monoclonal antibody reactive with the beta-2-microglobulin chain of the HLA complex. Lee et al (1984) suggested that immune abnormalities in recipients of blood products might be related to contamination with HLA-associated beta-2-microglobulin.

Contradicting earlier work by Solheim et al(1976), Morito et al (1978) suggested that antibodies against beta-2-microglobulin could inhibit EA rosette formation. The IgG preparations tested for inhibition of platelet aggregation had no evidence of cytotoxic anti-HLA activity. It is possible that the Fcγ-receptor blocking activity in and the inhibition

of platelet aggregation by IgG preparations from transfused subjects is associated with antibodies against beta-2-microglobulin.

The experiments described in this chapter have demonstrated that IgG preparations from transfused subjects can affect a range of cell types and functions in vitro. These effects cannot all be attributed to Fc -receptor blocking activity, and their relevance to the immune response in vivo remains to be established.

5. Conclusions

i) IgG preparations from transfused subjects were shown to block Fcγ-receptors on T and B lymphocytes and polymorphs but not on monocytes.

ii) Fcγ-receptor blocking activity against peripheral blood lymphocytes could be removed by absorption of IgG prepatations with a variety of cells, with and without the receptor, suggesting that the antibody activity was not directed solely against the receptor itself. IgG from transfused subjects blocked EAC and NSE less readily than EA rosette formation.

iii) IgG preparations from transfused subjects affected several functional assays both related (inhibition of the mitogen response and platelet aggregation) and unrelated (inhibition of polymorph ingestion of Staphylococcus aureus) to the Fcγ-receptor. No effect was found on Killer or Natural Killer cell function.

CHAPTER 13

Fcγ-RECEPTOR BLOCKING ANTIBODIES IN RECIPIENTS OF CLOTTING FACTOR CONCENTRATES

1. Introduction

i) Haemophilia in history

Practical measures to deal with inherited coagulation disorders did not wait for understanding of the coagulation process. In the Egypt of the Pharaohs a woman was forbidden to bear further children if her first son bled to death from a minor wound. The Talmud barred circumcision in a family if two successive sons had suffered fatal haemorrhages (Rosner, 1969).

Constraints on childbearing did not extend to the family of Queen Victoria, through whom haemophilia extended to most of the royal houses of Europe. Lack of appreciation of its hereditary pattern was, to the geneticist JBS Haldane, "A symptom of the divorce between royalty and reality" (quoted by Massie, 1968). The Spanish royal family put its haemophiliac sons in padded suits and padded the trees in the park where they played. The last Tsar and Tsarina of Russia turned to God and faith healing to protect their son. The undue influence bestowed on the faith healer Rasputin has been implicated in the failure of the Russian autocracy to retreat into constitutional monarchy (Massie, 1968). The high mortality rate in affected royalty, not entirely due to haemophilia, precluded reassessment of the disorder in the light of understanding of coagulation systems.

ii) Current management of haemophilia

The concept of "classical" haemophilia antedated unravelling of coagulation factors. Although the term is still used to denote deficiency of coagulation Factor VIII, also described as "haemophilia A", it may in the past have encompassed deficiency of Factor IX, or "haemophilia B". The clinical features are indistinguishable (Biggs et al, 1952).

The least uncommon of the inherited disorders of coagulation, the incidence of haemophilia A has been estimated at 0.5 per million. The incidence of haemophilia B is around one-fifth of this. Both types of haemophilia are transmitted as sex-linked recessive gene abnormalities, leading to male patient and female carrier populations (Bloom, 1981). The severity of illness is reflected by the percentage of the normal level of clotting factor in the patient's plasma.

Treatment is by replacement of the deficient factor when bleeding episodes occur. This can be achieved using a variety of preparations (Rizza, 1981):

a) Plasma - now largely superseded by

b) Cryoprecipitate

By fractionating plasma in the cold, a concentrate is obtained which contains approximately 3% of the original plasma protein, 20-85% of the original Factor VIII and large amounts of fibrinogen. There is insufficient Factor IX to make cryoprecipitate useful for the treatment of Factor IX deficiency. The reconstitution before use of

cryoprecipitate is laborious and it must be stored at -20°C or below. These drawbacks make cryoprecipitate less convenient for home use than

c) Freeze-dried human anti-haemophilic factor concentrate

Several varieties are available commercially (hence lyophilized preparations are sometimes described as "commercial Factor VIII"). They may be imported from, for example, the United States. Fractionation is achieved by one or more of cryoprecipitation or precipitation with ethanol, aminoacid or polyethylene glycol. Most of the materials used in the United Kingdom are of intermediate purity, containing up to 0.5 iu Factor VIII/mg protein and 40-60% fibrinogen.

d) Concentrates containing Factor IX

Most of the concentrates containing Factor IX are made from the supernatant remaining after cryoprecipitation of Factor VIII from plasma. Almost all the Factor IX concentrate used in the United Kingdom is supplied by National Health Service Laboratories, rather than commercial firms (Biggs, 1977).

iii) Immune function in haemophilia

Therapeutic clotting factors are derived from blood donations; to achieve a high concentration of the factor multiple donors are required. There has been considerable recent interest in the long-term immunological consequences of exposure to material from large numbers of donors.

The problem in reviewing recent work on immune function in haemophilia is re-interpretation of abnormalities in the light of subsequent evidence on the prevalence of retrovirus infection. Doubt has been cast on data derived in the United States since 1980, the period during which the prevalence of antibody indicating exposure to the apparent causative virus of the Acquired Immunodeficiency Syndrome (AIDS) has escalated to include some two-thirds of the haemophiliac population (Lederman et al, 1985).

Before the recognition of AIDS, evidence to link haemophilia to susceptibility to infection was slim (Froebel et al, 1983). At an anecdotal level, Beddall, Hill and George (1983) reported an outbreak of tuberculosis in a British paediatric ward in 1981. The incidence in children with haemophilia was almost as high as in children immunosuppressed by neoplasia and chemotherapy, and higher than in other children in the ward. Although interesting, this report could do no more than hint at some predisposing immune abnormality.

In 1983 came reports of decreased T helper/T suppressor lymphocyte ratios in haemophiliacs, a finding particularly prominent in users of commercial lyophilized Factor VIII rather than cryoprecipitate or Factor IX (Goldsmith et al, 1983; Kessler et al, 1983; Lederman et al, 1983; Menitove et al, 1983). Lymphocytes from these patients also showed diminished mitogen responses. Recall antigen testing was particularly poor in patients using commercial concentrates (Tsoukas et al, 1983).

Lee et al (1983) suggested that abnormalities in lymphocyte subsets were a consequence of exposure to HLA proteins in plasma products, and alluded to the blood transfusion effect on renal allograft survival. Measurement of the amount of beta-2-microglobulin in different preparations

was assumed to provide an estimate of HLA protein contamination. This was greatest in commercial Factor VIII and least in National Health Service Factor IX preparations.

Froebel et al (1983) showed that both American and Scottish Factor VIII preparations inhibited the mitogen response directly if added to normal lymphocytes. Lederman et al (1984) confirmed that American Factor VIII preparations could inhibit the PHA response and alter lymphocyte subsets in culture.

Although a firm association is now apparent between the use of American concentrates and seropositivity for human T-cell leukaemia virus Type III (HTLV-III) (Melbye et al, 1984), not all immune abnormalities in haemophiliacs can be explained by the virus. At the time when the experiments described below were performed, the association between the virus and AIDS was poorly defined and no antibody screening test was available. However, the relative self-sufficiency of the Scottish National Blood Transfusion Service supply of clotting factors, and consequent relative rarity of use of American products was taken to indicate that virus infection was rare (Carr et al, 1984).

This assumption has been borne out by subsequent studies (Melbye et al, 1984; Ludlam et al, 1985), underlining the importance of the findings of Froebel et al (1983) in Glasgow and Carr et al (1984) in Edinburgh that T-cell subset abnormalities were present not only in patients likely to have been exposed to American pathogens. The suggestion from Ludlam et al (1985) that prior depression of the T helper subset might predispose to seroconversion to HTLV-III was appropriately tentative.

The humoral response to repeated infusion of clotting factor con-

centrates has been little explored, although increased immunoglobulin production has been reported (Menitove et al, 1983; Moffat et al, 1985). Proud et al (1979) found that plasma from haemophiliacs who had received both whole blood and Factor VIII preparations could inhibit the lymphocyte response to purified protein derivative of Mycobacterium tuberculosis. Inhibitory activity was attributed by these authors primarily to alpha-2-macroglobulin, but was also present in the plasma fraction containing IgG.

iv) Aims of the study

Fcγ-receptor blocking antibodies have not been described in recipients of clotting factor concentrates. However, the evidence presented in earlier chapters that blood transfusion may be associated with the development of such antibodies, and the possibility that the antibody production is a histoincompatibility response, lead to consideration of the production of Fcγ-receptor blocking antibodies in response to alloantigens contaminating clotting factor preparations. The experiments described below were designed to answer the following questions:

a) Are Fcγ-receptor blocking antibodies present in patients who have received Factor VIII and Factor IX?
b) If so, do they exhibit the same properties in vitro as those previously demonstrated in recipients of blood?
c) Do any antibodies found cross-react with antibodies from multiply transfused uraemic subjects?
d) If such antibodies are produced, can they be used to remove and characterise the inducing antigen(s) from preparations of Factor VIII?

2. Materials and Methods

i) Subjects studied

Serum samples were taken from 39 male patients, 30 with Factor VIII and 9 with Factor IX deficiency (full details are given in Chapter 7:4). Further serum samples were obtained from 1 normal and 3 uraemic transfused subjects, all of whom were male.

ii) Methods used

IgG preparations were obtained from the serum samples, using DEAE chromatography as previously described (8:3:iv).

All IgG preparations were tested, using an EA rosette inhibition assay at both 4°C and 37°C (8:4:i), for their capacity to block Fcγ-receptors on peripheral blood lymphocytes from normal donors. IgG preparations from 27 subjects were also tested for their capacity to inhibit phytohaemagglutinin-induced blastogenesis of normal allogeneic lymphocytes (8:4:iv).

Using IgG from patients with strong Fcγ-receptor blocking activity attempts were made to extract and characterise the relevant antigen(s) from a lyophilized preparation of Factor VIII by affinity chromatography and SDS-PAGE gel electrophoresis. Full details of these methods are given in Chapter 8:9;8:10). The "antigen" fractions obtained by affinity chromatography from patients and controls were used for further experiments.

Three 120µl aliquots of each IgG preparation were taken. To the

first of each trio was added an equal volume of phosphate buffered saline. To the second was added "antigen" from the normal subject. To the third was added "antigen" from one of the haemophiliac subjects. IgG preparations from the haemophiliac subjects were also tested using autologous "antigen" in a separate experiment using similar controls. All samples were incubated at 37°C for 1 hour, then tested for EA rosette inhibition at 37°C using peripheral blood lymphocytes from an unrelated normal subject.

IgG preparations from the 4 haemophiliacs were tested, in parallel with autologous "antigen" and the "antigen":IgG combinations, for inhibition of phytohaemagglutinin-induced blastogenesis (8:4:iv).

"Antigen" fractions were run over SDS-PAGE gels to try to identify the molecular weight of the "antigen(s)".

3. Results

Figure 29 shows the extent to which IgG preparations from the 39 patients with haemophilia inhibited EA rosette formation by peripheral blood lymphocytes from a normal donor. Using the value of 35% as the upper limit of inhibition by IgG from normal subjects, as defined in Chapter 9, 18 patients had Fcγ-receptor blocking activity at 4°C and 24 at 37°C. As was noted in transfused dialysis patients (Chapter 10) no patient had antibody activity at 4°C only.

Figure 30 shows the same results, subdivided by transfusion history. Twenty patients had received clotting factor preparations only, and 19 had had previous blood transfusion. The difference in results between these two groups did not reach statistical significance, although when the assay was performed at 4°C there did appear to be a trend towards

higher levels of rosette inhibition in the "transfused" group. Eleven out of 19 subjects in this group, and only 7 of 20 who had not had blood, had results outwith the normal range. (Mean ± standard deviation percentage inhibition of EA rosette formation: 38 ± 28% "transfused", 23 ± 22% "untransfused").

The main point from the results, however, is that Fcγ-receptor blocking activity is not dependent on blood transfusion in patients already receiving clotting factor preparations. No relation was found between Fcγ-receptor blocking activity at either temperature and the type of haemophilia (Figure 31), severity of disease (Figure 32), or amount of clotting factor used during the previous year (Figure 34). Twenty-four patients with Factor VIII deficiency had used commercial concentrates before 1979. Six out of 24 patients tested had antibody detected against HTLV-III. IgG Fcγ-receptor blocking activity was not associated with either the use of commercial concentrates (Figure 35) or HTLV-III antibody (Figure 36). (All results illustrated are those for the assay performed at 37°C; nor were any associations between Fcγ-receptor blocking activity and the factors mentioned noted at 4°C).

Figure 37 shows the results for inhibition of phytohaemagglutinin-induced T cell activation by IgG preparations from 27 patients with haemophilia. The results from 11 normal subjects are those already discussed in Chapter 12. Eighteen out of the 27 IgG preparations from patients with haemophilia gave levels of inhibition outwith the range for normal subjects (for the normal group, mean + 2 standard deviations = 38% maximum value obtained 39%) ($P < 0.001$, X^2 test with Yates correction). Six patients with haemophilia had levels of inhibition greater than 80%. The relation between inhibition of the phytohaemagglutinin response and

use of blood and clotting factors is illustrated in Figures 38 and 39. The extent of inhibition appeared independent of Fcγ-receptor antibody or HTLV-III antibody status, previous blood transfusion, type and severity of disease, use of commercial concentrates and the amount of clotting factor consumed in the previous year.

The demonstration of Fcγ-receptor blocking activity and inhibition of the phytohaemagglutinin response by IgG preparations from patients who had received only clotting factor preparations rather than blood led to attempts to remove the antigen(s) assumed to be inducing these antibodies from Hemofil, a Factor VIII preparation. First, Hemofil reconstituted in distilled water was ultracentrifuged over a discontinuous sucrose gradient, using the method described in Chapter 8 (8:3:iii). Six fractions of graded molecular weight were obtained and dialysed into phosphate buffered saline. The protein concentration of each fraction was measured by spectrophotometry. From Figure 40 it can be seen that the peak protein concentration was found in Hemofil fraction 3. None of the 6 fractions inhibited EA rosette formation by peripheral blood lymphocytes from a normal subject.

Affinity chromatography performed using Hemofil and IgG preparations from 2 normal male subjects and 4 haemophiliac patients yielded preparations empirically described here as "antigen".

Figure 41 shows the protein concentration of substrate eluted from Sepharose beads after incubation with Hemofil and with IgG from one haemophiliac and one normal subject. A protein peak is seen within the first few mls of buffer passed through the chromatography column; this was taken to contain the putative "haemophiliac antigen" for the purposes of subsequent experiments. "Antigen" preparations from neither

the normal nor the haemophiliac subjects inhibited EA rosette formation by peripheral blood lymphocytes from an unrelated normal subject.

The results of experiments to find the effect of incubation with "antigen" on IgG preparations from normal, haemophiliac and transfused dialysis patients are shown in Figures 42 to 46.

Figure 42 shows removal of Fcγ-receptor blocking activity from IgG from a haemophiliac subject by autologous "antigen" but not by phosphate buffered saline nor by "antigen" derived using a normal subject. The same pattern was found with all 4 haemophiliacs tested (Figure 43). Phosphate buffered saline and "normal antigen" had no effect in this or subsequent experiments (results not illustrated). When IgG preparations from the 4 haemophiliacs were incubated with "antigen" derived from one of them, Fcγ-receptor blocking activity was removed from all 4 (Figure 44). Moreover, Fcγ-receptor blocking activity was removed by the same "antigen" from IgG from 3 dialysis patients (Figure 45) This "antigen" had been derived from Hemofil using IgG from a patient with Factor VIII deficiency who had never received blood or commercial concentrates. All the dialysis patients had had blood but none had had clotting factor preparations. None of the 4 haemophiliac "antigen" and "antibody" preparations inhibited the phytohaemagglutinin response of normal lymphocytes (Figure 46). However, when autologous "antigen" and "antibody" were incubated together beforehand,each combination was inhibitory.

SDS-PAGE gel electrophoresis of "antigen" derived from normal and haemophiliac subjects yielded no consistent pattern of protein banding. However, several protein bands were identified from "antigen" from haemophiliac but not normal subjects. Using dalton markers, these were estimated to have molecular weights of 25,000, 66,000 and 74,000.

4. Discussion

The demonstration of Fcγ-receptor blocking antibodies in patients receiving blood products because of haemophilia is of interest in conjunction with the work linking these antibodies with renal allograft survival (MacLeod et al, 1982a and b), and with the literature on immunosuppression in haemophilia (mentioned in the Introduction above; for example, Lederman et al, 1983 and 1984; Froebel et al, 1983; Ludlam et al, 1985).

IgG preparations from the patients studied here not only had Fcγ-receptor blocking activity but also were shown to inhibit the mitogen response to phytohaemagglutinin *in vitro*. Patients with haemophilia have raised plasma immunoglobulin levels (Menitove et al, 1983; Moffat et al, 1985), but IgG preparations used in these experiments were all adjusted for use to a concentration of Img/ml in phosphate buffered saline.

No similar work has previously been published. The response to phytohaemagglutinin of lymphocytes from patients with haemophilia has been reported to be normal (Froebel et al, 1983) or reduced (Moffat et al, 1985), particularly in those treated with lyophilized Factor VIII preparations (Lederman et al, 1983).

Shenton et al (1979) found that the IgG-containing plasma fraction from patients with haemophilia inhibited the response of autologous and homologous peripheral blood lymphocytes to purified protein derivative of *Mycobacterium tuberculosis*. All Shenton's patients had however received at least 10 units of blood in addition to clotting factor preparations.

Like the studies of lymphocyte subpopulations in haemophiliacs reported by Froebel et al (1983), Lederman et al (1983) and Moffat et al (1985), the results described here show no relationship between the amount of clotting factor consumed and the immune abnormalities detected. Nor were the effects of the IgG preparations tested associated with other clinical parameters such as severity of disease, nature of the deficient clotting factor, use of commercial Factor VIII or HTLV-III antibody status.

Interestingly, there was no difference in results from IgG preparations between those patients who had received blood as well as blood products and those who had received blood products alone. Blood products may be effective in inducing the production of antibodies which block Fcγ-receptors and inhibit the mitogen response, with the relevant antigen(s) being present in preparations of Factor VIII and Factor IX.

The hypothesis that the antigen(s) were present in clotting factor preparations led to the experiments aimed at extraction and characterisation of the antigen(s). Fcγ-receptor blocking activity was removed by incubation with Hemofil-derived haemophiliac "antigen" from IgG preparations not only from the patient with whose IgG the antigen had been prepared, but also from IgG from other patients with haemophilia who had received blood products but never blood, and from IgG from renal patients who had received blood but never blood products. This suggests that Fcγ-receptor blocking antibodies are produced against antigens common to blood and clotting factor concentrates.

HLA products contaminate clotting factor concentrates. Lee et al (1984) suggested that long-term massive exposure to HLA antigens in

Factor VIII preparations led to immune abnormalities, and linked the low concentration of HLA-associated beta-2-microglobulin in Factor IX concentrates to the low incidence of T cell subset abnormalities in patients with haemophilia B (Kessler et al, 1983; Moffat et al, 1985). The production of antibodies to beta-2-microglobulin from Factor VIII preparations could be associated with Fcγ-receptor blocking activity (Morito et al, 1978) but the presence of Fcγ-receptor blocking antibodies in patients with Factor IX deficiency makes less likely this explanation for the results presented here.

Lee et al (1984) confirmed, through elution chromatography of Factor VIII concentrate, that beta-2-microglobulin was present in association with proteins of a mass compatible with that of HLA Class I products (55,000 daltons), rather than in free form (mass (12,000 daltons). Gel electrophoresis of "antigen" preparations showed no evidence of protein bands corresponding to the molecular weights of free or HLA-associated beta-2-microglobulin. The absence of a consistent banding pattern in the haemophiliac "antigen" preparations may suggest that the method used was not sufficiently sensitive to demonstrate the antigen(s) responsible for antibody production, particularly as "antigen" preparations removed Fcγ-receptor blocking activity from homologous as well as autologous IgG preparations.

5. Conclusions

i) IgG preparations from patients with haemophilia were found to possess Fcγ-receptor blocking activity and to inhibit the response of normal lymphocytes to phytohaemagglutinin.

ii) These properties were independent of a history of blood transfusion, severity and type of disease, amount and type of clotting factor

concentrates used, and antibody status against Human T-cell Leukaemia Virus Type III.

iii) Fcγ-receptor blocking activity in IgG preparations from recipients of blood and of blood products appeared to be directed against common antigen(s) represented in Factor VIII concentrate.

iv) Although full characterisation of the antigen(s) was not achieved, Factor VIII concentrate provides a convenient substrate for further attempts to identify the antigen(s) responsible for Fcγ-receptor antibody production in recipients of blood and of blood products.

CHAPTER 14

Fcγ-RECEPTOR BLOCKING ANTIBODIES AND THE ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS)

1. Introduction

i) Clinical features of AIDS

In 1981 came the first reports of a fatal acquired immunodeficiency syndrome in homosexual American man (Gottlieb et al, 1981; Masur et al, 1981). Since then, there has been a vast amount written, in both medical and lay press, about this syndrome, empirically defined by the American Centres for Disease Control for surveillance purposes as the presence of a reliably diagnosed disease at least moderately predictive of an underlying cellular immunodeficiency. Such diseases include Kaposi's sarcoma in a patient under 60 years old, Pneumocystis carinii pneumonia, or other opportunistic infections. There must be absence of any known causes of the underlying immunodeficiency or host defence defect such as immunosuppressive therapy or lymphoproliferative malignancy (Fauci, 1985). The syndrome has been characterised by a number of cellular and humoral immune abnormalities, recently reviewed by Lane (1985) and discussed later in this chapter in relation to the author's experimental findings.

ii) Evidence for a viral aetiology

In 1983, when the experiments described later in this chapter were designed, there were doubts about the role of virus infection in the aetiology of AIDS (Woodruff and van Rood, 1983). These have been dispelled by recent research. Early epidemiological evidence showed

that the syndrome in the United States was concentrated in the homosexual community, but subsequently identified risk groups include intravenous drug abusers, immigrants from Haiti, recipients of blood or blood products, and heterosexual partners of any of these groups. Patterns of spread and case clusters suggest a viral aetiology (Fauci, 1985).

Identification and characterisation of an AIDS-related virus has been rapid. In 1983, Barre-Sinoussi et al in Paris isolated a T-lymphotropic retrovirus from a patient at risk of AIDS. The following year Gallo et al (1984) in the United States linked AIDS to a retrovirus akin to the previously described human T-cell leukaemia virus (Schupbach et al, 1984). Although the nomenclature used by the two groups differs, with the French describing lymphadenopathy-associated virus (LAV) and the Americans human T-cell leukaemia virus Type III (HTLV-III), their evidence has tended to converge. Differences beyond nomenclature have delayed American acceptance of evidence from Feorino et al (1984) equating the viruses.

Seroepidemiological evidence, detecting antibodies to LAV/HTLV-III, points strongly to the link with AIDS (Sarngadharan et al, 1984; Fauci, 1985). Moreover, infectious retrovirus has been isolated from Factor VIII concentrates (Levy, Mitra and Mozen, 1984), and the transmission of the virus in transfused blood has been charted in donor-recipient pairs (Feorino et al, 1984; Markham, 1985). The relation between the presence of IgG antibody to the virus in a community and the subsequent incidence of the clinical syndrome is unknown (Fauci, 1985).

Backing the epidemiological evidence is extensive research in the molecular biology of the implicated retrovirus. It appears to attach selectively to the T4 antigen (Dalgleish et al, 1984), giving a selective

deficiency in the ability of the T4 lymphocyte to respond to soluble antigen (Lane, 1985) and ultimately causing cell death (Klatzmann et al, 1984 b). By *in vitro* passage Montagnier et al (1984) have adapted the virus also to infect B cell lines transformed by Epstein-Barr virus.

iii) The role of pre-existing immunosuppression

The firm evidence which has accrued to link LAV/HTLV-III with AIDS does not preclude a role for pre-existing host immune status in determining the clinical outcome of exposure to the virus. On the basis that virus infectivity may be enhanced in proliferating T4 cells, Kalish and Schlossman (1985) have suggested that synergy with other infective agents may explain observations such as the relatively low rate of infection among transfusion recipients and health care personnel exposed to the virus (Hirsch et al, 1985; Jones et al, 1985).

It has been suggested that chronic immune stimulation by chronic infection may interfere with the host's capacity to eliminate infectious agents (Virgin and Unanue, 1985). This has been advanced as an explanation for the association between malaria and the development of Burkitt's lymphoma in response to Epstein-Barr virus infection (Dalldorf, 1982). Malaria has been shown to cause a transient inversion of the T helper/suppressor ratio and non-specific B cell proliferation (Whittle et al, 1984).

Biggar et al (1985) studied a community in rural Zaire, thought to be an endemic focus of the AIDS-related virus (Fauci, 1985). They found that the prevalence in healthy subjects of antibody against HTLV-1, -II and -III correlated strongly with the level of immune complexes and antibodies against *Plasmodium falciparum*, and considered the possibility

that malaria could affect the host response to retrovirus infection.

Levy and Ziegler (1983) proposed that AIDS was an opportunistic infection in subjects already immunocompromised by repeated infection. Infection with sexually transmitted pathogens such as cytomegalovirus and hepatitis B is increasingly prevalent in the semen of homosexual men in American cities (Sonnabend, Witkin and Purtilo, 1985).

It is interesting that repeated immune stimulation from viral infection has not been widely regarded as an explanation for the transfusion affect on renal transplantation (Chapter 5). Similarly, it is interesting how little discussion of the immunosuppressive effects of transfusion has contributed to the literature on AIDS, even in studies specifically focusing on acquired immunodeficiency following transfusion (Curran et al, 1984).

In response to Curran's 1984 paper, Shearer and Levy (1984) suggested that DR-mismatched blood transfusion might be particularly prone to dampen the immune response and predispose to infection with the then-speculative AIDS agent. Indeed, Shearer has consistently advocated exposure to alloantigens of the major histocompatibility complex as a cause or contributing factor to the development of AIDS (Shearer, 1983; Shearer and Levy, 1985).

Exposure to alloantigens through repeated homosexual contact has been postulated to cause immune suppression and likened to acquired immune tolerance of an allogeneic renal allograft (Mavligit et al, 1984). Seminal plasma may have immunosuppressive properties (Lord, Sensabaugh and Stites, 1977; Marcus et al, 1978; James and Hargreave, 1984). Murgita et al (1978) suggested that embryonic antigens, as expressed

on sperm, could suppress T cell proliferation in vitro. Hurtenbach and Shearer (1982) showed that germ-cell inoculation in mice led to impaired cellular immunity.

Unspecified differences between the mucosal linings of the genital and gastro-intestinal tracts have been cited to try to explain the consequences of rectal rather than vaginal insemination. Rectally inseminated rabbits have been shown to develop anti-sperm antibodies, immune complexes and serum inhibitors of lymphocyte proliferation (Richards, Bedford and Witkin, 1984; Witkin et al, 1985).

In homosexual men the same workers have demonstrated antibodies against sperm antigens (Witkin and Sonnabend, 1983; Witkin et al, 1983). Following the demonstration by Mathur et al (1981) of cross-reactivity between antigens on sperm and T lymphocytes, Sonnabend, Witkin and Purtilo (1983) proposed that such anti-sperm antibodies would cross-react with and inactivate T lymphocytes. This was amplified by Hsia et al (1984) who saw virus- and/or sperm-specific anti-idiotypic antibodies as a possible cause for AIDS. Rubinstein, Small and Bernstein (1985) have now demonstrated autoantibodies to T cells in patients with AIDS.

iv) Aims of the study

In 1983, at the start of this study, the evidence was thus in favour of a specific aetiological agent for AIDS, but with no firm indication as to which. The role of exposure to multiple alloantigens was uncertain. However, work on the blood transfusion effect on renal allograft survival and the studies, mainly in animals, of immune suppression by spermatozoa, suggested a field for study.

The presence of Fcγ-receptors on spermatozoa in both man and mice had been shown by Sethi and Brandis (1980). The experiments presented below were designed to test the hypothesis that repeated exposure to alloantigenic spermatozoa by homosexual contact would lead to the production of antibodies which would cross-react with Fcγ-receptors on spermatozoa and lymphocytes, and that these antibodies could be shown in vitro to be potentially immunosuppressive.

2. Materials and Methods

i) Subjects studied

Serum samples were obtained from:

a) 7 patients with AIDS (Chapter 7:6)

b) 33 patients attending a Genitourinary Clinic (Chapter 7:5)

c) 39 patients with haemophilia (Chapter 7:4)

d) 54 renal dialysis patients who had previously received at least 5 units of blood (Chapter 7:3)

e) 4 patients (2 male) with previous blood transfusion but no history of renal disease (Chapter 7:2)

f) 17 heterosexual normal subjects (9 male) with no history of transfusion or pregnancy (Chapter 7:1)

g) 10 normal multiparous women and 5 normal pregnant women (Chapter 7:1)

ii) Methods used

Serum samples from the patients attending the Genitourinary Clinic were tested for inhibition of EA rosette formation by peripheral blood lymphocytes from a normal subject (Method 8:4:i).

IgG preparations were obtained from serum samples (method 8:3:iv). Spermatozoa were obtained from anonymous healthy male volunteers, and IgG preparations from all study groups were tested for anti-sperm antibodies using a tray agglutination test (method 8:8).

IgG preparations from homosexual men with and without clinical evidence of AIDS were tested for inhibition of EA rosette formation (method 8:4:i) and for inhibition of phytohaemagglutinin-induced blastogenesis (method 8:4:iv) using peripheral blood lymphocytes from a normal subject.

IgG preparations from 3 subjects with haemophilia were absorbed (method 8:3:v) with human thymocytes (method 8:2:ii), platelets (method 8:1:iv and erythrocytes (method 8:1:v). Pre- and post-absorption IgG preparations were tested for inhibition of EA rosette formation of normal peripheral blood lymphocytes (method 8:4:i) and for anti-sperm antibodies (method 8:8).

3. Results

Figure 47 shows the results for inhibition of EA rosette formation by serum from patients attending the Genitourinary Clinic. No details of obstetric history were available for the female patients. The difference between values for the male heterosexual and homosexual groups was statistically significant ($P < 0.01$, Mann-Whitney U test).

On the basis of these preliminary results it was decided to proceed to separate IgG from serum from the homosexual group. Two serum samples were too small for this, so IgG results for the homosexual group are

available on only 9 patients instead of the original 11.

Figure 48 shows inhibition of EA rosette formation by IgG preparations from 15 male homosexual subjects, 9 of whom had no clinical evidence of AIDS and the other 6 with clinically defined AIDS. Four out of 9 "asymptomatic" men and all 6 with AIDS had IgG Fcγ-receptor blocking activity outwith the normal range (i.e. greater than 35% as defined in Chapter 9). IgG from the heterosexual man with AIDS gave 90% inhibition of EA rosette formation. The difference between results for the two groups depicted was significant ($P<0.01$, Mann-Whitney U test).

Figure 49 illustrates the titres of anti-sperm antibody activity found in IgG preparations. Agglutination of normal human spermatozoa was found with 3 out of 17 of normal IgG preparations (from men or nulliparous women) compared with 25 out of 54 from multiply transfused renal patients, 34 out of 39 from haemophiliacs, 5 out of 9 from asymptomatic homosexuals and by IgG from all of the six homosexual AIDS patients, as well as from the heterosexual AIDS patient (result not illustrated). (X^2 test: all test groups significantly different from the normal group). Two multiparous and 1 pregnant normal women also had detectable anti-sperm acitivity, but in none of the total of 32 IgG preparations from normal subjects did agglutination of spermatozoa occur beyond a titre of 1:8.

In none of 4 subjects transfused for reasons other than renal failure was anti-sperm antibody detected.

The results for all subjects tested are considered in Table 5 in comparison with results previously obtained for Fcγ-receptor blocking

activity (Chapters 9, 10 and 13; and Figure 48). The association between the presence of anti-sperm antibody (detected at any titre) and Fcɣ-receptor blocking antibody (greater than 35% EA rosette inhibition at 37°C on testing with normal peripheral blood lymphocytes) was statistically signficant (X^2 test, $P<0.01$).

Absorption of three IgG preparations with human thymocytes, or platelets was found to remove completely both anti-lymphocyte and anti-sperm activity (Figure 50). Absorption with human erythrocytes had no effect (Figure 50).

IgG preparations from 6 out of 9 "asymptomatic" homosexual men and from all 6 homosexual AIDS patients inhibited the phytohaemagglutinin response to an extent greater than seen in normal subjects (Figure 51 and Chapter 12). IgG from the one heterosexual AIDS patient also markedly inhibited the response (result not illustrated).

4. Discussion

Although a wide range of immune abnormalities has been demonstrated in patients with AIDS (reviewed by Lane 1985), the presence of Fcɣ-receptor blocking antibodies is a new finding. Raised total IgG concentrations (Lane et al, 1983) were unlikely to explain the results as all IgG preparations were tested at a standard concentration. Although the antibodies could be a non-specific consequence of polyclonal B cell activation (Lane et al, 1983), there are several points in favour of the hypothesis that anti-lymphocyte Fcɣ-receptor blocking antibodies are associated with homosexual contact with allogeneic spermatozoa independent of infection with HTLV-III.

No screening test for HTLV-III antibody was available in 1983 when samples were collected from the subjects attending a Genitourinary Clinic. One of the homosexual men tested in this study had been sexually active in North America but the others had no such history. Mortimer et al (1985) assessed the prevalence of HTLV-III antibody in homosexual men attending British Genitourinary Clinics in 1984. In London 34% of 282 patients were antibody-positive, but the figure for 5 centres outwith London was only 5% of 955 patients. It therefore seems unlikely that the results here were attributable to HTLV-III infection. Use of a control group of heterosexual men with genitourinary symptoms reduced the likelihood that other common sexual pathogens were responsible for serum Fcγ-receptor blocking activity.

The association between anti-sperm antibodies and Fcγ-receptor blocking antibodies was true not only for homosexual men but also for patients transfused with blood or blood products. It would be interesting to reassess the strength of the association using sperm and lymphocytes from the same donor. No attempts were made to assess anti-sperm antibody activity across a donor panel.

Absorption of IgG preparations with thymocytes and platelets, but not erythrocytes, removed antibody activity against both peripheral blood lymphocytes and spermatozoa. This provides further evidence for the association between the antibodies. The absence of Fcγ-receptors on thymocytes (Janossy 1981; confirmed by failure of the thymocytes used here to form EA rosettes) suggests that the anti-sperm antibodies are, like the anti-lymphocyte antibodies, not directed against the Fcγ-receptor itself, although Fcγ-receptors are expressed on spermatozoa (Sethi and Brandis 1980).

It has been suggested that Fcγ-receptor blocking antibody activity against B lymphocytes is directed against an allelic alloantigen system other than the defined HLA-A, B and DR systems (MacLeod et al, 1982b). Daar et al (1984b) failed to demonstrate Class II antigens on spermatozoa using a monoclonal antibody which detected a monomorphic determinant common to the known Class II antigen groups. Class I antigens were detected with a monoclonal antibody against the heavy chain of HLA-A, B and C antigens on sperm in the testis but not in the epididymis (Daar et al, 1984a). These monoclonal techniques may have failed to detect relevant undefined HLA systems. Alternatively, the antibodies described here may be directed against non-HLA determinants on spermatozoa.

A connection between AIDS and anti-sperm anti-lymphocyte Fcγ-receptor blocking antibodies is suggested by the occurrence of these antibodies in patients with AIDS, and at risk of AIDS through homosexual contact, blood or blood products (Fauci, 1985). The consequences of exposure to HTLV-III may be exacerbated by pre-existing immunosuppression (Ludlam et al, 1985). There is some theoretical backing for the contention that Fcγ-receptor blocking antibodies may depress the host response to infection or neoplasia.

Organisms expressing Fcγ-receptor-like structures include bacteria (for example, some Staphylococci) and parasites (such as Schistosoma) (Witz and Ran, 1985). Cells infected with cytomegalovirus may express Fcγ-receptors (Keller, Peitchel and Goldman, 1976). There is, to the author's knowledge, no published work on Fcγ-receptor expression on cells infected with HTLV-III. Malignant transformation of tumour cells has been linked to increased Fcγ-receptor expression (Witz and Ran, 1985).

The biological advantage of Fcɣ-receptor expression to infecting organisms and neoplastic cells is not known. Sonnabend et al (1985) suggested that circulating immune complexes could bind to Fcɣ-receptors on cytomegalovirus-infected cells and prevent virus antigen target recognition by cytotoxic lymphocytes. A benefit to malignant cells proposed by Witz and Ran (1985) was that Fcɣ-receptors could bind IgG and mask recognition of other surface antigens. Fcɣ-receptor blocking antibodies associated with transfusion or homosexual contact could depress the host response by a similar mechanism.

IgG preparations from patients with AIDS, homosexual men and transfusion recipients have been shown in this and previous chapters to depress the mitogen response of normal lymphocytes in vitro. Cunningham-Rundles, Michelis and Masur (1983) documented antibodies to T cells and suppression of in vitro lymphocyte activation by serum from patients with AIDS.

Witkin et al (1985) claimed that rectal insemination of rabbits led to the formation of anti-T cell and anti-sperm antibodies. Serum from inseminated rabbits inhibited the phytohaemagglutinin response of lymphocytes from normal rabbits. Absorption of serum with protein A to remove IgG did not abolish inhibition, suggesting that IgG was not responsible. Yet the use of IgG preparations here confirms that IgG can inhibit the mitogen response in vitro.

Interaction with T lymphocyte Fcɣ-receptors may stimulate suppressor function(Moretta et al, 1979) and result in the release of soluble suppressor factors. So-called "Soluble Immune Suppressor Substance T" (Greene et al, 1981) is produced in increased quantities by stimulated lymphocytes from patients with AIDS (Laurence, Gottlieb and Kunkel,1983).

If Fcγ-receptor blocking antibodies are also associated with suppression of T cell responses *in vivo*, they may contribute to the development of AIDS after exposure to HTLV-III.

5. Conclusions

i) Fcγ-receptor blocking IgG class antibodies were found in patients with AIDS and in homosexual men with no clinical evidence of the syndrome.

ii) Anti-sperm antibodies were detected in patients with AIDS, homosexual men, and patients who had received transfusion of blood or blood products.

iii) Evidence for an association between anti-sperm and anti-lymphocyte Fcγ-receptor blocking antibodies was provided by absorption experiments.

iv) IgG preparations from patients with AIDS and homosexual men inhibited the phytohaemagglutinin response of normal lymphocytes *in vitro*.

v) Experimental results were compatible with the hypothesis that homosexual contact may lead to the development of sperm-lymphocyte cross-reacting Fcγ-receptor blocking antibodies, which may predispose to the development of AIDS.

CHAPTER 15

THE RELATION BETWEEN PRETRANSPLANT Fcγ-RECEPTOR BLOCKING ANTIBODIES AND RENAL ALLOGRAFT SURVIVAL

1. Introduction

i) Background

The association found in the previous chapters between alloantigen exposure and Fcγ-receptor blocking IgG class antibodies with possible immunosuppressive actions *in vitro* leads to the consideration of the clinical situation of renal transplantation. The original work by MacLeod and her colleagues linking renal allograft survival to Fcγ-receptor blocking antibodies was published in 1982 (MacLeod et al, 1982 a and b). No other centre has yet confirmed the findings in this retrospective analysis.

ii) Aims of the study

During the period of the other experimental work reported in this volume, a prospective study was also in progress to compare renal allograft survival with pretransplant Fcγ-receptor blocking IgG class antibody in recipient serum.

2. Materials and Methods

i) Subjects studied

Serum samples were obtained during the day prior to transplantation

from 63 renal allograft recipients (36 male, mean age 35, range 16 - 65 years). This group included 7 patients receiving second and 2 receiving third cadaveric allografts. All patients had received at least 5 units of third party whole blood or packed cells.

Tissue match placed the emphasis on DR match, followed by the B and then the A loci. A positive T cell crossmatch on current or historical sera precluded transplantation, but a positive B cell crossmatch did not.

All patients were treated peri- and postoperatively with Prednisolone, initially in a dose of 20 mg daily, tapering to 10 mg daily at one year. All studies in this chapter were also treated with Azathioprine, in a starting dose of 3mg/kg/day adjusted to keep the white cell count above 4×10^9/ml. Rejection episodes, diagnosed on clinical, biochemical and where possible histological criteria, were treated with increased doses of Prednisolone.

For analysis of the results, a patient was classed as a "transplant acceptor" if the allograft were functioning one year following transplantation. Three kidneys were thought to have infarcted for non-immunological reasons; results for these patients were not excluded from the analysis.

ii) Methods used

IgG preparations were obtained by DEAE chromatography (method 8:3:iv) from pretransplant serum samples and used for all experiments unless otherwise stated. The EA rosette inhibition assay (method 8:4:i) was performed using the following Fcγ-receptor bearing cells:

a) Peripheral blood lymphocytes from normal subjects.

IgG preparations from all 63 transplant recipients were tested at both 4°C and 37°C.

b) Peripheral blood lymphocytes from the kidney donor. Blood was taken from the cadaveric donor shortly before donor nephrectomy.

It was decided to use ultracentrifuged pretransplant serum (method 8:3:ii) rather than IgG in these experiments to allow the cells to be used within several hours of harvesting.

Serum from 18 transplant recipients was tested at 4°C and 37°C.

c) B lymphocytes from normal subjects.

IgG preparations from 26 transplant recipients were tested against B enriched lymphocyte preparations (method 8:1:ii). Because of the relatively small proportion of B lymphocytes available in peripheral blood, the assay was performed at only one temperature (4°C).

d) B lymphocytes obtained from the spleen of the cadaveric kidney donor and frozen until use (method 8:1:ii; 8:2:i).

IgG preparations from 31 transplant recipients were tested, again at 4°C only.

3. Results

Where IgG preparations were tested with third party rather than donor cells, the results are, except where otherwise stated, the mean values for inhibition of EA rosette formation of lymphocytes from 3 to 6 normal subjects. All groups of "acceptors" and "rejectors" studied were (fortuitously) comparable in age, sex, primary renal disease and duration of dialysis. Any differences in tissue match or transfusion history are detailed beside the relevant experimental results.

Figure 52 shows the results when IgG preparations from all 63 subjects were tested against normal peripheral blood lymphocytes at both temperatures. There was no evidence for an advantage to graft survival in the presence prior to transplantation of Fcγ-receptor blocking antibody whether detected at $4^{o}C$ or $37^{o}C$.

Indeed, the converse appeared to be true. Table 6 shows the same results divided into "antibody-positive" (greater than 35% inhibition) and "antibody-negative". The presence of Fcγ-receptor blocking antibody at $4^{o}C$ was associated with graft failure ($P<0.05$, X^2 test). At $37^{o}C$ a greater proportion of patients had antibody and the association with graft failure did not reach statistical significance (X^2 test).

Even in those patients who had functioning transplants after one year there was a highly significant correlation between pretransplant antibody detected at $4^{o}C$ and the serum creatinine at one year (Figure 53, $r = 0.64$, $P<0.001$).

Another way of looking at the results derived from a panel is not to take the mean result but to regard any patient with antibody activity against any one of the panel as positive. When the results are expressed in this way, 34 patients (26 with a functioning kidney after one year) were antibody-negative and 29 (14 with a functioning kidney) were antibody-positive. The association between antibody and graft failure remained significant ($P<0.05$, X^2 test).

This finding could not be explained by difference in the prevalence of anti-HLA antibodies in the groups studied. Nor was any difference noted in the number of pretransplant blood transfusions (mean number

of units: "acceptors" = 9 "rejectors" = 11) nor in the closeness of HLA matching between kidney donor and recipient (Table 7).

Figure 54 shows the relation between one-year renal allograft survival and inhibition of EA rosette formation of donor peripheral blood lymphocytes by ultracentrifuged pretransplant recipient serum. Inclusion in this group necessitated the harvesting of fresh peripheral blood lymphocytes from a local cadaveric donor. Even where this was practicable, the proportion of EA rosette forming lymphocytes was at times as low as 5% and so not acceptable for use in the assay. Only 18 subjects were studied, and only 4 of these had lost graft function within a year. It is, therefore, difficult to draw conclusions from the results depicted.

Figure 55 gives the results for Fcγ-receptor blocking antibody against normal B lymphocytes. No correlation is seen with one-year renal allograft survival.

Figure 56 illustrates the relation between one-year renal allograft survival and pretransplant IgG antibody activity against B lymphocytes derived from the spleen of the cadaveric donor. Only one of the patients studied was found on routine crossmatching to have cytotoxic antibody against donor B cells; that graft was rejected within a few days of transplantation. Six out of 9 "rejectors" had pretransplant IgG Fcγ-receptor blocking activity against donor B cells and 15 out of 22 "acceptors" did not. There was, therefore, no evidence that antibody was associated with improved graft survival.

Donor-recipient HLA matching was marginally worse in the "rejector" group (Table 8). The "acceptors" had had a significantly greater

number of units of blood ($P<0.05$, mann-Whitney U test). The mean $\pm$ standard deviation units of blood was 13 $\pm$ 15 for the "acceptors" and 6 $\pm$ 1 for the "rejectors".

4. Discussion

The factors affecting the outcome of renal transplantation are many, and even the association with HLA typing may be unclear unless thousands of patients are studied (Opelz, 1985b). The failure here to detect an association between pretransplant Fcγ-receptor blocking antibody and renal allograft survival could reflect simply the small number of patients studied. However, the original work proposing the association was based on similar numbers of patients, with correlations found between one-year renal allograft survival and pretransplant Fcγ-receptor blocking activity against donor splenic B lymphocytes, panels of normal B cells and panels of B cells from patients with chronic lymphatic leukaemia (MacLeod et al, 1982 a and b).

The results in this chapter reflect attempts to look in a variety of ways at the relation between allograft survival and Fcγ-receptor blocking IgG class antibody. The experiments in Chapters 10 and 11 showed an association between blood transfusion and IgG antibodies detected by their capacity to block Fcγ-receptors on normal human peripheral blood lymphocytes. Although this finding was similar to that of MacLeod et al (1982b and 1983) using B lymphocytes, no association with allograft survival was found. The converse appeared to be true. Fcγ-receptor blocking activity at $4^{o}C$, but not at $37^{o}C$, was associated with allograft rejection. Also, in patients with functioning transplants after one year, serum creatinine correlated with pretransplant antibody activity.

It has been suggested that patients with strong activity widespread across a panel may fall into a different category than those with selective activity against lymphocytes from one or two donors (MacLeod et al, 1982a). Even patients whose antibody activity was selective had poorer transplant survival in the present study.

IgG preparations were not tested for cytotoxicity against the peripheral blood lymphocytes used in the panels. However, all were screened by the routine Tissue Typing service, and only 6 out of 63 had antibody activity against more than 30% of the donor panel. It seems unlikely that this low prevalence of anti-HLA antibodies could explain the failure to confirm the reported association with renal allograft survival. Although cytotoxicity against donor cells was excluded before transplantation, the experiments using peripheral blood lymphocytes from the cadaveric kidney donor contributed little further.

The results using third party B lymphocytes failed to substantiate the possibility that antibody blocking B cell, rather than peripheral blood lymphocyte, Fcγ-receptors is important to renal allograft survival. Using B lymphocytes prepared from donor splenic tissue the reported association between transplant function and Fcγ-receptor blocking activity was not confirmed.

Explanations for the discrepancy between these and previous results were sought in experimental techniques. There is no obvious reason for the chicken EA technique used here (Sandilands et al, 1980) to give different answers than the ox-EA technique used by MacLeod et al (1982a and b). Where cells were plentiful, the EA rosette inhibition assay was performed at both $4^{o}C$ and $37^{o}C$. Experiments using peripheral

blood lymphocytes were therefore performed at both temperatures. $4^{o}C$ was chosen for the B cell experiments to facilitate comparison with the Aberdeen assay at that temperature.

MacLeod et al attributed their findings to Fcγ-receptor blocking IgG. The majority of their experiments used recipient serum ultracentrifuged for 90 minutes at 100,000g to remove immune complexes which might also block the receptor. IgG separated from serum by DEAE chromatography was used for almost all of the experiments discussed here. This process is in standard use and there is no evidence that it would remove the relevant IgG antibodies. IgG preparations were found to be pure by radial immunodiffusion and gel electrophoresis, and were ultracentrifuged after thawing prior to use. It appears unlikely that the method of preparation of IgG eliminated an association between IgG Fcγ-receptor blocking activity and renal allograft survival.

Untracentrifuged serum from 18 patients was tested against peripheral blood lymphocytes from the cadaveric kidney donor but the small number of patients, particularly in the "rejector" group, hindered interpretation of the results. The question as to whether serum Fcγ-receptor blocking factors other than IgG might be relevant to renal allograft survival is explored further in Chapter 16.

The possibility that the patients studied were in some way different from those studied by MacLeod et al (1982a and b) has not been excluded. Both studies were however conducted in Scotland. The population of the West of Scotland, while larger than that of the Grampian area, is not strikingly cosmopolitan. Most of the patients here were born of local parents, lived all their lives locally and

received blood from local donors. Transfusion policies in the two areas were similar. Patients with a spectrum of renal disease were studied here, and there was no evidence that primary disease affected antibody production or outcome in patients who received transplants. The prevalence of HLA-DRw6, suggested by Hendriks et al (1983) to indicate strong immune responders, was similar in the "acceptor" and "rejector" groups.

Few of the patients studied in this chapter had had previous transplants or were highly sensitized. Three recipients whose IgG preparations were tested against donor splenic B lymphocytes had a positive anti-donor B cell crossmatch. These patients were included in the analysis; their exclusion did not alter the lack of correlation between Fcγ-receptor blocking antibody and transplant outcome.

The renal allografts studied by MacLeod et al (1982a and b) did not have pretransplant donor-recipient tissue matching for Class II antigens. Those studied here were matched preferentially for DR, then the B loci, with relatively little attention to the A loci. This policy is reflected in the figures for mismatches depicted in the Results section, and may reflect a genuine difference between the donor-recipient pairs studied in the two centres.

Fcγ-receptor blocking antibody may have more relevance to the protection of renal allografts where the match for Class II antigens is less favourable. There have been a number of reports of antibodies in transplant recipients apparently directed against non-DR Class II antigens (for example d'Apice and Tait 1980; Nunez, McPhaul and Stastny, 1983). Evidence linking anti-Class II and Fcγ-receptor

blocking antibodies is discussed in Chapter 6. MacLeod et al (1985a) have suggested that Fcγ-receptor blocking antibodies following transfusion may be produced against B lymphocytes from the blood donor and his family in a pattern suggesting HLA linkage, but representing an as yet undefined antigen system (MacLeod et al, 1982a and b).

Against matching for Class II antigens as an explanation for the differing results is the finding in Glasgow that a degree of DR match does not appear to make a difference in transplant outcome of sufficient magnitude to account for the discrepancy (Watson, personal communication).

The failure to confirm the Aberdeen results echoes the findings of Suthanthiran et al (1978) and Soulillou et al (1978), discussed in more detail in Chapter 6. If no correlation is seen between renal allograft survival and pretransplant Fcγ-receptor blocking IgG antibody, other non-IgG serum factors removed by DEAE chromatography could be contributing to previous reports of a correlation. This topic is examined further in the next chapter.

5. Conculsions

i) No correlation was found between one-year renal allograft survival and the presence in recipient serum before transplantation of IgG class antibody with Fcγ-receptor blocking activity against normal peripheral blood or B lymphocytes, nor against donor peripheral blood or splenic B lymphocytes.

ii) No obvious explanation for the discrepancy between this and previous work was found.

NON-IgG Fcγ -RECEPTOR BLOCKING FACTORS AND RENAL ALLOGRAFT SURVIVAL

1. Introduction

i) Background

The failure reported in Chapter 15 to confirm an association between Fcγ-receptor blocking IgG antibody and renal allograft survival led to consideration of possible reasons for the discrepancy between these results and those of MacLeod et al (1982 a and b). The use in the majority of experiments reported here of IgG preparations, rather than ultracentrifuged serum as in most of the previous work, represented a difference in technique. It was decided to look further not at IgG but at other serum Fcγ-receptor blocking factors.

ii) Aims of the study

The experiments described in this chapter were designed to answer the following questions:

a) Is there a difference in Fcγ-receptor blocking activity between ultracentrifuged serum and IgG prepared from the same serum sample?

b) Is renal allograft survival associated with the presence in pretransplant serum of Fcγ-receptor blocking factors other than IgG?

2. Materials and Methods

i) Subjects studied

Serum samples were obtained from:

a) 53 (36 male) patients on renal replacement therapy. The mean age was 42 (range 19 - 57)years. At the time of sampling 18 patients had functioning renal transplants, 24 were on haemodialysis and 10 were on continuous ambulatory peritoneal dialysis. All had received at least 5 units of blood. Transplanted subjects were maintained on Prednisolone and Azathioprine.

b) 78 (48 male) renal allograft recipients during the day before transplantation. The mean age was 37 (range 16 - 65) years. All patients had received at least 5 units of third party whole blood or packed cells. Priority was given to donor-recipient histocompatibility at the DR, then B then A loci. A positive T cell crossmatch on current or historical sera precluded transplantation, but a positive B cell crossmatch did not.

All patients were treated peri- and postoperatively with Prednisolone, initially in a dose of 20 mg daily, tapering to 10 mg daily at one year. Fifty-five patients were also given Azathioprine, in a starting dose of 3mg/kg/day adjusted to keep the while cell count above 4×10^9/ml. Because the Renal Unit immunosuppression protocol changed during the period of study, the other 23 patients were given Cyclosporin in an initial once daily dose of 15mg/kg adjusted to achieve optimum serum creatinine. Rejection episodes, diagnosed on clinical, biochemical and where possible histological criteria, were treated with increased doses of Prednisolone.

ii) Methods used

Each of the 53 serum samples from patients on renal replacement therapy was divided. One aliquot was ultracentrifuged (method 8:3:ii) and the other was used to prepare IgG at a concentration of Img/ml in phosphate buffered saline (method 8:3:iv). The preparations were then tested in parallel at $4^{o}C$ for inhibition of EA rosette formation by peripheral blood lymphocytes from the same normal donor (method 8:4:i).

Six samples of ultracentrifuged serum which were found to have Fcγ-receptor blocking activity at standard concentration (N) were retested in doubling dilutions to N/32.

Serum taken shortly before transplantation from 45 subjects was ultracentrifuged (method 8:3:ii) and tested at $4^{o}C$ for inhibition of EA rosette formation of normal peripheral blood lymphocytes. All these subjects were treated with Azathioprine rather than Cyclosporin.

Pretransplant serum from 50 subjects was fractionated over discontinuous sucrose gradients (method 8:3:iii). Each serum fraction obtained was tested at $37^{o}C$ for inhibition of EA rosette formation of normal peripheral blood lymphocytes (method 8:4:i). Twenty-seven of these subjects were treated with Azathioprine and 23 with Cyclosporin.

3. Results

Figure 57 shows the correlation between the Fcγ-receptor blocking activities of ultracentrifuged serum and IgG, when tested simultaneously with the same lymphocytes. There is a highly significant correlation

between the two values obtained ($r = 0.64$, $P<0.001$). Where 35% inhibition is taken as the upper limit for a "negative" result for IgG (Chapter 9) and 30% for serum (Bakkaloglu et al,1980) the paired values for 9 out of the 53 samples are not concordant. Eight are "positive" with ultracentrifuged serum but "negative" with IgG.

Preparation of IgG as described gives a concentration of IgG lower than that in serum (method 8:3:iv). That dilution was unlikely to explain loss of Fcγ-receptor blocking activity following preparation of IgG from serum is shown in Figure 58. Six out of the 8 samples of ultracentrifuged serum previously found to be "positive" but with "negative"IgG were tested. All maintained Fcγ-receptor blocking at all dilutions to N/32.

Figure 59 shows no association between one year renal allograft survival and pretransplant Fcγ-receptor blocking activity in ultracentrifuged serum when normal peripheral blood lymphocytes were used as the Fcγ-receptor bearing cells. This result was derived using lymphocytes from a single normal donor; Figure 54 in Chapter 15 showed no association where lymphocytes from the kidney donor were used.

The results for serum fractions collected from discontinuous sucrose gradients will be considered separately for the 27 patients treated with Azathioprine and the 23 treated with Cyclosporin. The reason for this is the influence of Cyclosporin on renal allograft survival, even where relatively small numbers of patients are considered. Figure 60 shows the cumulative renal allograft survival for patients transplanted in Glasgow in 1983 and 1984, with 71 patients given Cyclosporin compared to 71 historical controls given Azathioprine. If non-immunological failures were excluded, 85% of kidneys in the

Cyclosporin group and 66% in the Azathioprine group were functioning after one year ($P<0.05$, X^2 test).

Figure 61 shows inhibition by serum fractions from Azathioprine-treated patients of EA rosette formation of peripheral blood lymphocytes from a normal donor. Within the results for each fraction, values are divided into those from the 17 patients with functioning transplants after one year ("acceptors") and those from the 10 whose transplants failed ("rejectors").

If one looks first at the results for fraction 4, which contains the 7S, or IgG peak, one can see no difference between the values for the "acceptors" and "rejectors". Moving towards the higher molecular weight fractions the two groups diverge. In fraction 1, 16 out of 17 acceptors and none out of 10 rejectors had levels of inhibition greater than the 30% regarded as the upper limit of normal for serum (Bakkaloglu et al, 1980) ($P<0.001$, X^2 test with Yates correction).

The results for the Cyclosporin-treated group were less clearcut (Figure 62). There was no significant difference between results for the 14 "acceptors" and 9 "rejectors" in fraction 4 (X^2 test and Mann-Whitney U test). Fcγ-receptor blocking activity in both fraction 3 and fraction 1 was associated with transplant function after one year (for both, X^2 test with Yates correction, $P<0.01$).

The results for all 50 patients from serum fraction 1 are compiled in Figure 63. The pattern that Fcγ-receptor blocking activity was associated with renal allograft survival was followed in all but 5 cases ($P<0.001$, X^2 test with Yates correction).

Transfusion histories were similar for the different groups of patients (Table 9). HLA matching between kidney donors and recipients was slightly poorer in the Cyclosporin-treated group but there was no apparent association in this small group of patients between histocompatibility and graft function.

No association was found in these 50 patients between the presence of Fcγ-receptor blocking activity in serum fraction 1 and the patient's age, sex, primary renal disease, mode of dialysis, time on dialysis, total number of units of blood received, or tissue type. Nor was any association noted with serum urea, creatinine or phosphate levels.

The results of skin testing with Dinitrochlorobenzene were available for 44 of the patients (Tsakiris, personal communication). The response to Dinitrochlorobenzene was graded from 0 to 15 with lower values indicating depressed cell-mediated immunity (Watson et al, 1979). There was a significant inverse correlation between the skin test score and the percentage inhibition of EA rosette formation by serum fraction 1 ($r = -0.41$, $P < 0.01$) (Figure 64).

4. Discussion

When IgG and ultracentrigued serum prepared from the same serum sample were tested in parallel against lymphocytes from the same normal donor, their capacities to inhibit EA rosette formation were in accord in all but 9 out of 53 cases. In 8 of these 9, ultracentrifuged serum was inhibitory but IgG was not, suggesting that preparation of IgG had removed serum Fcγ-receptor blocking factors which were not removed by ultracentrifugation. The use by MacLeod et al (1982a and b) of ultra-centrifuged serum, instead of IgG as used in the experiments in Chapter

15, might therefore contribute to the discrepancy between the results for the association between Fcγ-receptor blocking and renal allograft survival.

The decision to fractionate pretransplant serum over discontinuous sucrose gradients was taken for several reasons. First, it appeared necessary in view of the previous results to look at more than simply the IgG Fcγ-receptor blocking activity. Secondly, the technique was well developed within the laboratory (McLean et al, 1985).

Thirdly, when the technique was first in use in the laboratory in 1977 and 1978, pretransplant serum from renal patients had been fractionated in this way and the fractions tested for their Fcγ-receptor blocking activity against normal peripheral blood lymphocytes. The results are unpublished, but reproduced in Figure 65 (Sandilands and Peel, personal communication).

At that time, pretransplant transfusion was not routine. The 14 patients whose transplants were still functioning at the time of writing (8 years post-transplant) had received more pretransplant blood (mean 13.1, standard deviation 15.7) than had the 7 who rejected their grafts (mean 3.3, standard deviation 5.3). Levels of Fcγ-receptor blocking activity in the fraction containing the IgG peak (fraction 4) were higher in the group who rejected their transplants. Interestingly, those who retained functioning grafts had greater levels of inhibition in the higher molecular weight fractions.

This tentative finding was confirmed in the patients studied in 1983 and 1984. Not only was Fcγ-receptor blocking activity in serum fraction I (>19S) highly predictive of one-year renal allograft survival,

but it also correlated with diminished cell-mediated immunity, assessed by skin testing with Dinitrochlorobenzene. It is not possible with the data shown to exclude a coincidental connection between these two factors which may correlate independently with renal allograft survival.

The relation between these results and the findings of MacLeod et al (1982a and b) is uncertain. Ultracentrifugation would remove serum fraction I, although it is possible that the weaker association with transplant survival seen for serum fraction 3 could be explained by Fcγ-receptor blocking factors left in ultracentrifuged serum.

The Fcγ-receptor blocking factors described by MacLeod et al (1982a and b) and the Fcγ-receptor blocking IgG antibodies described in earlier chapters of this volume showed some degree of specificity against target lymphocytes. The strength of the correlation shown between serum fraction I Fcγ-receptor blocking factors and renal allograft survival suggests that the effect is non-specific, as the lymphocytes used were from normal staff members, not the kidney donor. The correlation with Dinitrochlorobenzene skin testing, an index of non-specific cell-mediated immunity (Watson et al, 1979; Bramwell et al, 1985), may also indicate that the effect is non-specific.

Likely candidates for the role of blocking factor(s) are immune complexes. Certain types of complexes are known to block the Fc - receptor (Sandilands et al, 1980). Carpentier et al (1982) claimed that renal allograft survival was associated with pretransplant circulating immune complexes, a finding which Keusch et al (1984) failed to reproduce.

Immune complexes have been reported to initiate suppressor T cell

function by blocking the Fcɣ-receptor (Moretta et al, 1979), to suppress cell-mediated immunity (Virgin and Unanue, 1985), to inhibit macrophage tumoricidal activity (Eparza, Green and Schreiber, 1983) and possibly to impair the response to tetanus toxoid after repeated booster immunization (Saxon et al, 1981). Caulfield et al (1983) claimed that antigen-antibody complexes induced idiotype specific suppressor T cells in mice, while studying the effects of pnemococcal polysaccharide.

Other possibilities for the blocking factor(s) are globulins of high molecular weight, such as alpha-2-macroglobulin. Alpha-2-macroglobulin is a major serum globulin, whose actions are reviewed by James (1980). To date there have been no reports of individuals lacking this protein, suggesting that absence of the appropriate genes may be a lethal deletion. Alpha-2-macroglobulin has a molecular weight of 725,000 daltons and is dissociable into 4 subunits of 185,00 daltons. It has a variety of potentially immunoregulatory actions, including binding to lymphokines and lectins, and inhibition of the mixed lymphocyte reaction. There are no reports, to the author's knowledge, of its action on Fcɣ-receptors.

The suggestion that alpha-2-macroglobulin might be relevant to transplantation was made by Kamrin (1959), who found that administration of alpha-2-macroglobulin was associated with prolonged skin graft survival in mice. This finding was confirmed by Mowbray (1963), but not by Davis and Boxer (1965). Ford, Caspary and Shenton (1973) suggested that alpha-2-macroglobulin could block antigen recognition by lymphocytes, Davis et al (1978) concurred, and Cooperbrand et al (1976) reviewed the evidence for such non-specific immunosuppressive substances in a variety of conditions.

Proud, Shenton and Smith (1979) assessed the capacity of plasma from previously transfused uraemic and haemophiliac subjects to inhibit the lymphocyte response to purified protein derivative of mycobacterium tuberculosis. After the plasma was fractionated according to molecular weight, the fraction containing IgG gave some inhibition of the lymphocyte response, but considerably more was found in the fraction containing macroglobulins.

Proud et al (1979) also claimed an association between the presence in pretransplant plasma of alpha-2-macroglobulin and renal allograft survival, and demonstrated prolongation of cardiac allograft survival in rats given alpha-2-macroglobulin. After a rush of papers from the same group (Shenton et al, 1979; Veitch et al, 1980), the proposed association between alpha-2-macroglobulin and renal allograft survival has lapsed into abeyance.

An immunosuppressive glycoprotein has been described in the serum of pregnant women. Suppression of mixed lymphocyte reactivity by serum from pregnant women (Bissenden, Ling and Mackintosh, 1980) may be related to the IgG antibodies described, for example, by Power et al (1983a). However, Fizet et al (1983) reported that an alpha-globulin, of molecular weight 210,000, reduced maternal lymphocyte responsiveness to paternal antigens and gave non-specific inhibition of the mixed lymphocyte reaction between unrelated third party lymphocytes.

Toge et al (1983) claimed that serum from patients with advanced gastric carcinoma induced suppressor cell proliferation by normal lymphocytes, but did not discuss the nature of the suppressor factor(s). There is therefore little evidence associating alpha-2-macroglobulin with immunosuppression outwith transplantation.

It is possible that neither immune complexes nor alpha-2-macroglobulin can explain the apparent correlation between high molecular weight serum Fcγ-receptor blocking factors and renal allograft survival. Experiments to try to establish the nature of the blocking factor(s) are described in the next chapter.

The response to skin testing with Dinitrochlorobenzene has been associated with blood transfusion and renal allograft survival (Watson et al, 1979 and 1981) and the correlation here with serum fraction I Fcγ-receptor blocking activity may indicate that blood transfusion may be relevant to the production of blocking factor(s). The relation between blocking factor(s) and transfusion is discussed in the next chapter.

5. Conclusions

i) Preparation of IgG by DEAE chromatography may exclude serum Fcγ-receptor blocking factors not removed by ultracentrifugation of serum.

ii) High molecular weight Fcγ-receptor blocking factors in pretransplant serum correlate with renal allograft survival.

CHAPTER 17

THE NATURE AND RELATION TO TRANSFUSION OF HIGH MOLECULAR WEIGHT SERUM FcγR-RECEPTOR BLOCKING FACTORS

1. Introduction

i) Background

The results presented in the last chapter show a correlation between renal allograft survival and the presence in pretransplant serum of high molecular weight (>19S) factor(s) detected by their capacity to block peripheral blood lymphocyte FcγR-receptors. The purposes of this chapter are to examine the nature and relation to blood transfusion of these factor(s).

ii) Aims of the study

The experiments described in this chapter were designed to answer the following questions:

a) Is the presence of high molecular weight serum FcγR-receptor blocking factor(s) associated with previous transfusion of blood or blood products?

b) Can the development of such factors be demonstrated over the course of an elective blood transfusion protocol in renal dialysis patients?

c) Do high molecular weight serum fractions from normal and transfused subjects inhibit phytohaemagglutinin-induced blastogenesis of normal lymphocytes?

d) What is the nature of the high molecular weight serum Fcγ-receptor blocking factors which appear to correlate with renal allograft survival?

2. Materials and Methods

i) Subjects studied

Serum samples were taken from the following groups of subjects:

a) 10 healthy laboratory staff. None had received transfusion of blood or blood products.

b) 4 (2 male) subjects with serum creatinine greater than 600 micromols/l, but never transfused. The mean age was 38 (range 21 - 59) years.

c) 6 (2 male) subjects without known renal disease but previously transfused at least 5 units of blood. The mean age was 40 (range 22 - 57) years.

d) 6 patients with haemophilia receiving blood products but not blood. All were male, with mean age 26 (range 19 - 38) years.

e) 6 (5 male) renal dialysis patients undergoing elective third party blood transfusion prior to entry to the pool awaiting cadaveric transplantation. The mean age was 38 (range 20 - 46) years. The serum samples used were taken i) before any blood was given (no patient had received previous transfusion) and ii) two weeks after transfusion of the fifth unit of packed cells.

f) 34 patients about to undergo renal transplantation. Samples were taken as before during the few hours prior to transplantation. All patients had received at least 5 units of third party blood. Tissue typing criteria were as in Chapters 15 and 16.

ii) Methods used

All serum samples were fractionated over discontinuous sucrose gradients (method 8:4:iii) and the fraction of highest molecular weight, designated fraction I, was used for subsequent experiments.

Fraction I preparations from serum from the following subjects were tested for their capacity to inhibit EA rosette formation at 37°C by peripheral blood lymphocytes from a normal subject (method 8:4:i).

a) 6 normal subjects
b) 4 untransfused uraemic subjects
c) 6 non-uraemic transfused subjects
d) 6 haemophiliac subjects
e) 6 uraemic subjects (both pre- and post-transfusion samples)

Fraction I preparations from serum from the following subjects were tested for their capacity to inhibit phytohaemagglutinin-induced blastogenesis of peripheral blood lymphocytes from a normal subject (method 8:4:iv).

a) 6 normal subjects
b) 6 haemophiliac subjects
c) 31 transfused uraemic subjects. Samples were taken immediately prior to transplantation. All patients were immunosuppressed with Prednisolone; in addition 18 received Azathioprine and 13 Cyclosporin. 16 patients had a functioning transplant one year later ("acceptors") and 15 had lost the kidney because of rejection ("rejectors").

Fraction I preparations from pretransplant samples from 3 "acceptors" and 3 "rejectors", and from samples from 3 transfused non-uraemic subjects were separated by SDS-PAGE gel electrophoresis (method 8: 10).

Pooled serum fraction I preparations were made for transplant "acceptors" (19 subjects), "rejectors" (6 subjects) and normal subjects (10 subjects). These pools were tested for inhibition of EA rosette formation and phytohaemagglutinin-induced blastogenesis of normal peripheral blood lymphocytes, before and after absorption with Sepharose beads, both alone and conjugated to Staphylococcal Protein A (methods 8:4:i; 8:4:iv; 8: 11). SDS-PAGE gel electrophoresis was performed on the absorbed and unabsorbed pools.

Alpha-2-macroglobulin, at a concentration of 250 mg/l in phosphate buffered saline, was tested for its capacity to inhibit EA rosette formation at 37°C by peripheral blood lymphocytes from 3 normal subjects.

3. Results

All the patients in Chapter 16 whose serum contained high molecular weight Fcγ-receptor blocking factors were renal dialysis patients who had received at least 5 units of blood. Figure 66 shows the results of experiments performed to see whether the presence of such factors is associated with previous transfusion of blood or blood products. No difference was found between Fcγ-receptor blocking activities of serum fraction I from normal and uraemic untransfused subjects. The differences between activities of serum fraction I from normal and non-uraemic transfused ($P<0.001$) and haemophiliac ($P<0.05$) subjects reached

statistical significance (Mann-Whitney U Test).

Figure 67 shows the results for inhibition of EA rosette formation by fraction I before and after transfusion of 6 dialysis patients. The samples were tested in parallel against peripheral blood lymphocytes from the same normal donor. In 3 out of 6 patients there was some increase in Fcγ-receptor blocking activity after transfusion; in 1 there was a marked decrease, and in the other 2 there was no change. The difference between the values before and after transfusion did not reach statistical significance (Wilcoxon Matched Pairs Signed Rank Test).

Figure 68 shows the extent to which serum fraction I preparations inhibited phytohaemagglutinin-induced blastogenesis of normal peripheral blood lymphocytes. The difference between the results for normal and haemophiliac subjects reached statistical significance at the 5% level (Mann-Whitney U Test). The difference between the results for the normal and transfused uraemic subjects was much more striking ($P<0.001$, Mann-Whitney U Test). There was no significant difference between the values obtained for the "acceptor" and "rejector" groups.

Fractions I and 4, alone and in combination, from 3 transfused subjects were tested for their capacity to inhibit the phytohaemagglutinin response of the same normal lymphocytes. As Figure 69 shows, the inhibitory activities of fractions I and and 4 (containing the IgG peak) appeared to be additive.

SDS-PAGE gel electrophoresis of serum fraction I from 3 "acceptors", 3 "rejectors" and 3 non-uraemic transfused subjects showed no obvious differences among the groups(gel not shown). Samples from all the non-

uraemic transfused subjects and from 2 of the "acceptors" were previously found to have Fcγ-receptor blocking activity.

Nor was any different pattern of protein banding noted on electrophoresis of the pools of normal, "acceptor" and "rejector" fraction I. Differing densities of the protein bands from the various pools mirrored differing protein concentrations (assessed as before by optical density at 280nm): normal 369 mg/l, "acceptor" 222 mg/l, "rejector" 132 mg/l.

The protein band corresponding to molecular weight 150,000 daltons (dalton markers not shown) was removed when the fraction I pool was absorbed with Sepharose-Protein A, but not with Sepharose alone. When the proteins removed by the absorption technique were run over similar gels, the protein band obtained was found to correspond to that formed by monomeric IgG (gels not shown).

When fraction I pools before and after absorption with Sepharose-Protein A were tested for Fcγ-receptor blocking activity against peripheral blood lymphocytes from 3 normal donors, the results shown in Figure 70 were obtained. Fcγ-receptor blocking activity was not fully removed by absorption.

Similarly, absorption did not remove the capacity to inhibit the phytohaemagglutinin response (Figure 71). On the contrary, the inhibitory activity of the "acceptor" pool was increased. This experiment was performed with a single normal lymphocyte donor, and requires confirmation using other donors.

Alpha-2-macroglobulin, at the concentration used, did not inhibit EA rosette formation by peripheral blood lymphocytes from 3 normal donors

(percentages inhibition 0, 0 and 22%).

4. Discussion

The finding in Chapter 16 that renal allograft survival correlated with pretransplant high molecular weight serum Fcγ-receptor blocking activity led to the experiments described here. Taken in conjunction with the results for transfused uraemic subjects in Chapter 16, the results here do suggest that the presence of high molecular weight serum Fcγ-receptor blocking factors may be associated with previous transfusion of blood or blood products, although the number of subjects studied here was relatively small. In particular, the failure of the pre- and post-transfusion samples to show a significant increase in Fcγ-receptor blocking activity after transfusion may reflect the number of patients rather than a lack of association with transfusion. Three of the 6 subjects did show an increase in fraction I Fc -receptor blocking activity after elective transfusion.

Like the pattern when IgG preparations rather than serum fractions were tested in earlier experiments, values were outwith the normal range for all the non-uraemic patients who had received blood transfusion, but for only a proportion of transfused subjects. This discrepancy was discussed in Chapter 10, and may reflect depressed responsiveness to alloantigens in uraemia.

The proportion of haemophiliacs whose serum fraction I had Fcγ-receptor blocking activity was not markedly different than the IgG results in Chapter 13. This contrasted with the results for inhibition of the phytohaemagglutinin response by fraction I, where the modest levels of

inhibition achieved by samples from haemophiliacs were outstripped by those from transfused uraemic subjects (Figure 68). The difference between results for recipients of blood and blood products may be genuine, but the absence of results for untransfused uraemic or transfused non-uraemic subjects makes this difference difficult to interpret.

There was certainly no evidence from the results shown that renal allograft survival was related to pretransplant fraction I inhibition of blastogenesis by lymphocytes from random normal donors. No association was seen between inhibition of EA rosette formation and the mitogen response by fraction 1 (results not illustrated). Inhibition of the phytohaemagglutinin response could, therefore, not be regarded as an in vitro model of T cell suppression associated with high molecular weight serum Fcγ-receptor blocking activity.

None of the experiments using gel electrophoresis gave an indication as to the nature of the fraction I Fcγ-receptor blocking factor(s), suggesting possibly that the gel method or staining procedures were not sufficiently sensitive to detect the relevant factor(s).

As discussed in Chapter 16, major contenders for the factor(s) appeared to be immune complexes and alpha-2-macroglobulin. Although there is extensive evidence for immunoregulatory roles for alpha-2-macroglobulin (reviewed by James 1980), there is no evidence that it can inhibit the Fcγ-receptor. The concentration of alpha-2-macroglobulin chosen for the experiments described here was chosen to approximate to the protein concentration found in the fraction I pool which had Fcγ-receptor blocking activity. It is possible that alpha-2-macroglobulin might inhibit EA rosette formation if used at a different concentration,

but it was not found to be inhibitory here.

It would be interesting to absorb the fraction I preparations with anti-alpha-2-macroglobulin antibody, and then retest them for Fcγ-receptor blocking activity. Absorption with antibody against alpha-2-macroglobulin of the serum fractions tested by the Newcastle group only partially removed their ability to inhibit the response to purified protein derivative of Mycobacterium tuberculosis (Proud et al, 1979; Veitch et al, 1980).

Absorption with Staphylococcal Protein A has been regarded as a method of removing IgG, in monomeric or complexed form (Burlingham et al, 1985b). Gel electrophoresis of serum fraction I showed in all groups of subjects a protein band of molecular weight 150,000 daltons. This corresponded to IgG, although immunodiffusion techniques gave no evidence of monomeric IgG suggesting that IgG was present in serum fraction I in the form of immune complexes.

Absorption of fraction I with Protein A appeared to remove these presumed complexes, as gel electrophoresis of absorbed fraction I preparations showed no corresponding band. Yet the failure to absorb out all the Fcγ-receptor blocking activity suggested the presence of Fcγ-receptor blocking factor(s) other than IgG-containing complexes. If the increased inhibition of the phytohaemagglutinin response after absorption were confirmed, this might suggest that immune complexes mask the inhibitory effect of other unspecified serum factor(s).

IgG preparations from transfused subjects were shown in Chapter 12 to inhibit the mitogen response, and it was suggested there that the

mechanism might be by interaction with the Fcγ-receptor, as already described for immune complexes (Moretta et al, 1979). The additive effect on inhibition of the mitogen response of serum fractions I and 4 (containing the IgG peak) may indicate that the inhibitory factors of differing molecular weights are acting by differing mechanisms.

The experiments described here fail to provide evidence for either alpha-2-macroglobulin or IgG immune complexes being responsible for the observed correlation between renal allograft survival and the presence in pretransplant serum of high molecular weight Fcγ-receptor blocking factors. Nor was any other compound implicated by the gel electrophoresis analysis of fraction I from different groups of patients although it is possible that more sensitive electrophoretic techniques may be required.

5. Conclusions

i) There appeared to be an association between transfusion of blood or blood products and high molecular weight serum Fcγ-receptor blocking activity, although no significant change in activity was found when 6 patients were followed over a course of elective transfusion.

ii) Inhibition of the phytohaemagglutinin response by pretransplant serum fraction I was not association with one-year renal allograft survival.

iii) The nature of the high molecular weight serum Fcγ-receptor blocking factors was not established.

CHAPTER 18

CONCLUSIONS

The experiments described in the preceding chapters confirmed the association between blood transfusion and the production of non-cytotoxic IgG class Fcγ-receptor blocking antibodies (MacLeod et al, 1982b),Chapters 9 to 11). IgG preparations from transfused patients reacted with a range of Fcγ-receptor bearing cells in addition to the B lymphocytes against which they were initially described. IgG Fcγ-receptor blocking activity was associated with the ability of IgG preparations to inhibit the mitogen response in vitro. IgG preparations from transfused subjects also affected polymorph and platelet function in vitro, although clinical correlates in vivo were not sought (Chapter 12).

IgG antibodies with Fcγ-receptor blocking activity and the ability to inhibit the mitogen response were also found in recipients of clotting factor concentrates (Chapter 13), in asymptomatic homosexual men and and in patients with the Acquired Immunodeficiency Syndrome (Chapter 14). The finding of antibodies with potentially immunosuppressive effects in vitro in association with Fcγ-receptor blocking activity may indicate a role for Fcγ-receptor blocking antibodies in the transfusion effect on renal allograft survival (MacLeod et al, 1982a and b), immune abnormalities in haemophilia (Froebel et al, 1983), susceptibility to the Acquired Immunodeficiency Syndrome (Fauci, 1985), and the treatment of autoimmune disease with pooled immunoglobulin (Templeton et al, 1985), all situations with multiple alloantigen exposure (Woodruff and van Rood, 1983).

Many factors contribute to renal allograft survival (Opelz, 1985a), so the failure in Chapter 15 to confirm the association with Fcγ-receptor blocking antibodies does not wholly exclude their immunosuppressive role. The strong association found in Chapter 16 between allograft outcome and the presence in pretransplant serum of high molecular weight Fcγ-receptor blocking factors is not necessarily related to the previous work implicating IgG. The correlation between high molecular weight Fcγ-receptor blocking activity and response in vivo to skin testing with Dinitrochlorobenzene suggests that these Fcγ-receptor blocking factors may be related to the mechanism of depression of cell-mediated immunity associated with previous blood transfusion and subsequent renal allograft survival (Watson, 1979 and 1981).

Although some evidence was provided in Chapter 17 for an association between transfusion of blood or blood products and the presence of high molecular weight serum Fcγ-receptor blocking factors, preliminary attempts to identify the factor(s) were unsuccessful. Future work in this area should concentrate, at an experimental level, on identification of the factor(s) and confirmation of the association with transfusion. At a clinical level, extension of the number of patients studied would confirm whether the presence in pretransplant serum of high molecular weight Fcγ-receptor blocking factors may be regarded as predictive of renal allograft survival.

The immunosuppressive effect of blood transfusion is of substantial benefit in renal transplantation, and the availability of clotting factor concentrates has transformed the lives of patients with haemophilia. Liberal use of blood or blood products may not only increase the risk of transmitted infection, but also lead to immune abnormalities. Where

transfusion is less crucial, sparing use of blood and blood products may minimise the uncertain long-term sequelae of exposure to multiple alloantigens.

"THE HUMORAL IMMUNOSUPPRESSIVE EFFECTS OF BLOOD TRANSFUSION IN RENAL TRANSPLANTATION"

SUMMARY

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January, 1986

SUMMARY

Renal transplantation offers the optimal quality of life for the patient requiring renal replacement therapy. The main reason for renal allograft failure remains immunological rejection. The discovery that pretransplant blood transfusion is associated with decreased graft loss through rejection led to the introduction of elective transfusion protocols for renal dialysis patients. Despite extensive research, the mechanism of the blood transfusion effect on transplant outcome remains unclear. Of the many suggested explanations, one proposed by MacLeod and her colleagues, claimed that Fcγ-receptor blocking antibodies were produced after blood transfusion and correlated with renal allograft survival. The initial aims of the work described in this volume were to confirm and extend these findings (MacLeod et al, 1982b).

Using IgG prepared from whole serum by standard chromatography, the association between Fcγ-receptor blocking activity and blood transfusion was confirmed for both uraemic and non-uraemic subjects. The development of Fcγ-receptor blocking activity was shown over the course of elective transfusion of previously untransfused renal dialysis patients.

IgG preparations from transfused subjects were shown to interact *in vitro* not only with B lymphocytes as previously described, but also with normal, allogeneic peripheral blood and T lymphocytes, thymocytes, polymorphs, platelets and spermatozoa. The pattern of reactivity did not correspond to the Fcγ-receptor bearing cell populations. The significance *in vivo* of inhibition by IgG preparations from transfused subjects of polymorph phagocytosis and platelet aggregation remains

uncertain. Perhaps relevant to attenuation of allograft rejection was inhibition of the mitogen response which was associated with IgG Fcγ-receptor blocking activity.

Confirmation of the presence of Fcγ-receptor blocking antibodies in patients potentially immunosuppressed by blood transfused was followed by similar experiments using IgG preparations from patients exposed to a wide range of alloantigens, although not necessarily in blood. Patients with haemophilia were chosen, as a group who received clotting factor concentrates derived from multiple donors, and in whom there were recent reports of impaired indices of immune function. Like the recipients of blood transfusion, patients with haemophilia yielded IgG which blocked Fcγ-receptors and inhibited the mitogen response of lymphocytes from normal subjects.

Only a small proportion of the haemophiliac subjects were later found to have antibody against Human T cell Leukaemia Virus Type III, thought now to mark potential development of the Acquired Immunodeficiency Syndrome (AIDS). The epidemiology of AIDS, while consistent with unifactorial viral aetiology, led to suggestions that pre-existing immunosuppression may predispose to the expression of the virus.

Reports of anti-lymphocyte antibodies in the serum of practising homosexual men, the risk group with the highest incidence of AIDS, stimulated experiments to assess the prevalence of Fcγ-receptor blocking antibodies in AIDS patients and in homosexual men with no evidence of the syndrome. IgG preparations from both groups blocked Fcγ-receptors and inhibited the mitogen response of normal lymphocytes. Cross-reactivity with spermatozoa may indicate that anti-lymphocyte antibodies

are induced by rectal insemination. Although these experiments were performed before identification of the virus, and anti-viral antibody status was not checked, the prevalence of infection in comparable populations at the time of testing (1983) has been regarded as too low to account for these findings.

Despite the demonstration of IgG Fcγ-receptor blocking activity in subjects with acquired immune abnormalities, no association was found between renal allograft survival and pretransplant IgG receptor blocking activity, using a variety of donor and third party Fcγ-receptor bearing cells. No conclusive explanation was found for the discrepancy between this and previous work (MacLeod et al, 1982a and b).

Lest the method of preparation of IgG eliminate the suggested association with transplant outcome, pretransplant sera were instead separated into fractions of graded molecular weight. There was a highly significant correlation between renal allograft survival and Fcγ-receptor blocking activity in the highest molecular weight fraction (>19S). Although the serum factor(s) responsible were not identified, their presence appeared to be associated with previous transfusion of blood or blood products, and with diminished response to skin testing with Dinitrochlorobenzene. This may indicate that high molecular weight serum Fcγ-receptor blocking factors are involved in non-specific depression of cell-mediated immunity following blood transfusion, and may be regarded as predictive of successful renal transplantation.

"THE HUMORAL IMMUNOSUPPRESSIVE EFFECTS OF BLOOD TRANSFUSION IN RENAL TRANSPLANTATION"

Two Volumes: Volume Two

References, Tables and Figures

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Test	% rosette-forming PBL	% inhibition of EA rosette formation by sample 1	by sample 2
1	19	87	0
2	21	89	0
3	20	92	7
4	20	73	2
5	20	70	0
6	21	88	12
7	21	94	0
8	21	88	0
9	22	77	9
10	20	77	0
Mean ± SD	20.5 ± 0.8	83.5 ± 8.0	3.0 ± 4.3

TABLE 1 : Variation in results obtained with the EA rosette inhibition assay and 10 simultaneous tests:

i) percentage of rosette-forming peripheral blood lymphocytes

ii) inhibition of rosette formation by "known positive" and "negative" IgG preparations

patient	HLA - A, B and DR type of patients			Peripheral blood lymphocyte donor			
	A	B	DR	A 1,2 B 5,8 DR 3,8	A 2,28 B 8,14 DR 3	A 11,24 B 8,35 DR 3	A 1,11 B 5,8 DR 2
1	2,3	35,62	1,4	0	0	27	0
2	1,3	7,16	2,6	21	55	66	31
3	3,11	7,35	2	0	21	17	15
4	1,28	37,40	2,4	0	27	17	0
5	2,29	44,50	2,7	57	75	70	40
6	2,30	13,18	2,7	65	37	76	80
7	2,32	13,27	2,3	50	27	53	65
8	2,28	40,44	4,5	0	9	0	0
9	1,2	14,15	7	56	62	78	93
10	2,11	35,44	2	0	12	7	8
11	1	8	3	20	57	50	34
12	24,11	7,27	4	0	74	68	74
13	3,28	7	2	5	66	67	60
14	2,29	44,50	2,7	55	34	50	59
15	1,2	8	3,4	4	0	0	0

TABLE 2: Variation with lymphocyte donor of results of the EA rosette inhibition assay (37°C) for IgG preparations from 15 transfused dialysis patients

	normal	uraemic untransfused
uraemic untransfused	$P<0.05$	-
uraemic transfused	$P<0.001$	$P<0.05$
transfused transplanted	$P<0.001$	$P<0.001$
total transfused	$P<0.001$	$P<0.001$

TABLE 3 : Statistical significance (Student's t test) of differences between groups of subjects in percentages of EA rosette-forming peripheral blood lymphocytes

Assay	Patient Number	% Inhibition of EA-rosette formation	% Inhibition of cytotoxicity
ADCC	1	61	1
	2	46	40
	3	44	0
	4	34	0
	5	27	0
NK	1	65	0
	2	53	15
	3	48	29
	4	46	31
	5	14	17
	6	65	22
	7	48	0
	8	44	5
	9	0	0

TABLE 4: Inhibition of killer (K) cell function (ADCC) and natural killer (NK) cell function by IgG from multiply transfused uraemic patients

Anti-sperm antibody	Fc γ-receptor blocking antibody +	Fc γ-receptor blocking antibody −
−	48	20
+	31	46

TABLE 5 : Comparison between Fcγ-receptor blocking and anti-sperm antibody activity in 145 subjects

a) $4^{o}C$

Antibody	Graft function −	Graft function +
−	12	33
+	11	7

b) $37^{o}C$

Antibody	Graft function −	Graft function +
−	7	22
+	16	18

TABLE 6 : Association between one-year renal allograft function and pretransplant IgG antibody blocking Fcγ-receptors on normal peripheral blood lymphocytes

		Mean number of HLA mismatches		
		A	B	DR
Acceptors	(40)	1.1	0.9	0.4
Rejectors	(23)	0.9	0.8	0.4

TABLE 7 : Donor-recipient HLA mismatches for 63 renal allografts

		Mean number of HLA mismatches		
		A	B	DR
Acceptors	(22)	1.0	0.8	0.2
Rejectors	(9)	1.2	0.8	0.4

TABLE 8 : Donor-recipient HLA mismatches for 31 renal allografts

		Number of patients	Mean number of units of blood	Mean number of HLA mismatches A	B	DR
Azathioprine	acceptors	17	9	1.3	1.1	0.3
	rejectors	10	12	0.8	0.7	0.3
Cyclosporin	acceptors	14	13	1.5	1.0	0.7
	rejectors	9	10	1.6	0.7	0.9

TABLE 9 : Transfusion and tissue matching in 50 renal transplant recipients

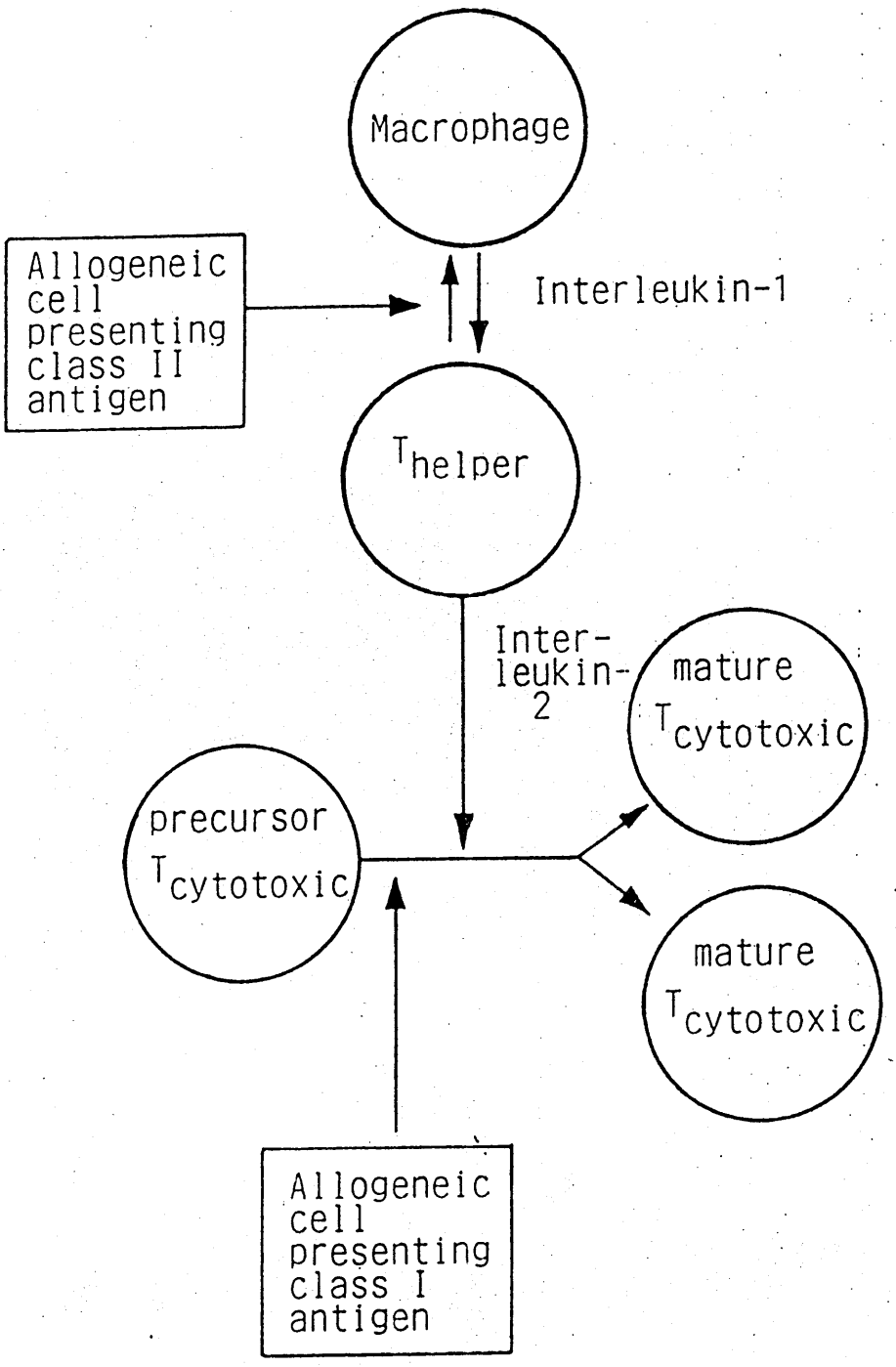


FIGURE 1: Mechanisms of rejection - cell-mediated cytotoxicity

Delayed - type Hypersensitivity

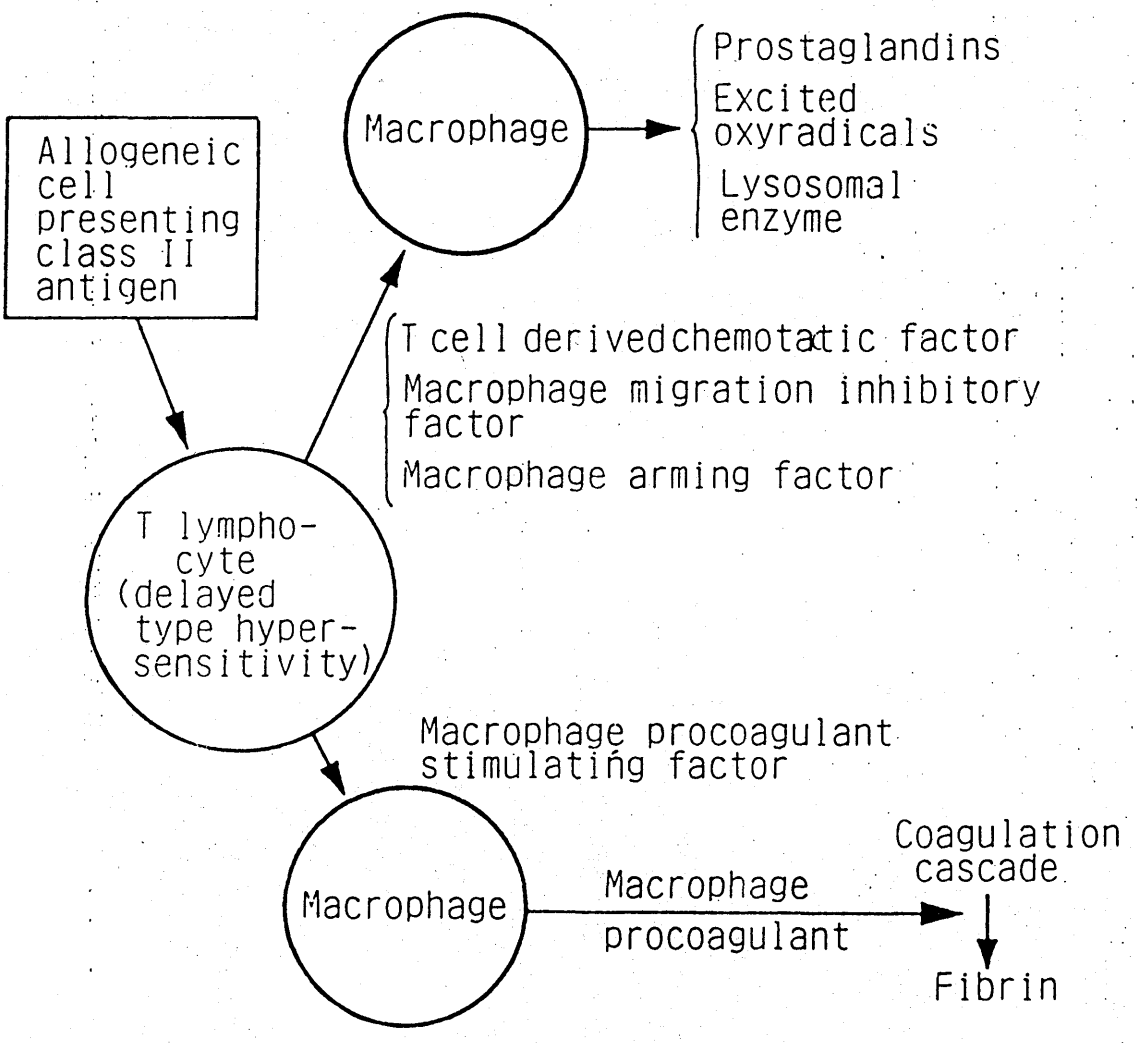


FIGURE 2: Mechanisms of rejection - delayed-type hypersensitivity

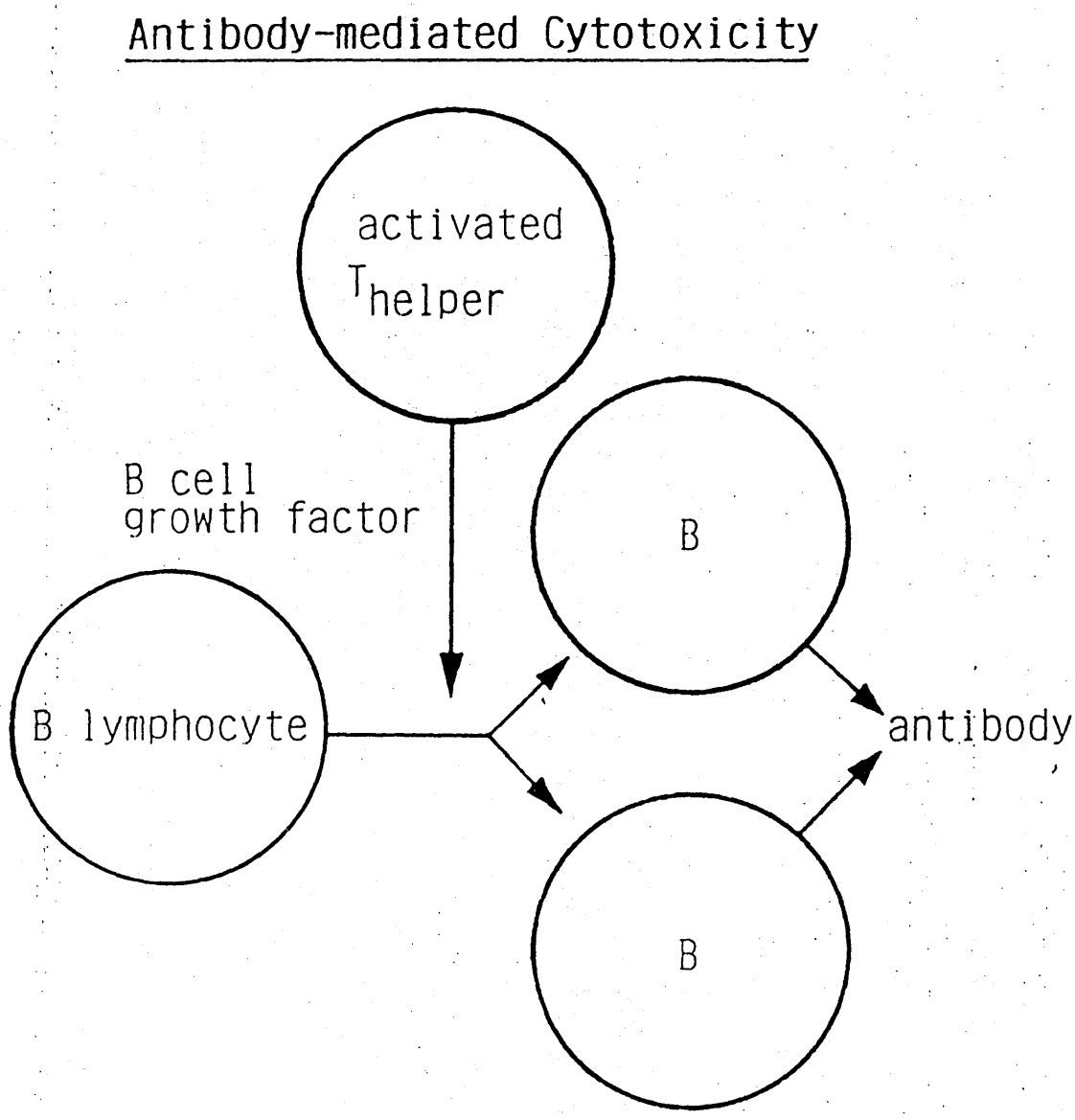


FIGURE 3: Mechanisms of rejection - antibody-mediated cytotoxicity

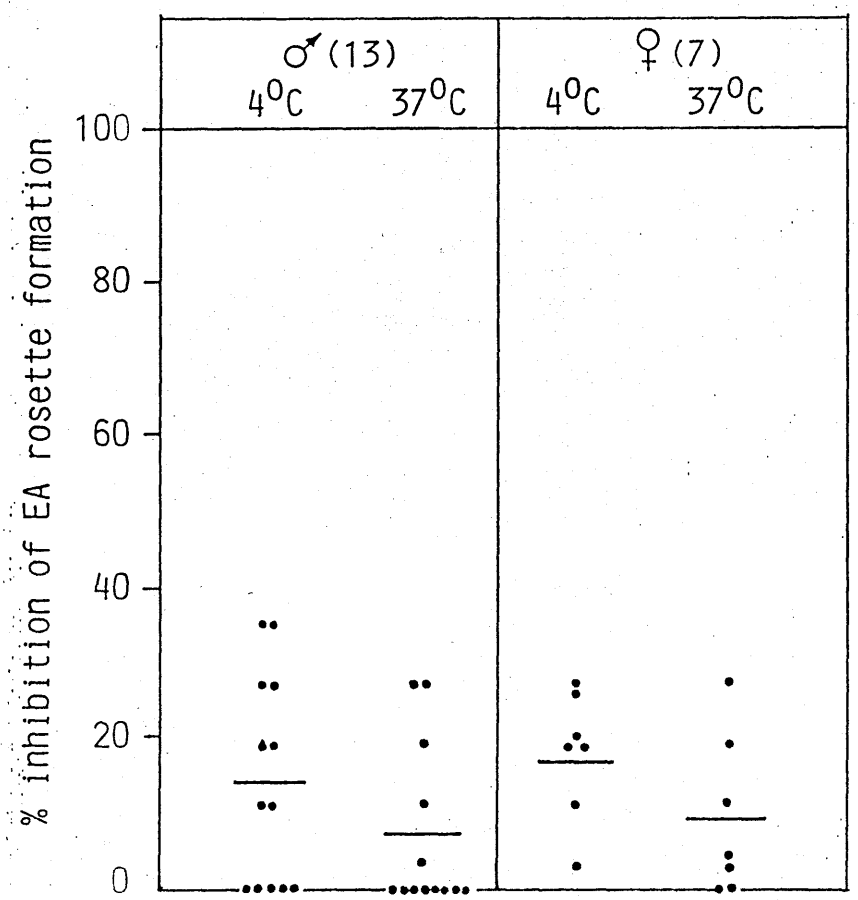


FIGURE 4 : Inhibition of EA rosette formation of peripheral blood lymphocytes by IgG preparations from 20 normal subjects

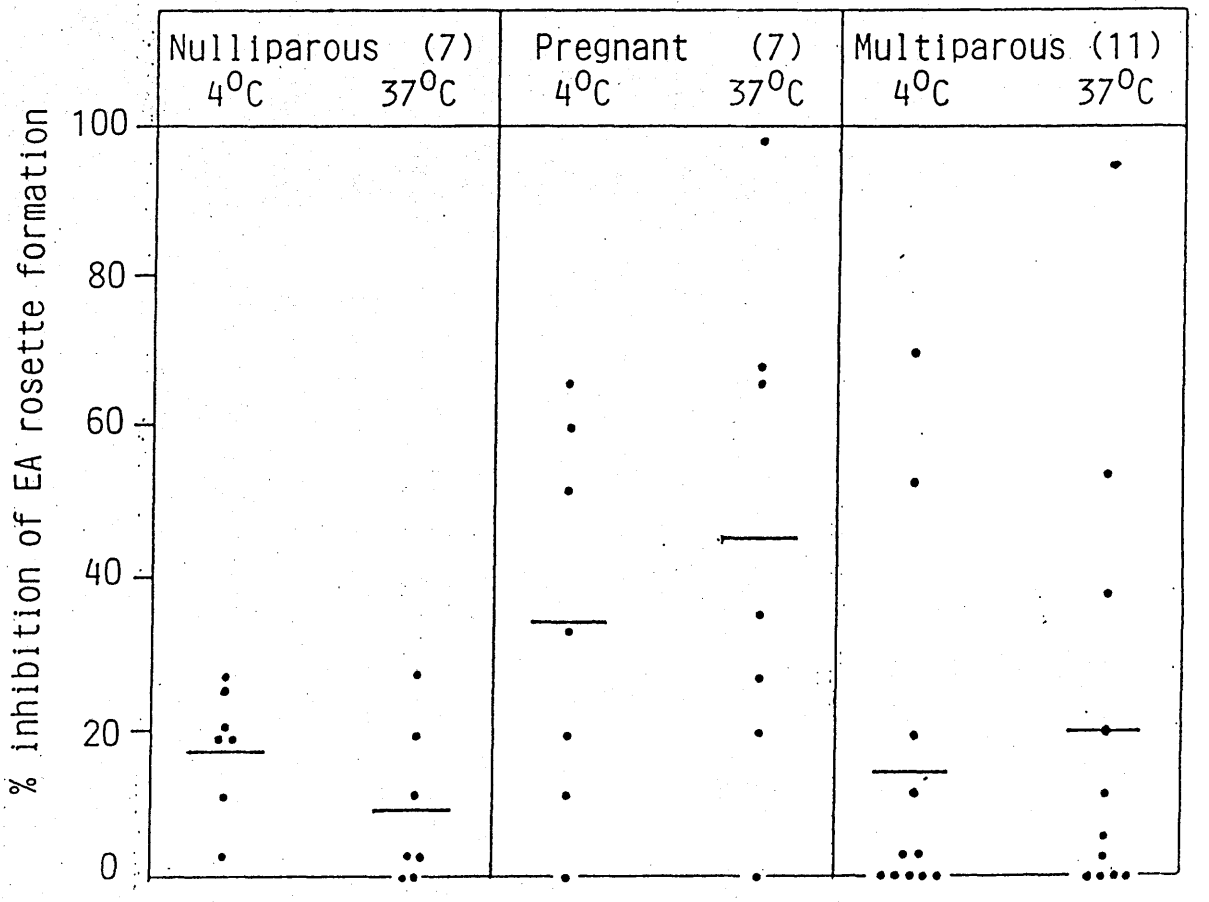


FIGURE 5 : Inhibition of EA rosette formation of peripheral blood lymphocytes by IgG preparations from 25 women

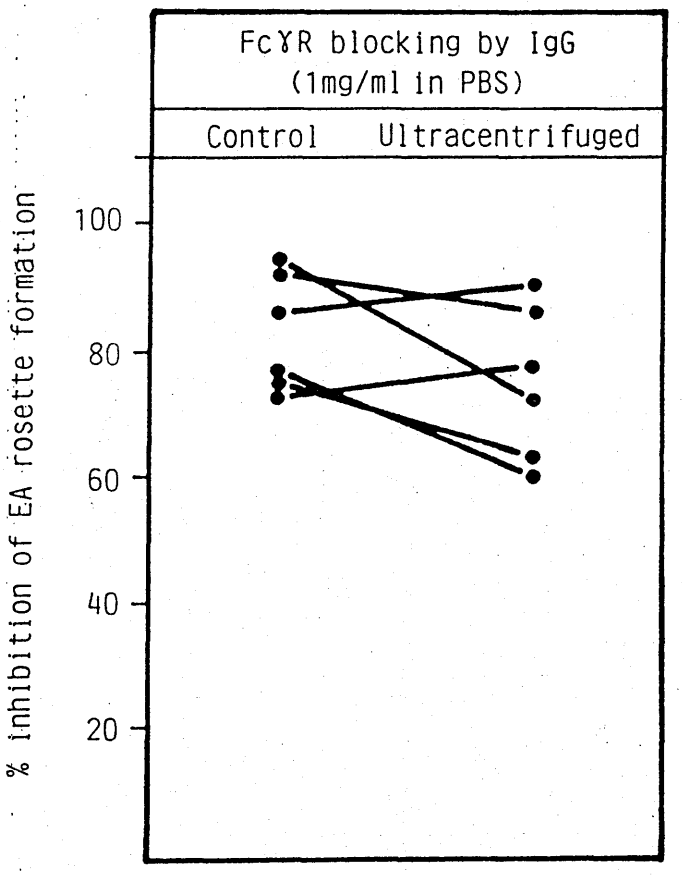


FIGURE 6: Effect of ultracentrifugation for 1 hour at 105,000g on Fcγ-receptor (FcγR) blocking activity of 6 IgG preparations

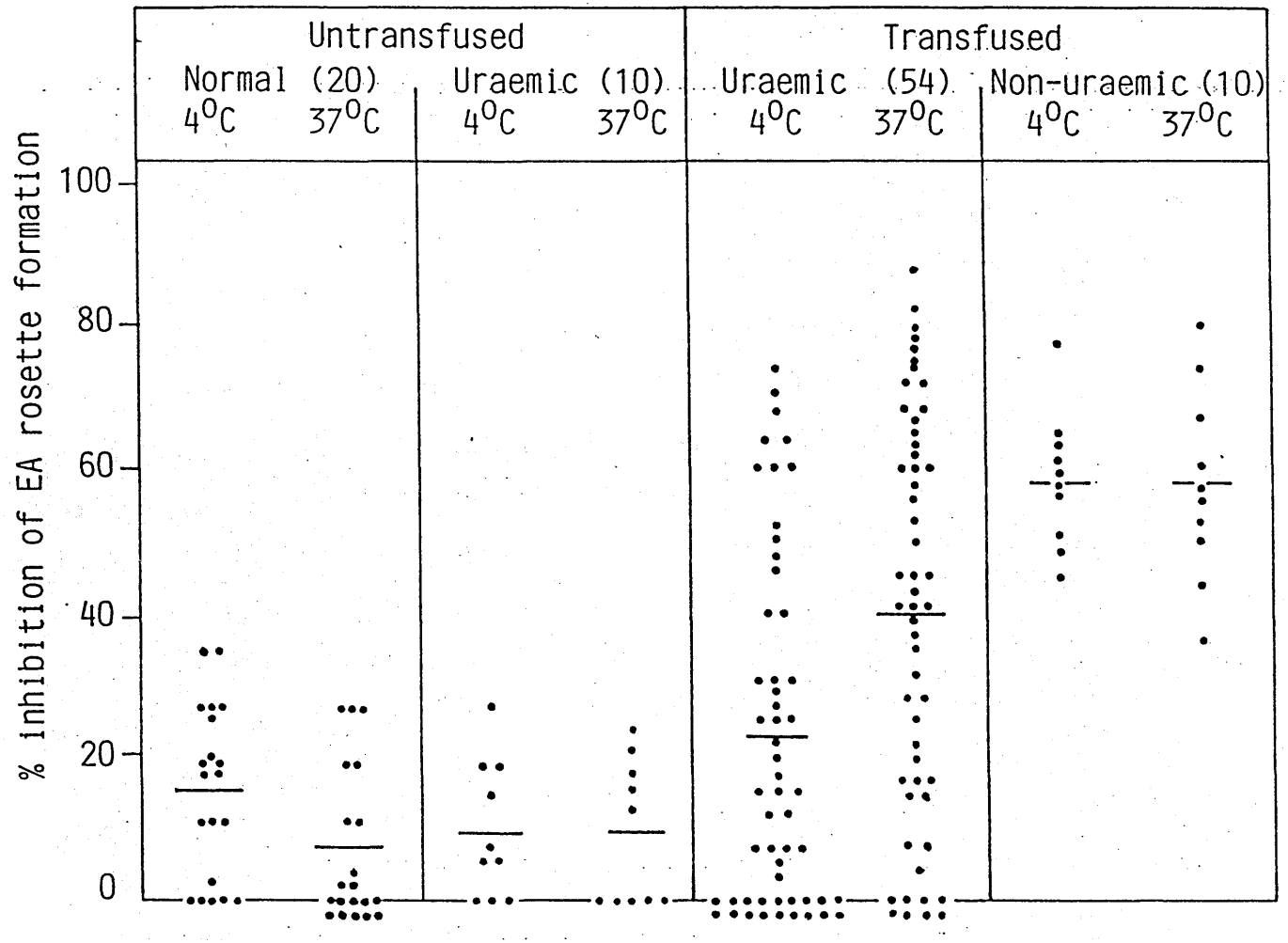


FIGURE 7: Inhibition of EA rosette formation of normal peripheral blood lymphocytes by IgG preparations from 94 untransfused and transfused subjects

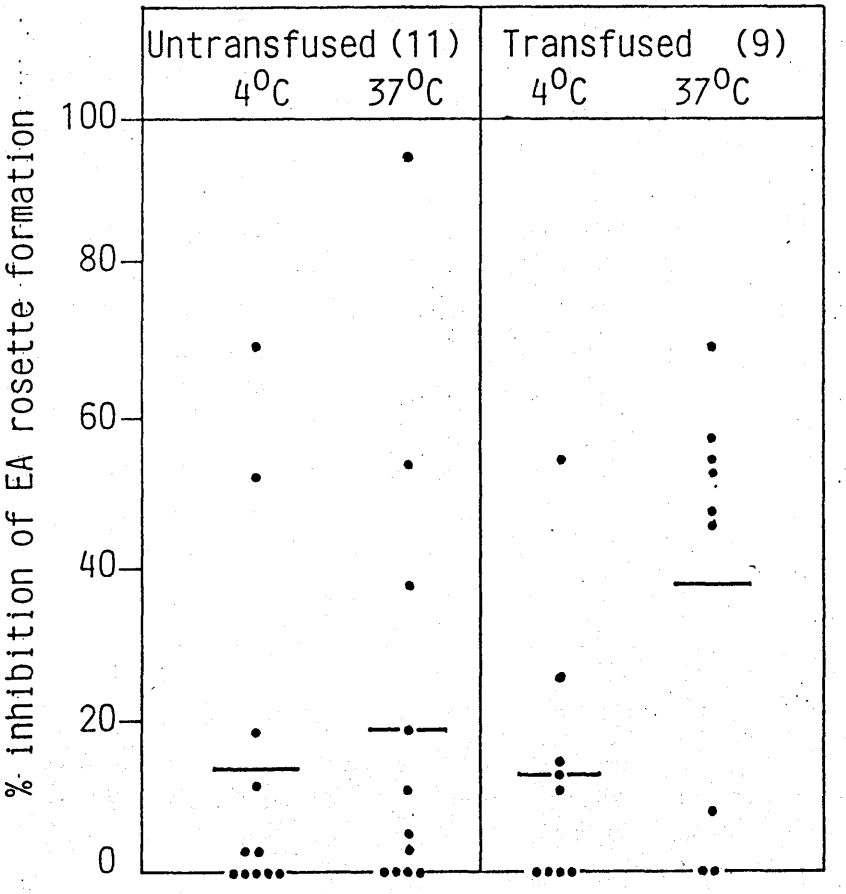


FIGURE 8: Inhibition of EA rosette formation of normal peripheral blood lymphocytes by IgG preparations from 20 untransfused and transfused multiparous women

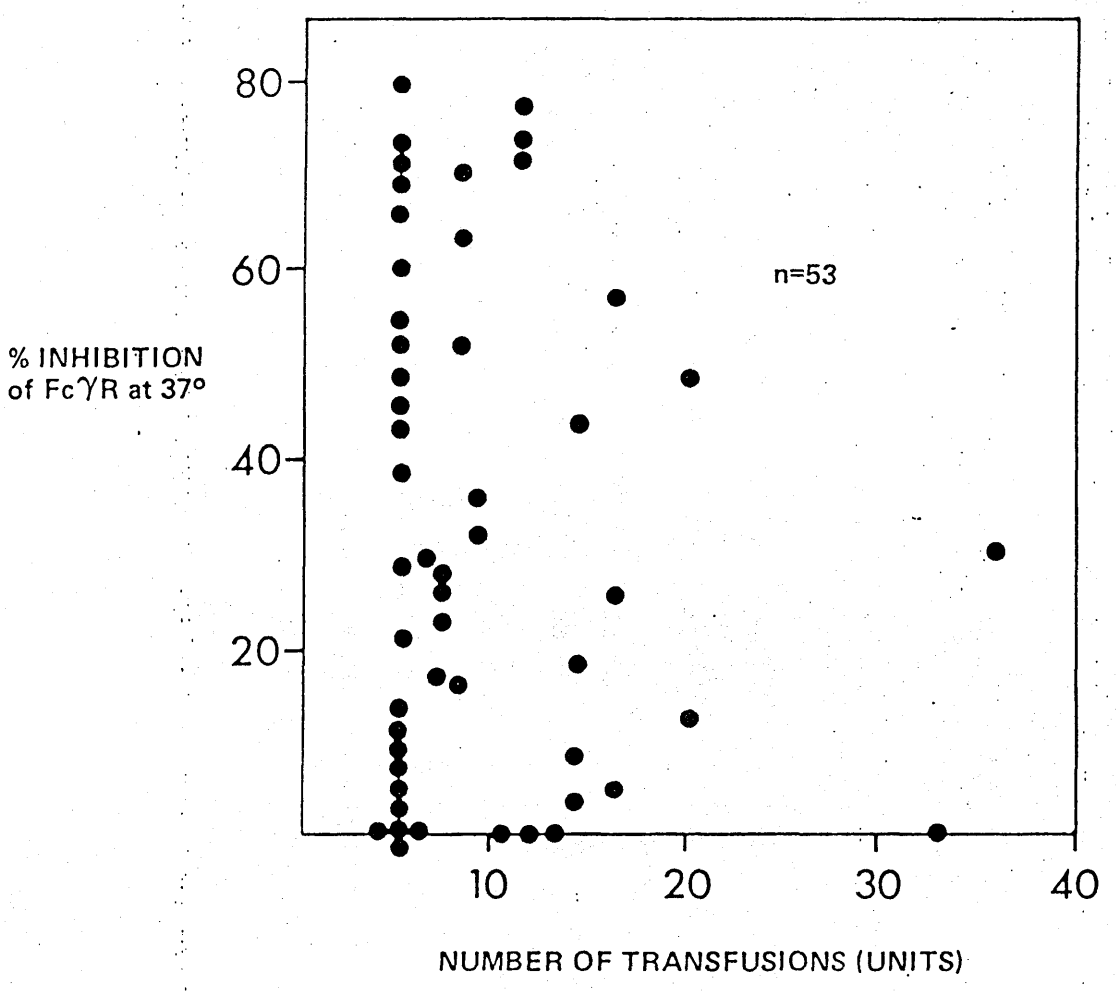


FIGURE 9 : Comparison between transfusion history and inhibition of EA rosette formation of normal peripheral blood lymphocytes by IgG preparations from 53 transfused dialysis patients.

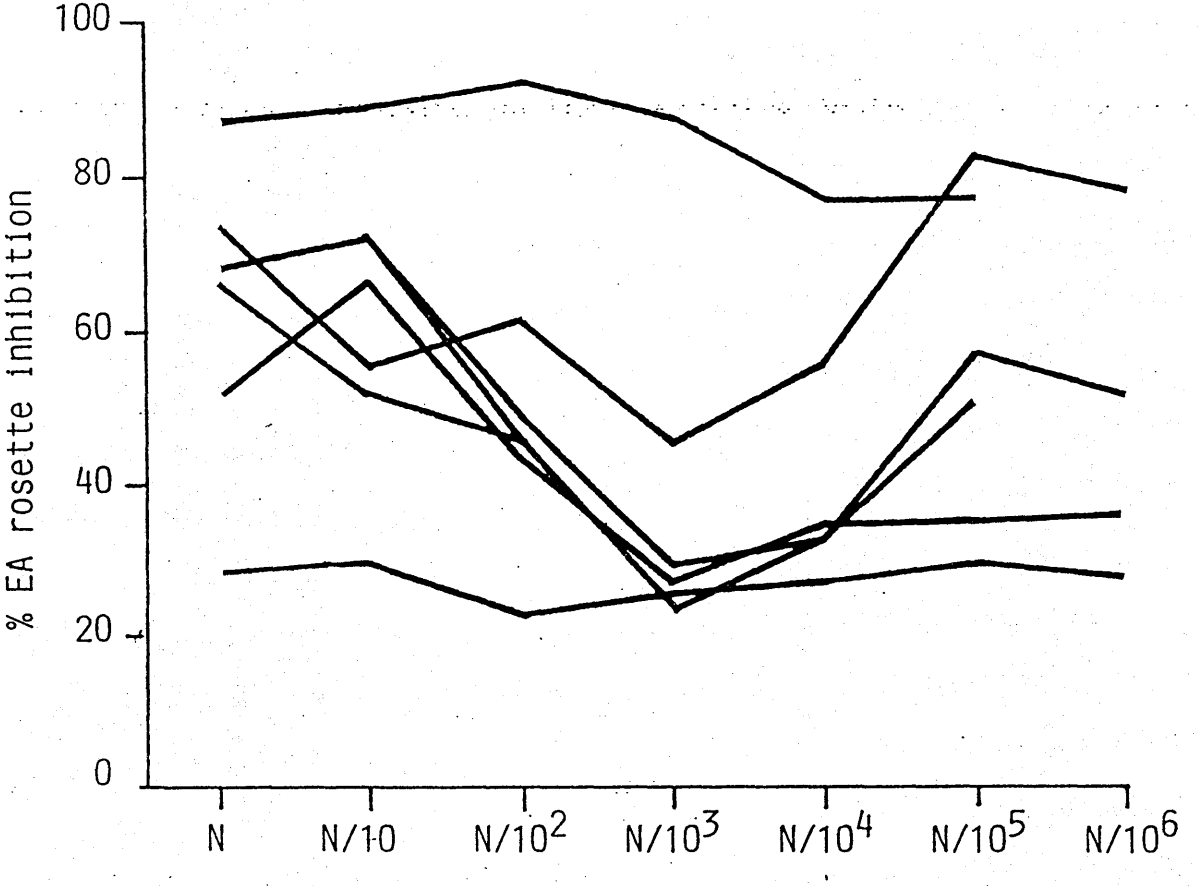


FIGURE 10 : Inhibition of EA rosette formation by IgG preparations at dilutions from N(1mg/ml) to $N/10^6$

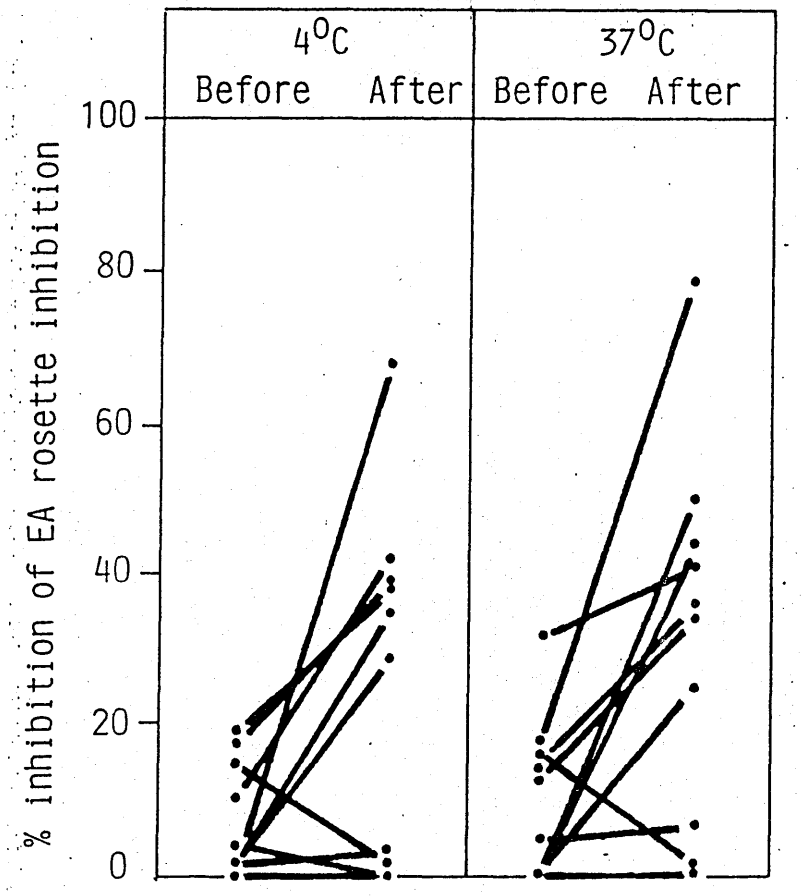


FIGURE 11: Inhibition of EA rosette formation of normal peripheral blood lymphocytes by IgG preparations from 10 subjects before and after elective third party transfusion

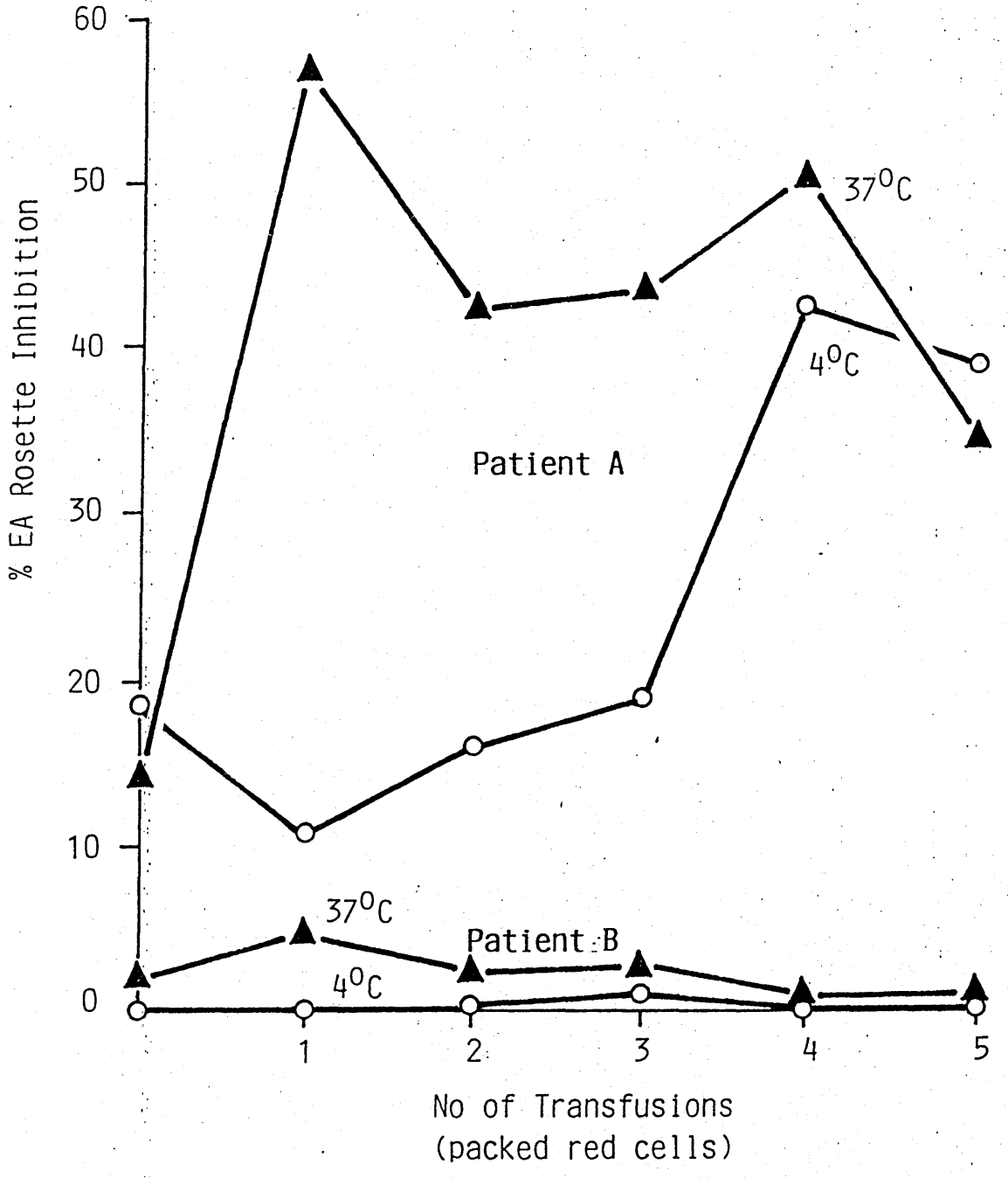


FIGURE 12 : Effect of elective third party transfusion on FcY-receptor blocking activity at 4°C and 37°C of IgG preparations from 2 patients

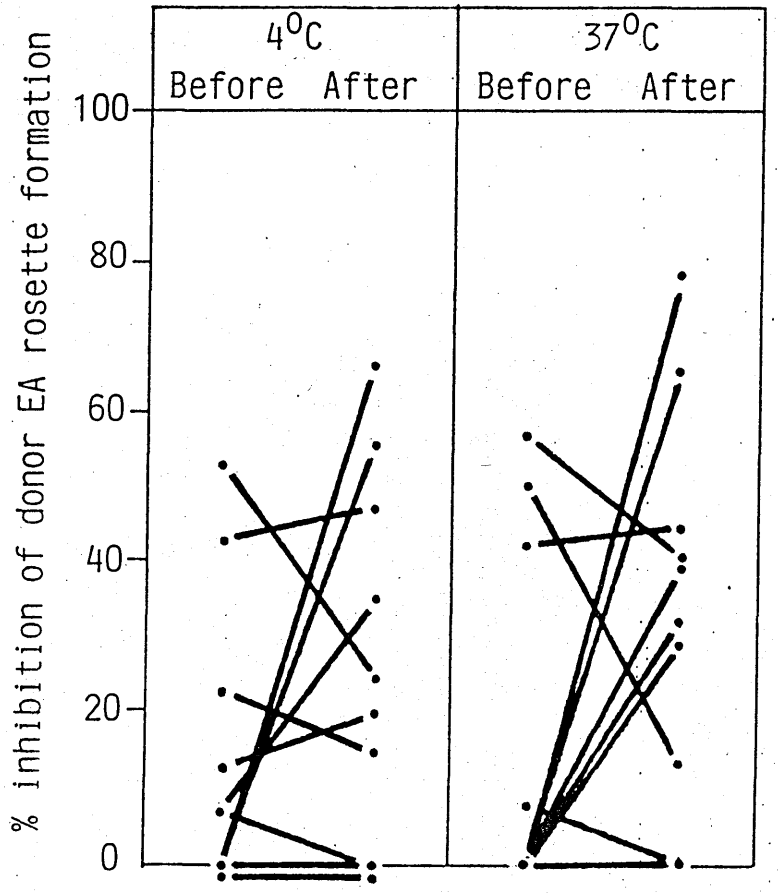


FIGURE 13 : Inhibition of EA rosette formation of donor peripheral blood lymphocytes by IgG preparations from 10 subjects before and after donor-specific transfusion

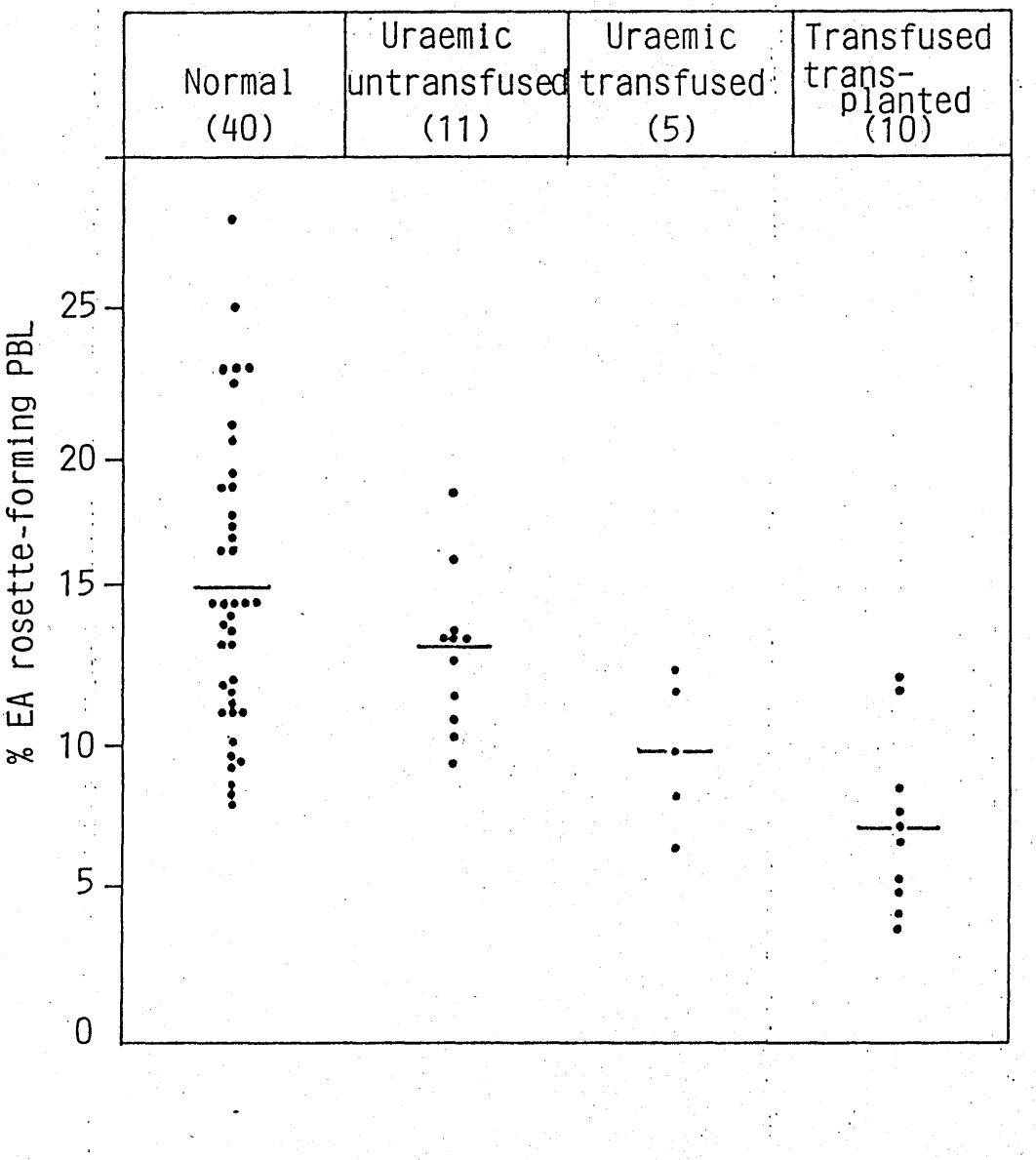


FIGURE 14 : Percentages of EA rosette-forming peripheral blood lymphocytes from 66 untransfused and transfused subjects

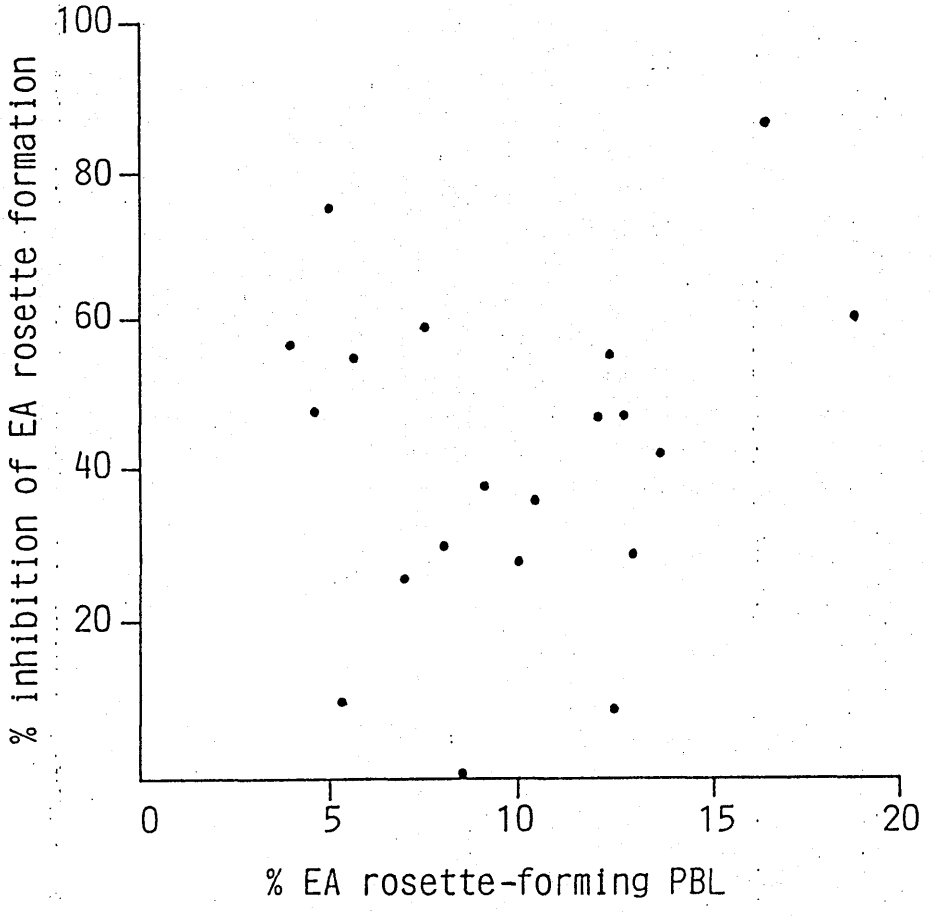


FIGURE 15 : Percentage of EA rosette-forming peripheral blood lymphocytes and percentage inhibition at 37°C of EA rosette formation of allogeneic peripheral blood lymphocytes by corresponding IgG preparations (n = 20)

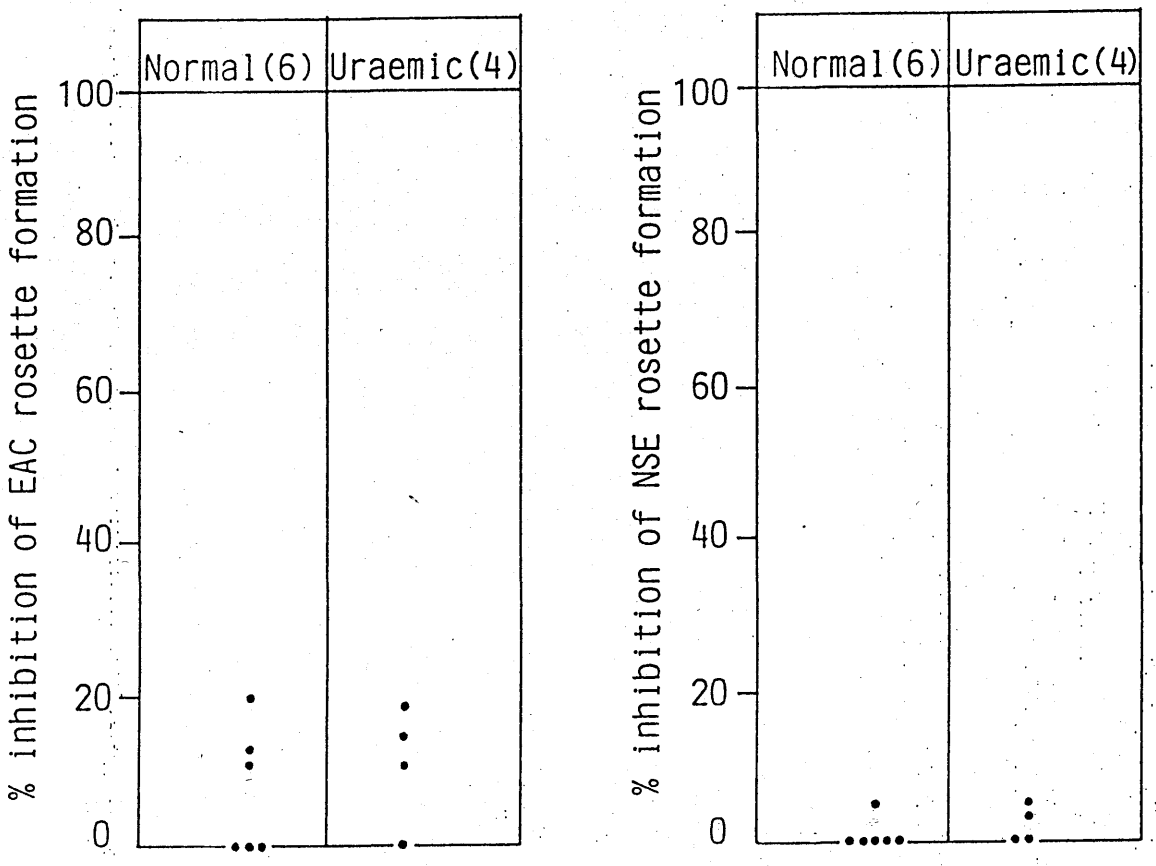


FIGURE 16 : Inhibition of EAC and NSE rosette formation of normal peripheral blood lymphocytes by IgG preparations from 10 untransfused subjects

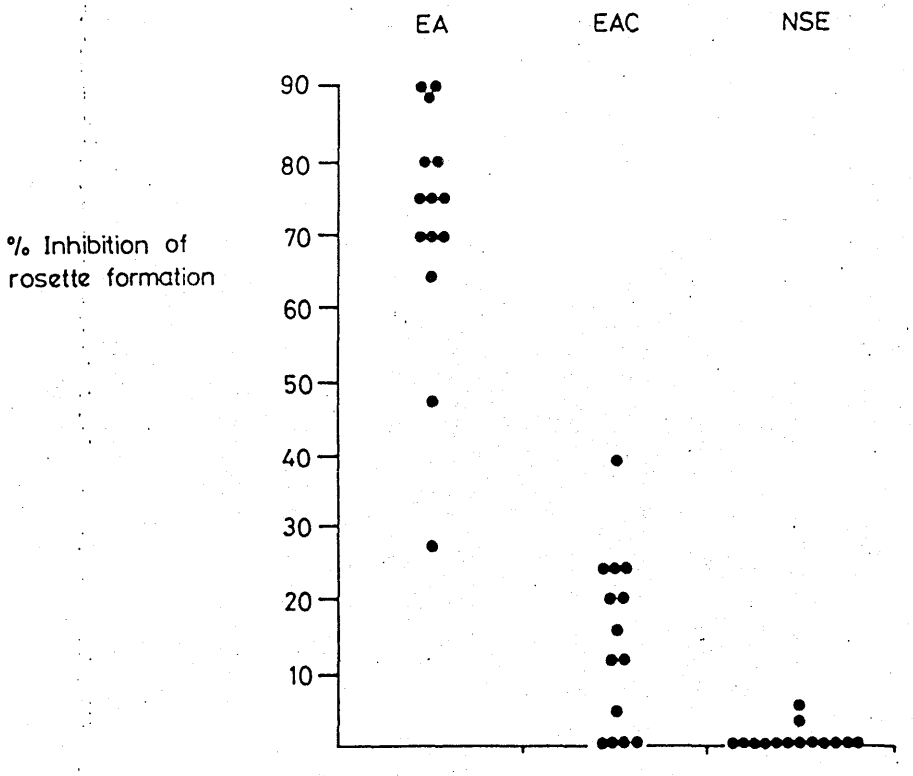


FIGURE 17: Inhibition by IgG preparations from 14 transfused dialysis patients of formation by normal peripheral blood lymphocytes of EA, EAC and NSE rosettes

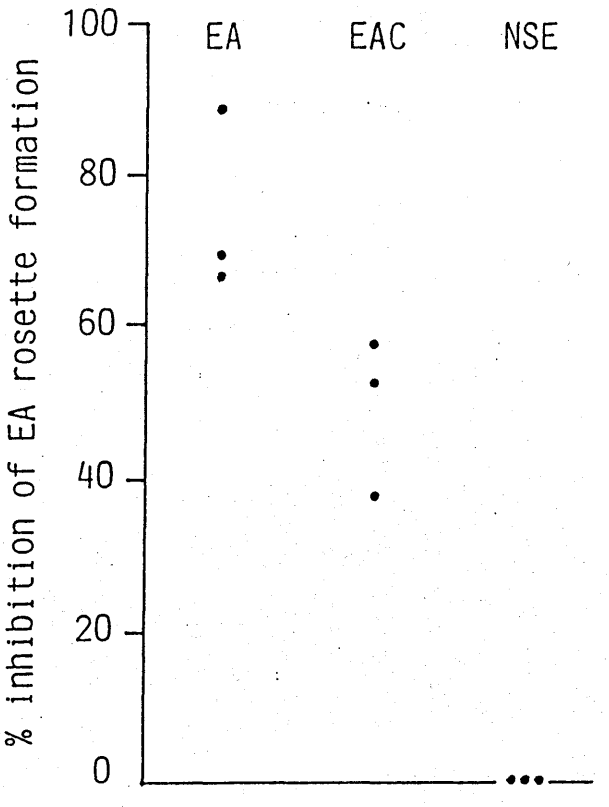


FIGURE 18 : Inhibition of EA, EAC and NSE rosette formation of paternal peripheral blood lymphocytes by IgG preparations from 3 pregnant women

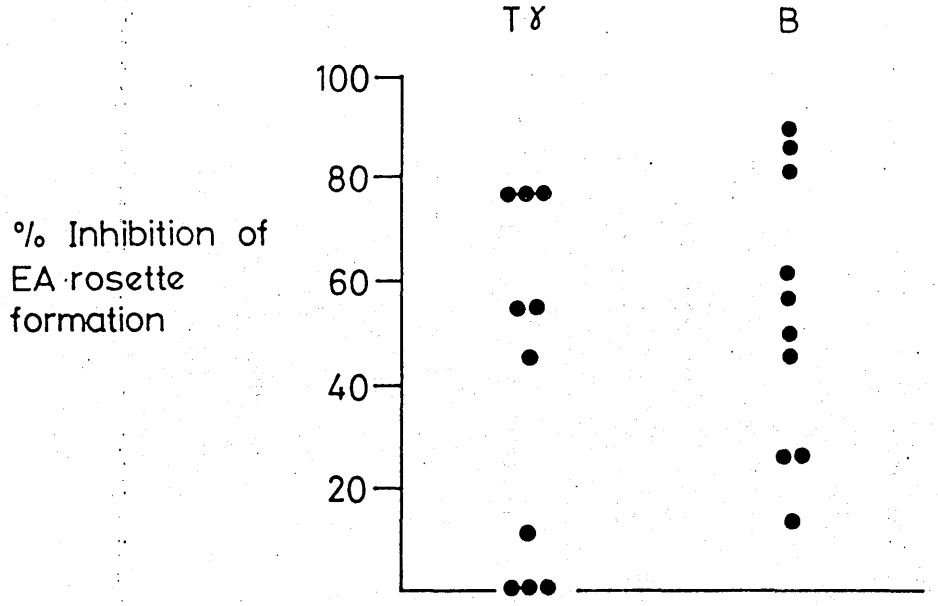


FIGURE 19 : Inhibition of EA rosette formation of Tγ and B lymphocytes from a normal donor by IgG preparations from 10 transfused dialysis patients

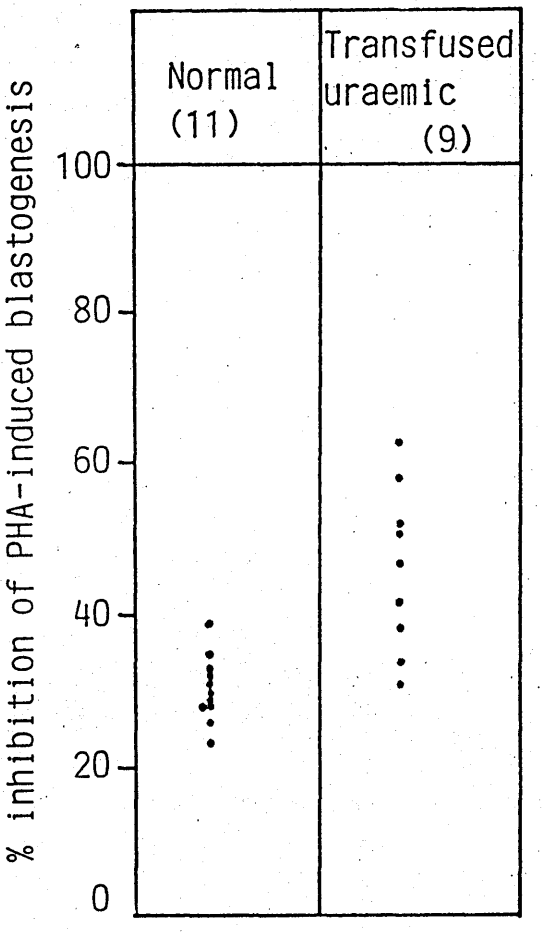


FIGURE 20: Inhibition of phytohaemagglutinin-induced blastogenesis of normal peripheral blood lymphocytes by IgG preparations from 20 untransfused and transfused subjects

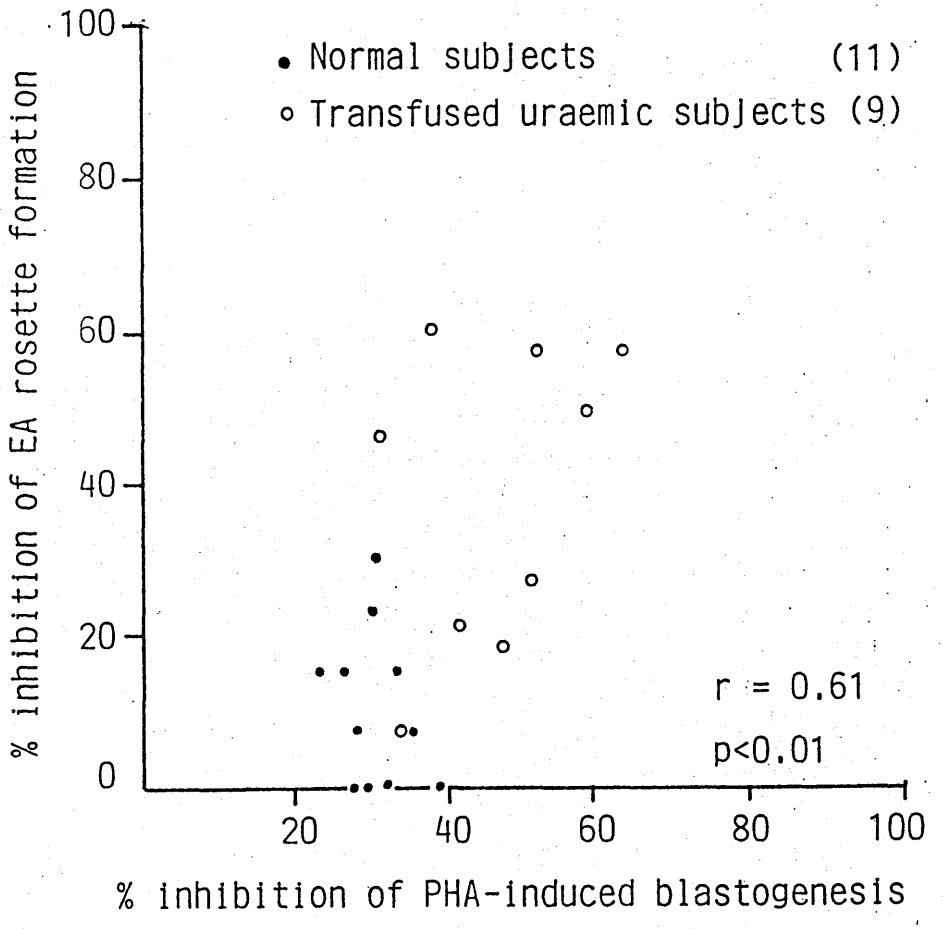


FIGURE 21: Relation between inhibitions of EA rosette formation and phytohaemagglutinin-induced blastogenesis of normal peripheral blood lymphocytes by IgG preparations from 20 subjects

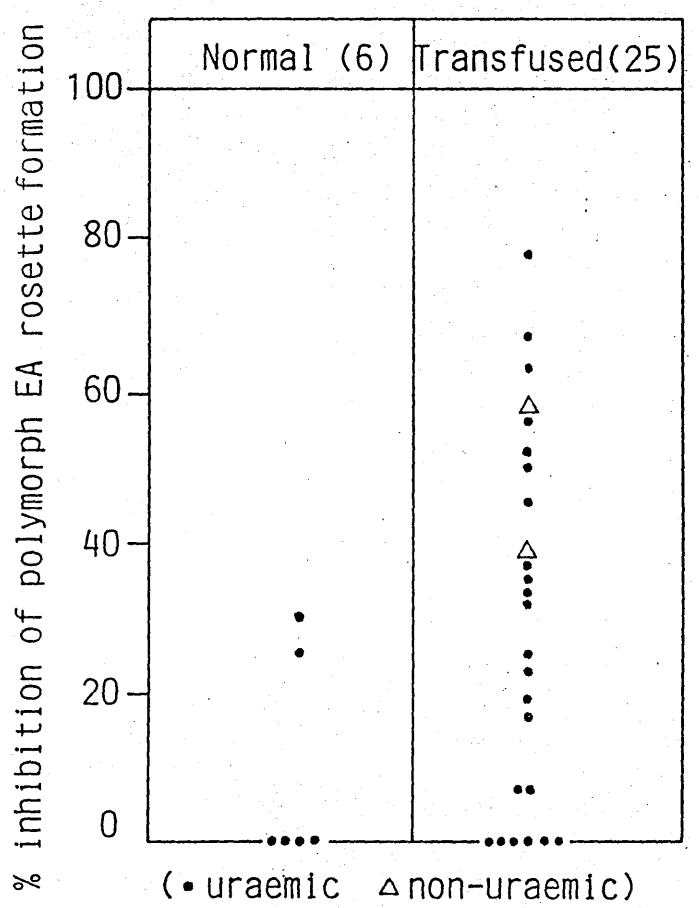


FIGURE 22: Inhibition of EA rosette formation of normal polymorphs by IgG preparations from 31 untransfused and transfused subjects

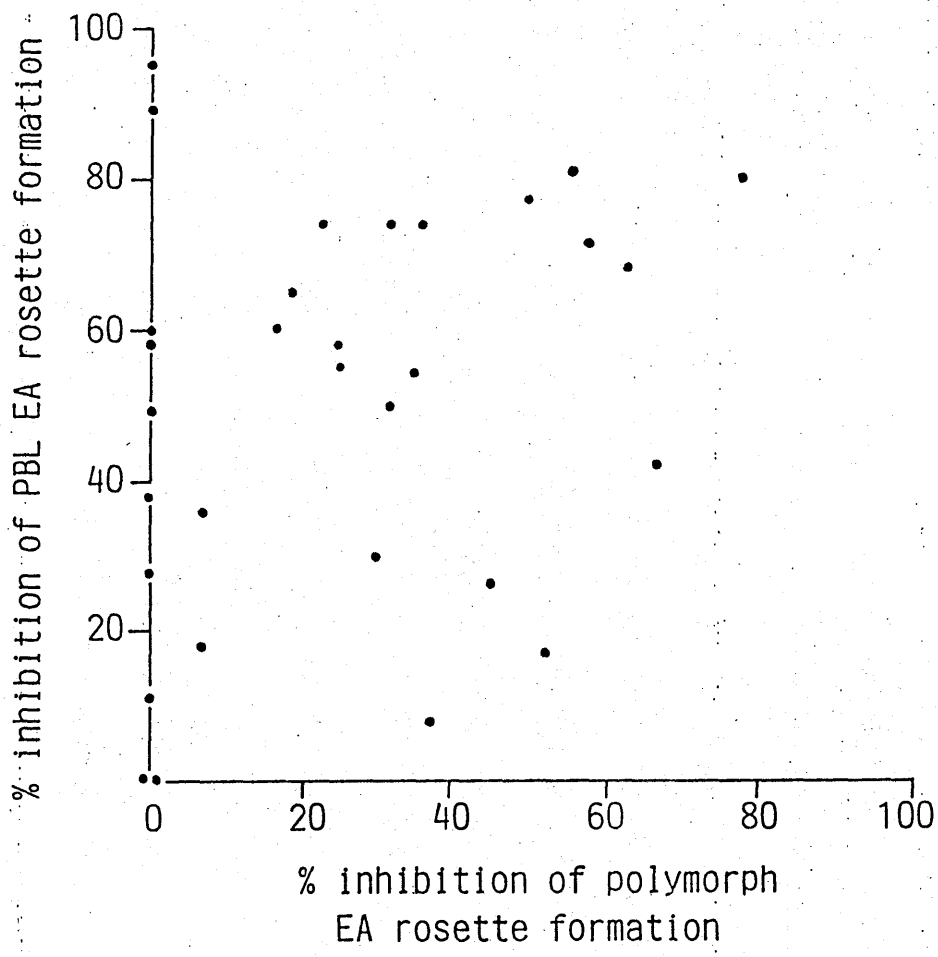


FIGURE 23 : Comparison between inhibition of polymorph and lymphocyte EA rosette formation by 31 IgG preparations at 37°C

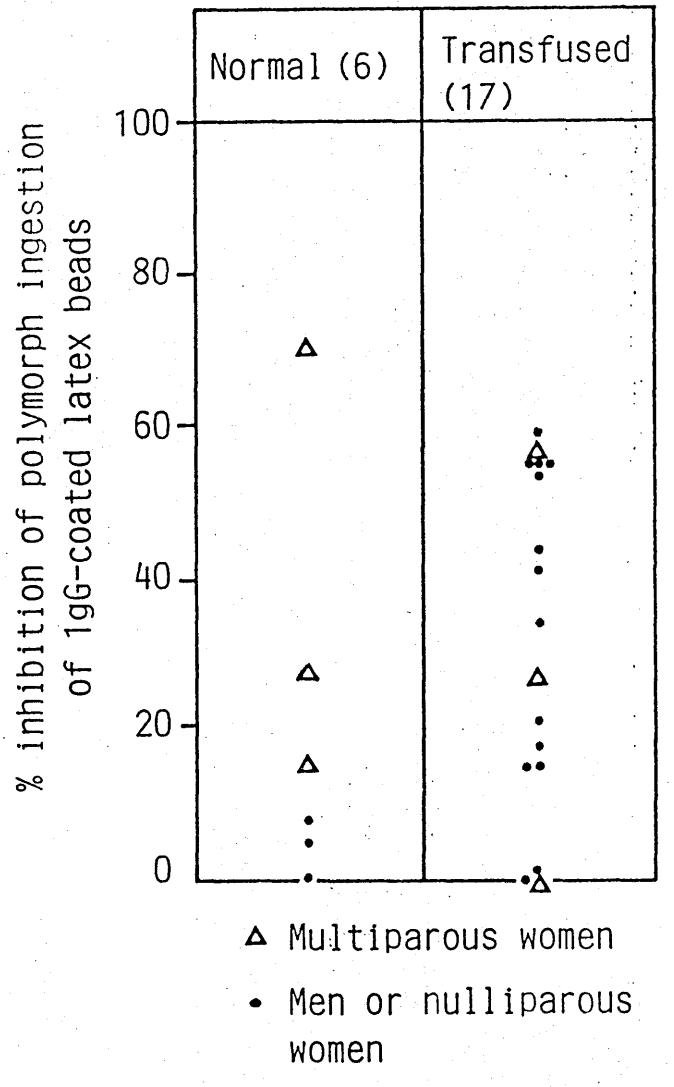


FIGURE 24: Inhibition by IgG preparations from 23 untransfused and transfused subjects of phagocytosis by normal polymorphs of IgG-coated latex beads

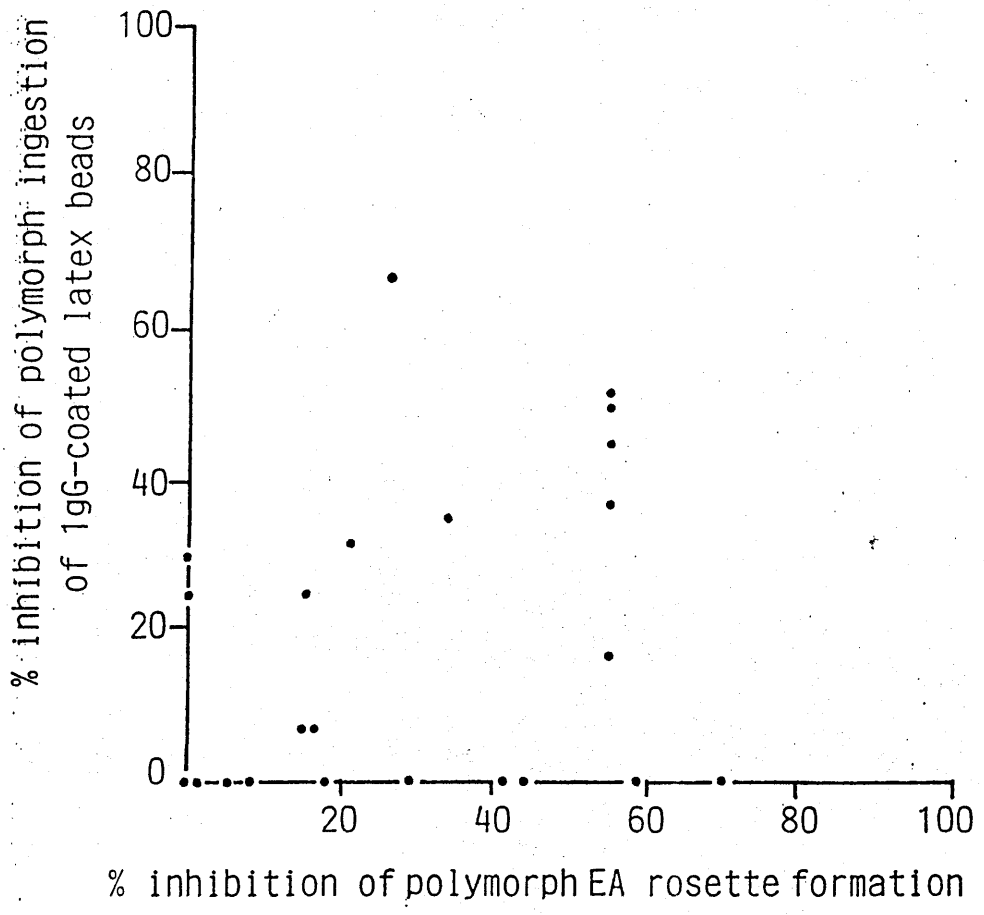


FIGURE 25: Relation between inhibitions of normal polymorph EA rosette formation and ingestion of IgG-coated latex beads by IgG preparations from 23 subjects

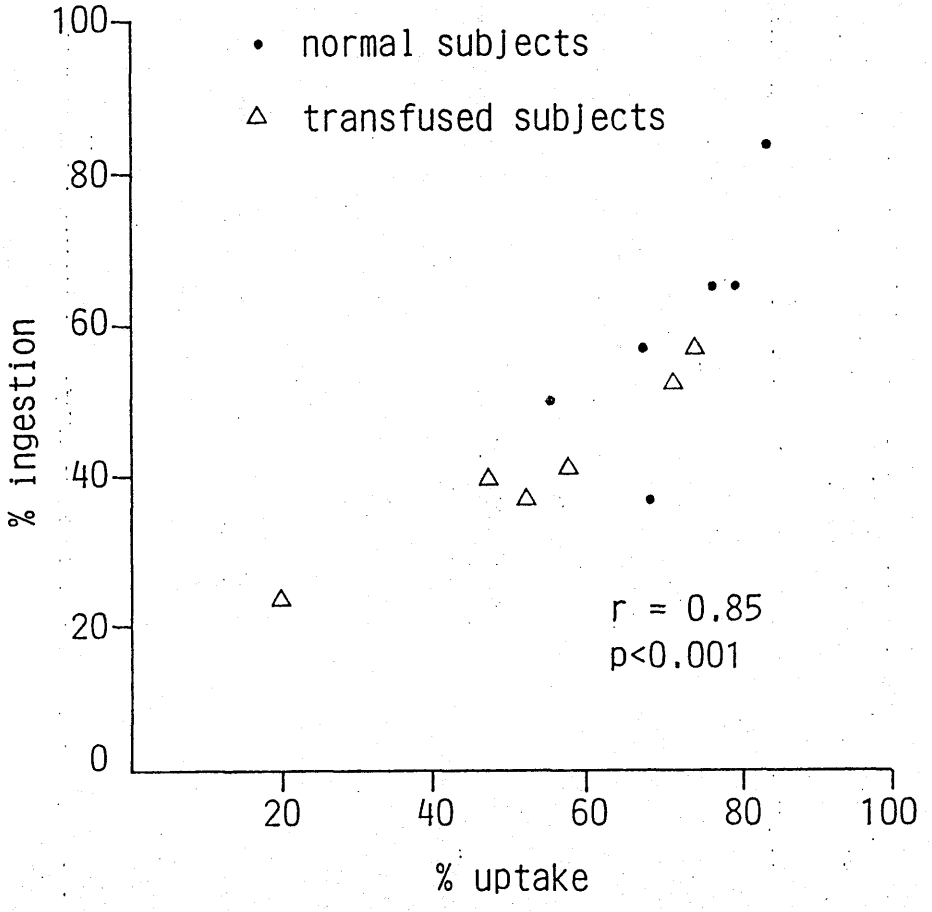


FIGURE 26 : Effect of IgG preparations from 12 untransfused and transfused subjects on uptake and ingestion by normal polymorphs of Staphylococcus aureus

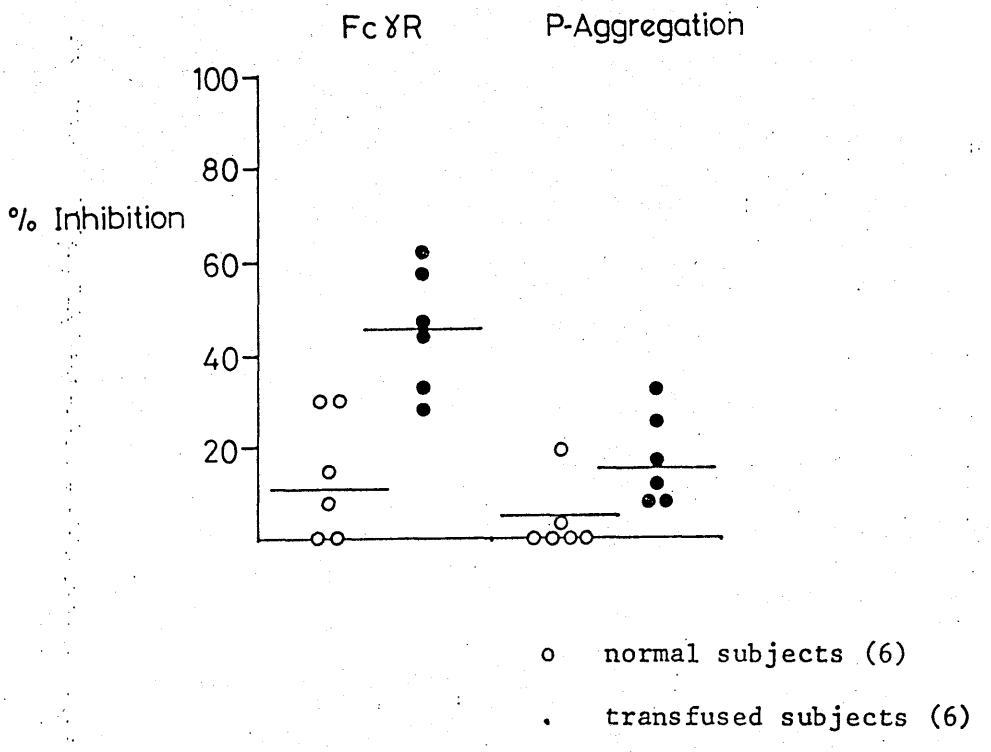


FIGURE 27 : Effect of IgG preparations from normal and transfused subjects on:

i) EA rosette formation by normal peripheral blood lymphocytes (Fc γR)

ii) Collagen-induced aggregation of normal platelets (P-aggregation)

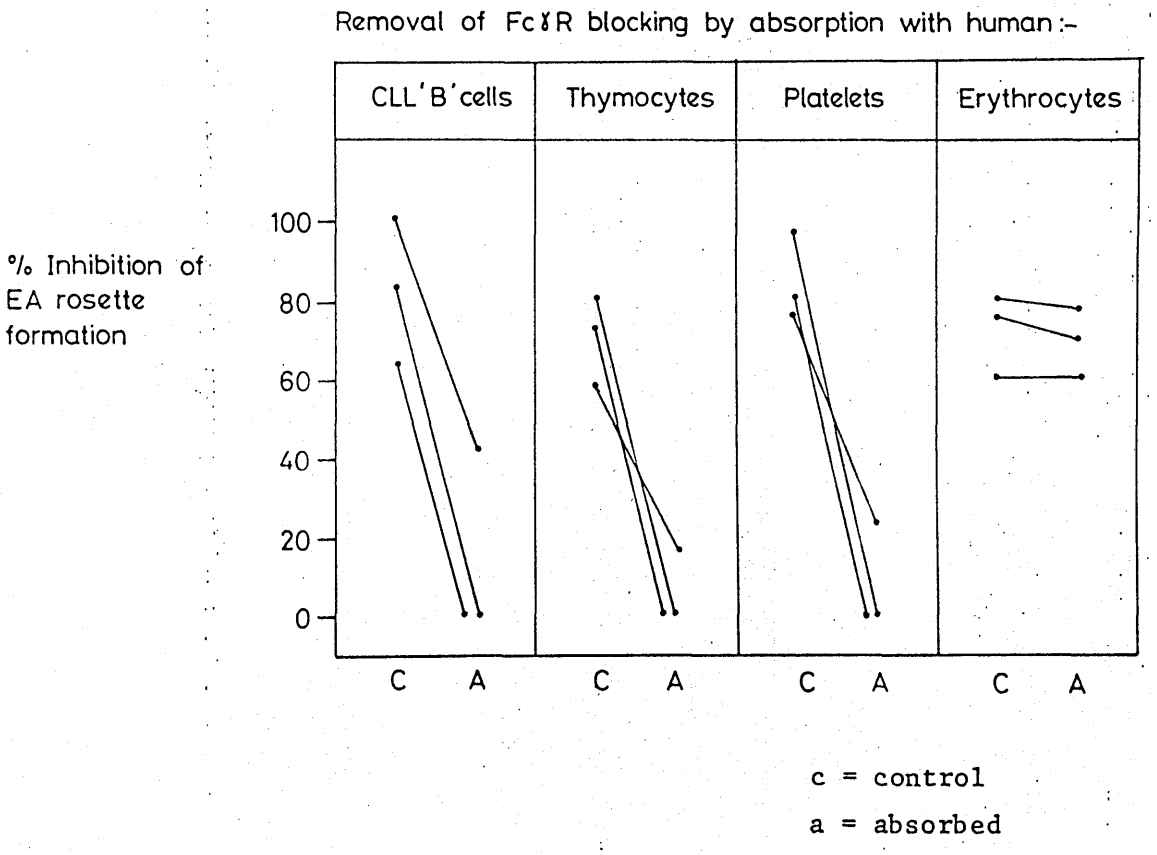


Figure 28 : Removal by absorption of Fcγ-receptor blocking activity from IgG preparations from 3 transfused subjects

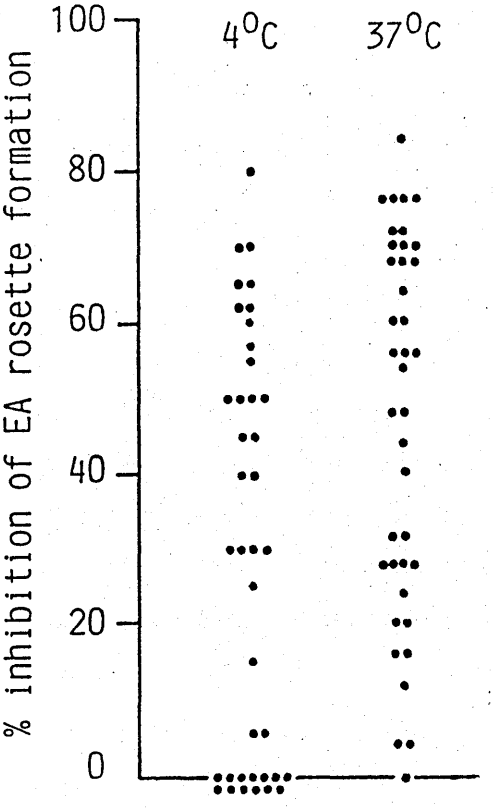


FIGURE 29: Inhibition of EA rosette formation of normal peripheral blood lymphocytes by IgG preparations from 39 haemophiliacs

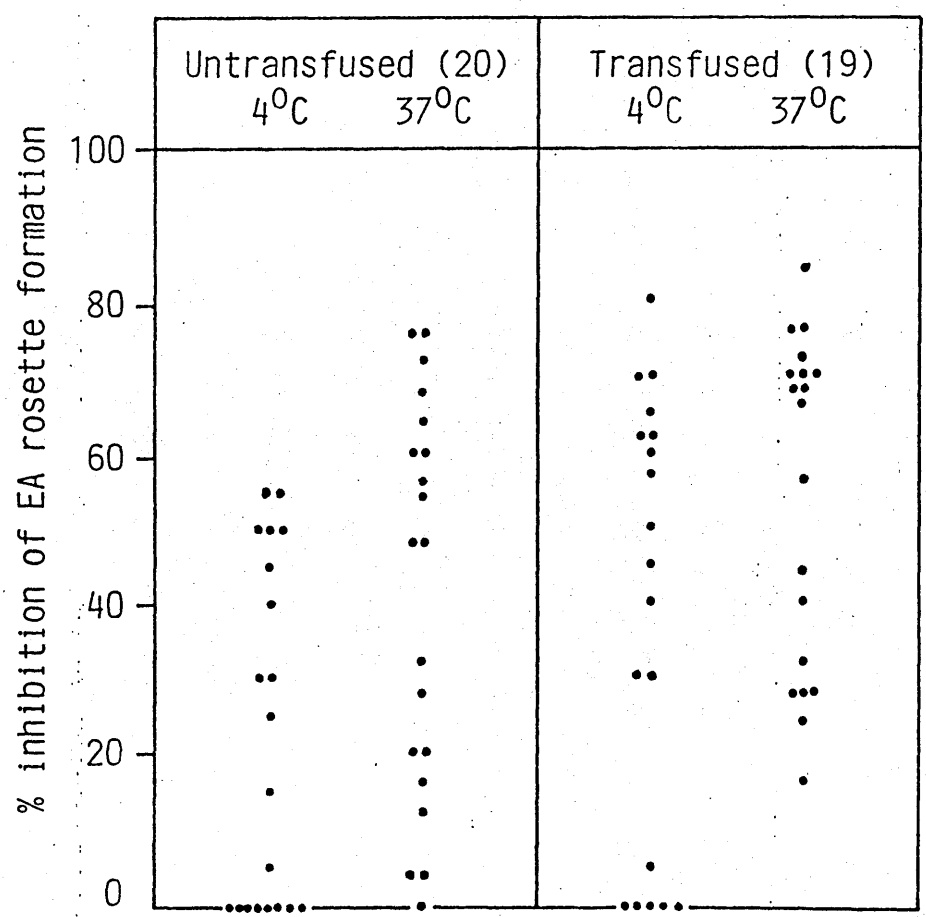


FIGURE 30: Inhibition of EA rosette formation of normal peripheral blood lymphocytes by IgG from 39 haemophiliacs according to blood transfusion history

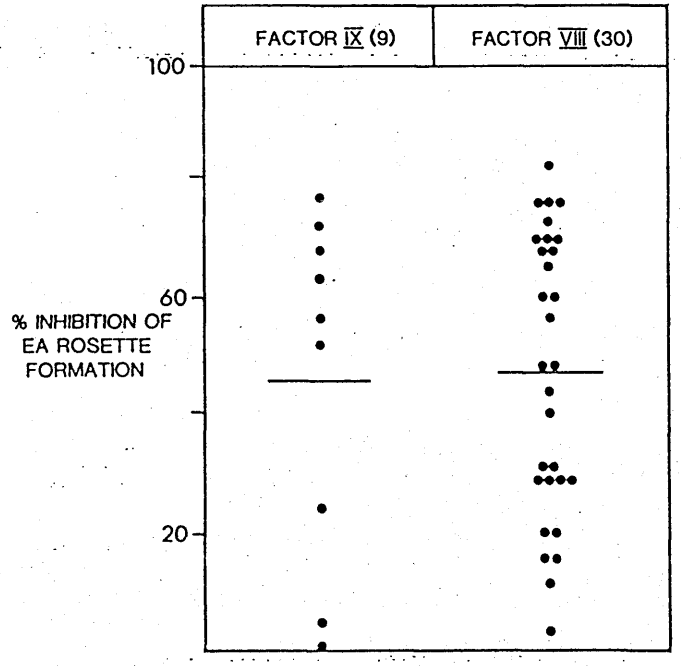


FIGURE 31 : Comparison of inhibition of EA rosette formation of normal peripheral blood lymphocytes by IgG preparations from 39 patients with Factor VIII and Factor 1X deficiency

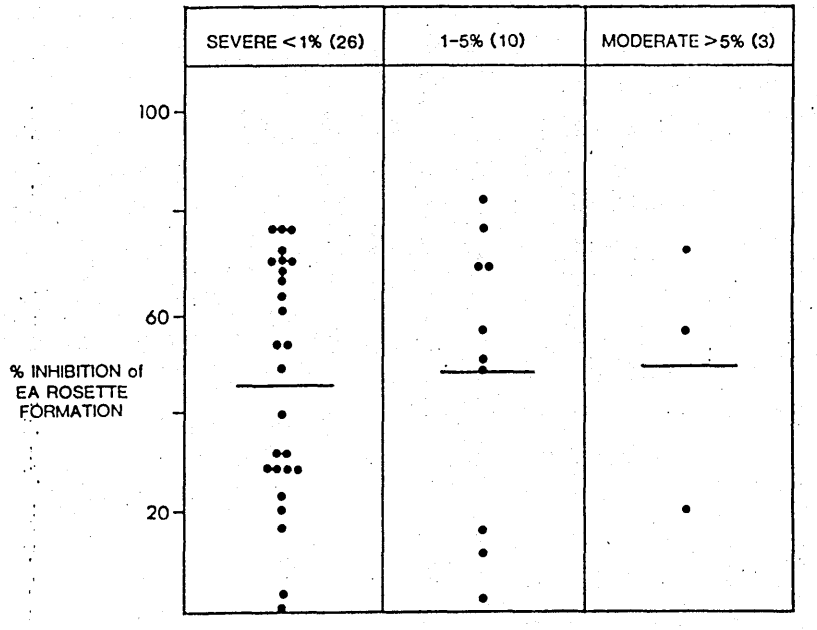


FIGURE 32 : Comparison between inhibition of EA rosette formation of normal peripheral blood lymphocytes by IgG preparations from 39 haemophiliacs and severity of disease (expressed as percentage of normal clotting factor activity)

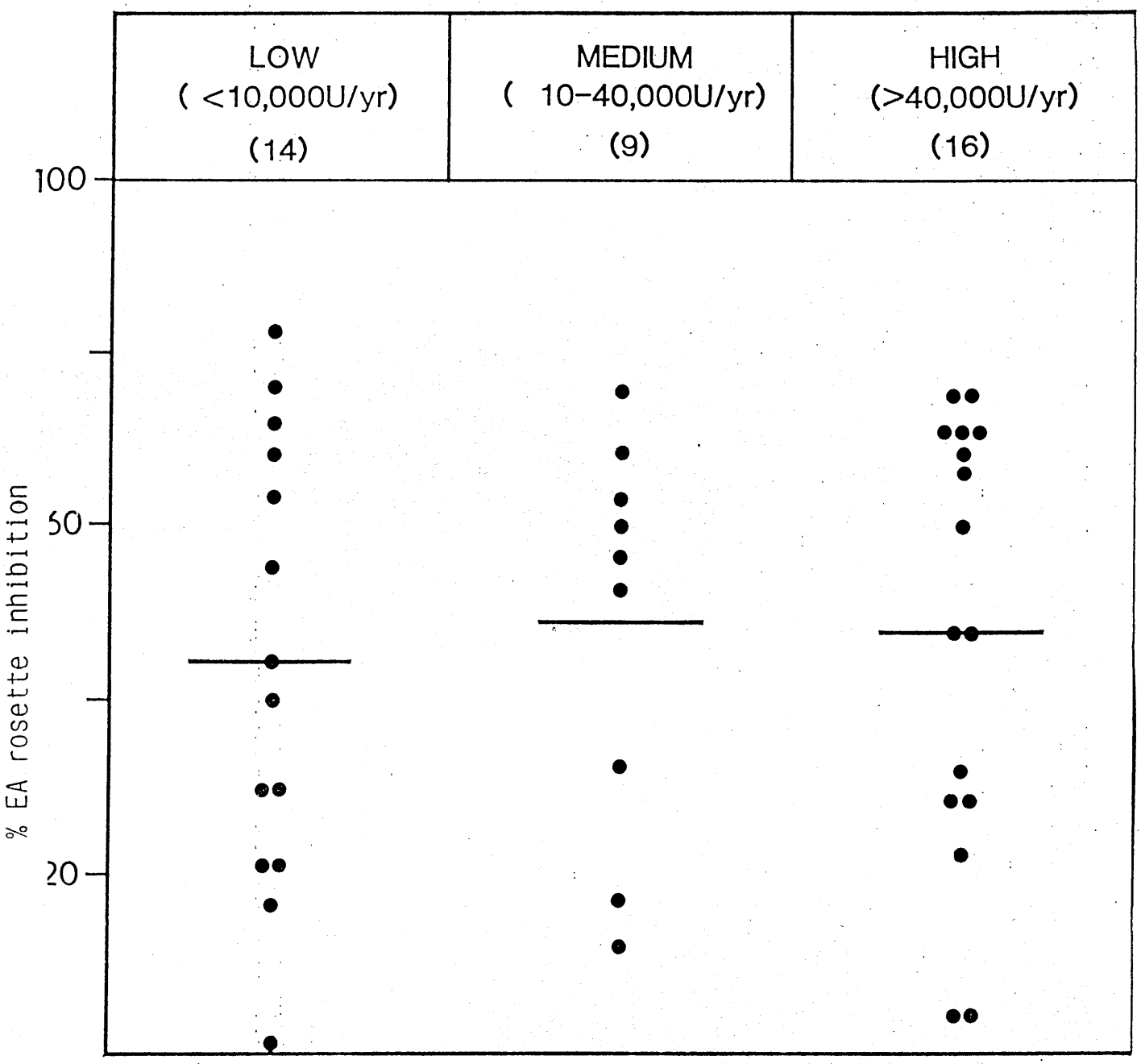


FIGURE 34: Relation between consumption of clotting factor concentrates and inhibition of EA rosette formation of normal peripheral blood lymphocytes by IgG preparations from 39 haemophiliacs

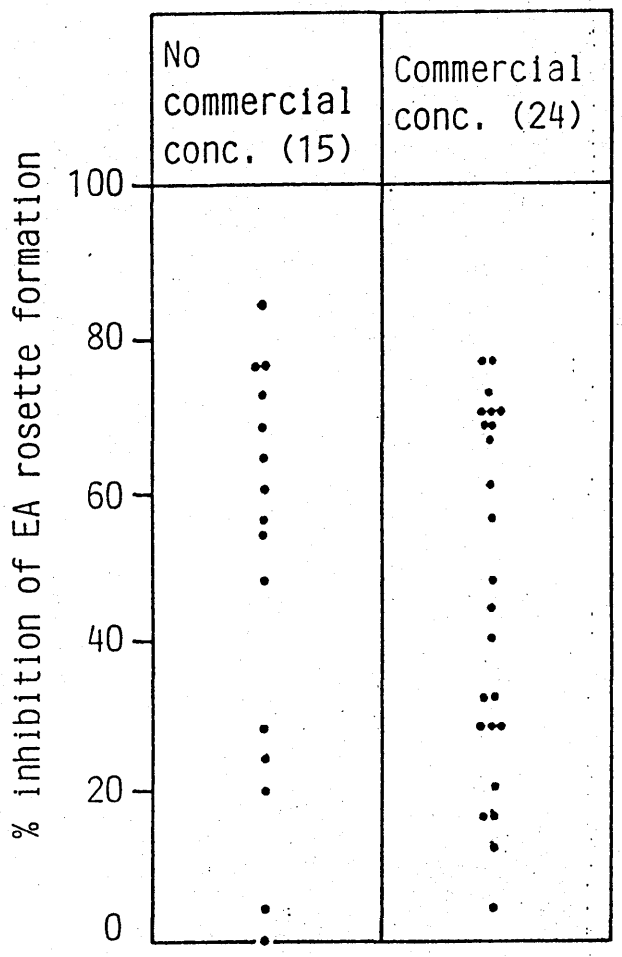


FIGURE 35: Relation between use of commercial clotting factor concentrates and inhibition at 37°C of EA rosette formation of normal peripheral blood lymphocytes by IgG preparations from 39 haemophiliacs

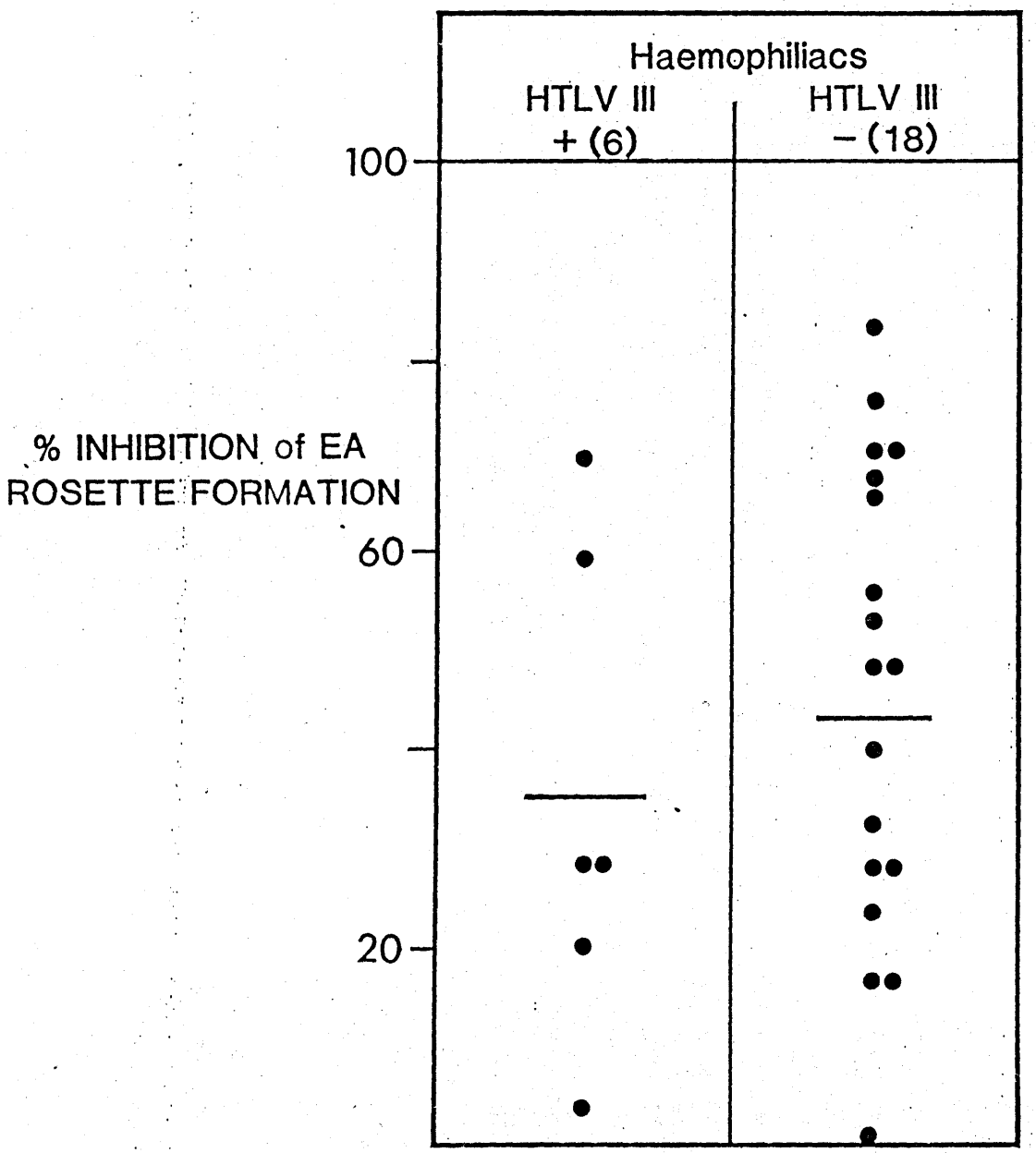


FIGURE 36 : Inhibition of EA rosette formation of normal peripheral blood lymphocytes by IgG preparations from haemophiliacs with (+) or without (-) antibody to human T-cell leukaemia virus Type III

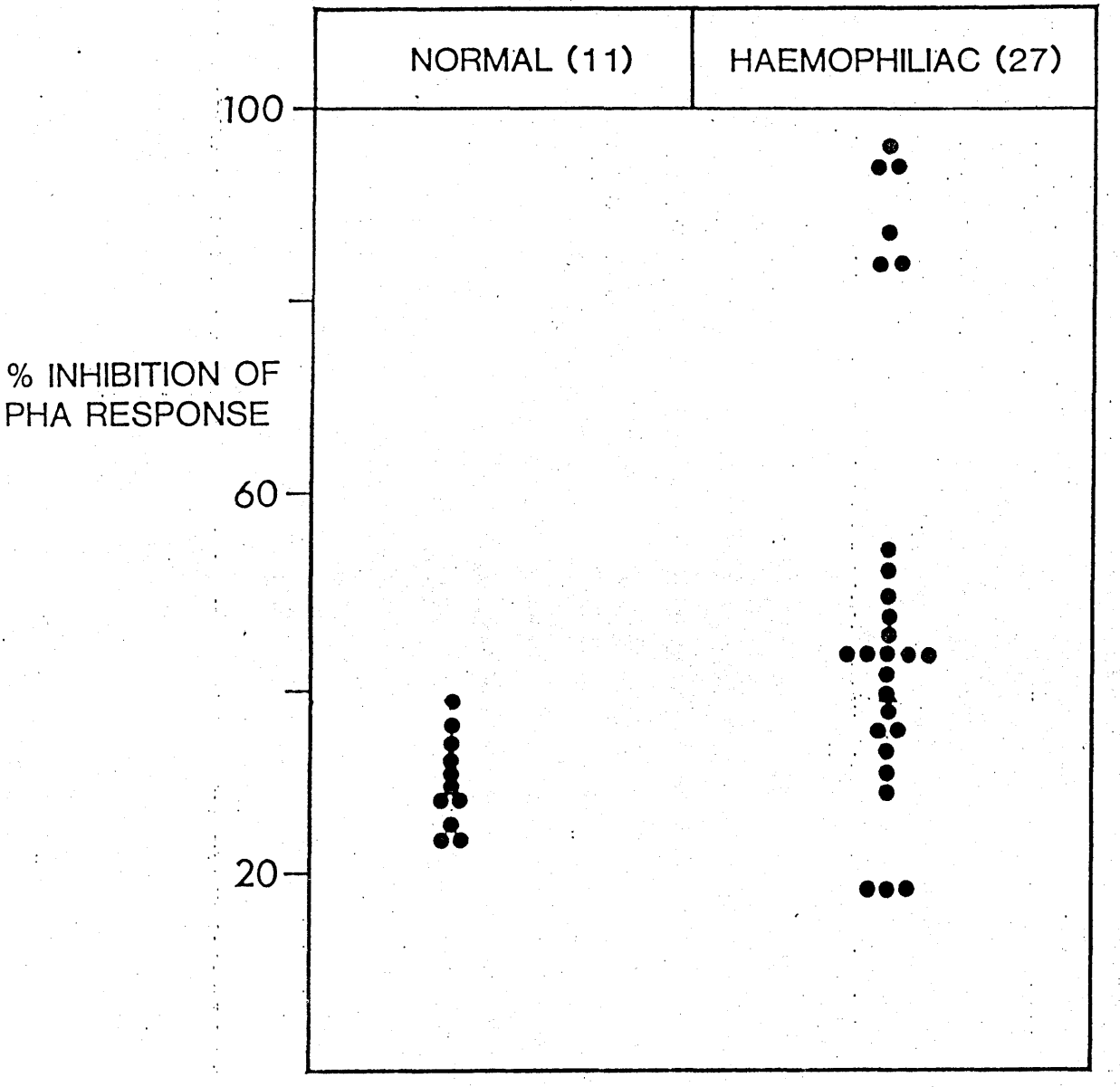


FIGURE 37 : Inhibition of phytohaemagglutinin-induced blastogenesis of normal peripheral blood lymphocytes by IgG preparations from normal subjects and patients with haemophilia.

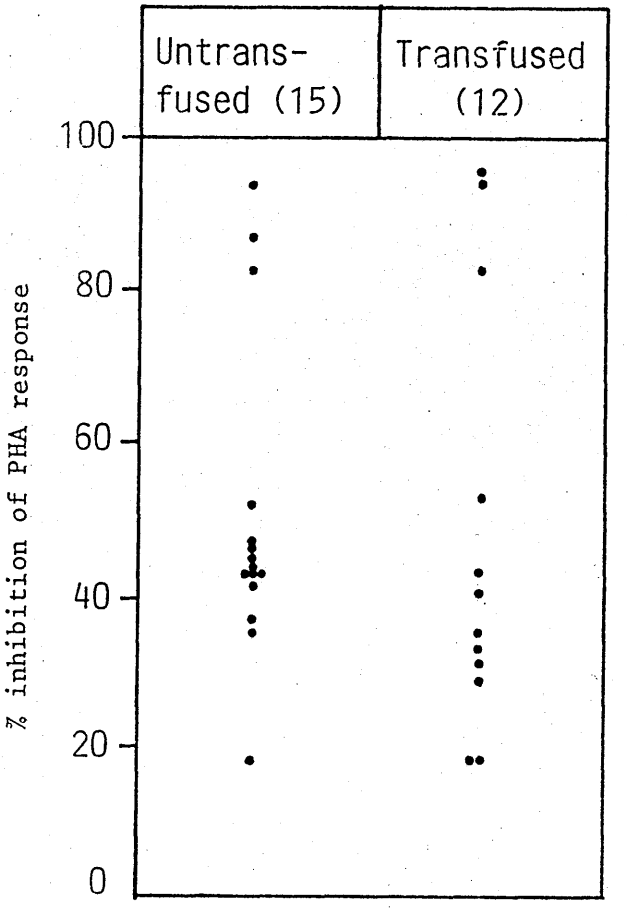


FIGURE 38: Comparison between blood transfusion history and inhibition of the phytohaemagglutinin response of normal peripheral blood lymphocytes by IgG preparations from 27 haemophiliacs

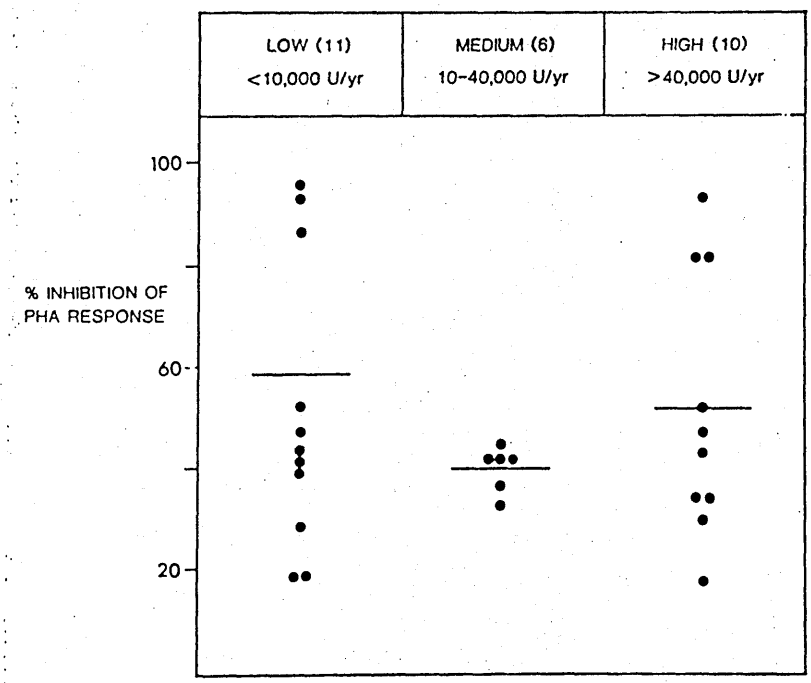


FIGURE 39 : Comparison between use of clotting factor concentrates and inhibition of the phytohaemagglutinin response of normal peripheral blood lymphocytes by IgG preparations from 27 haemophiliacs

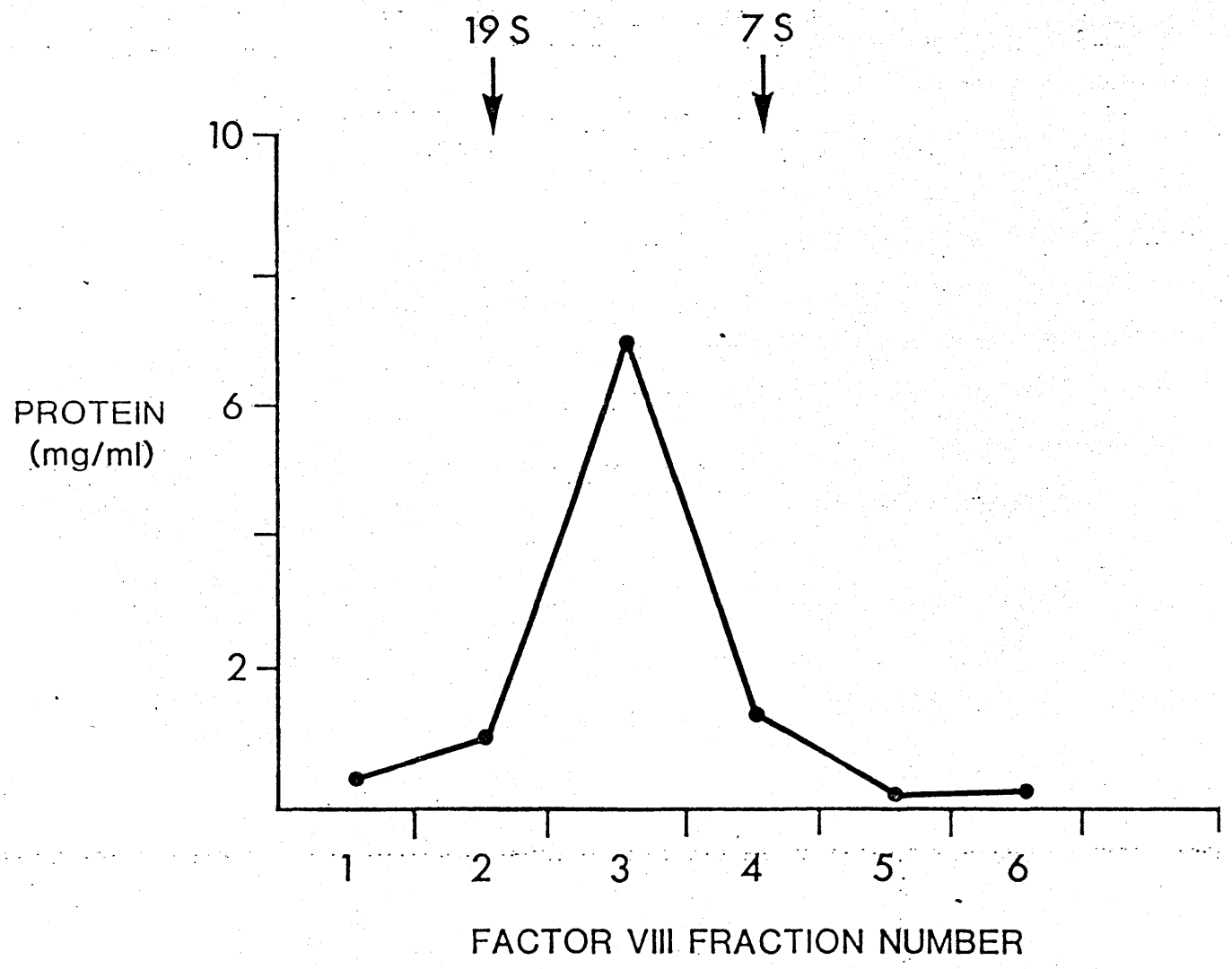


FIGURE 40 : Protein concentration of sucrose gradient fractions of Hemofil

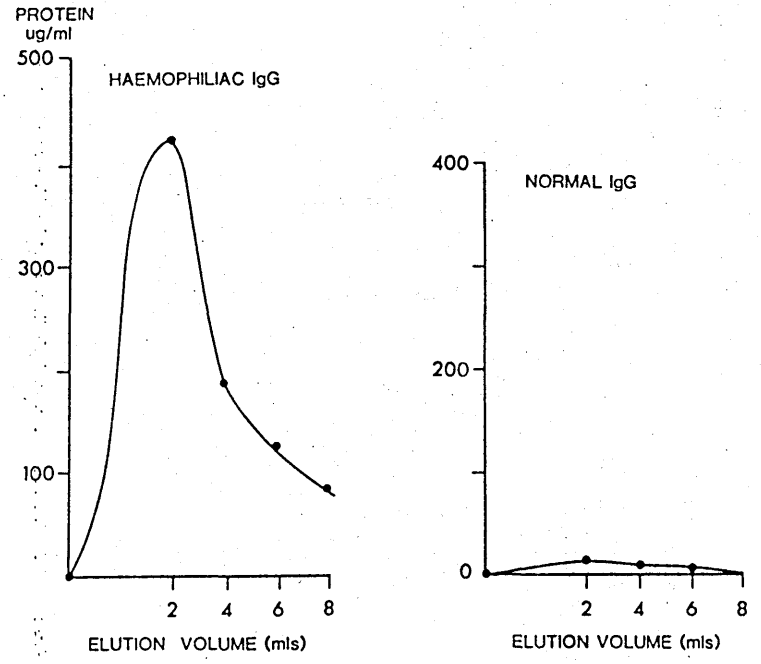


FIGURE 41 : Elution of Hemofil-derived "antigen" from Sepharose columns

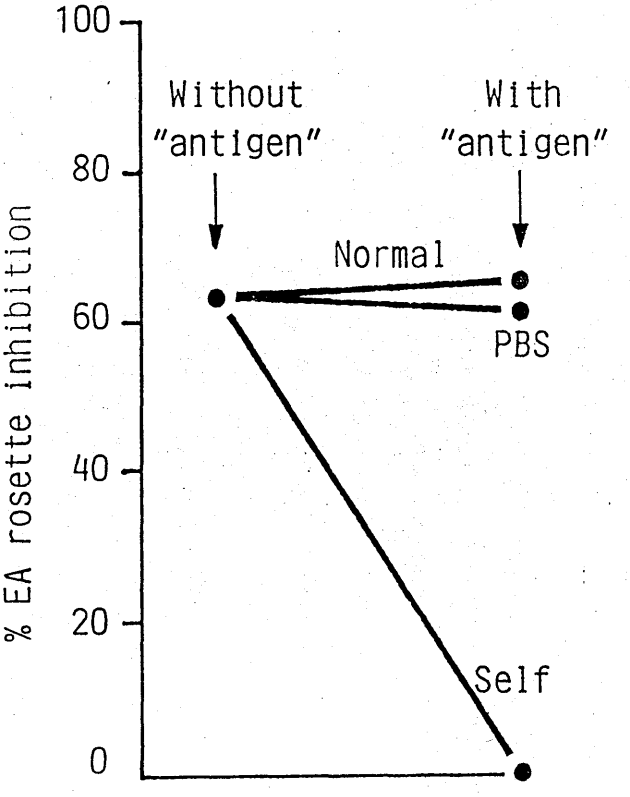


FIGURE 42 : Removal of Fcγ-receptor blocking activity from IgG from a haemophiliac by incubation with autologous Hemofil-derived "antigen"

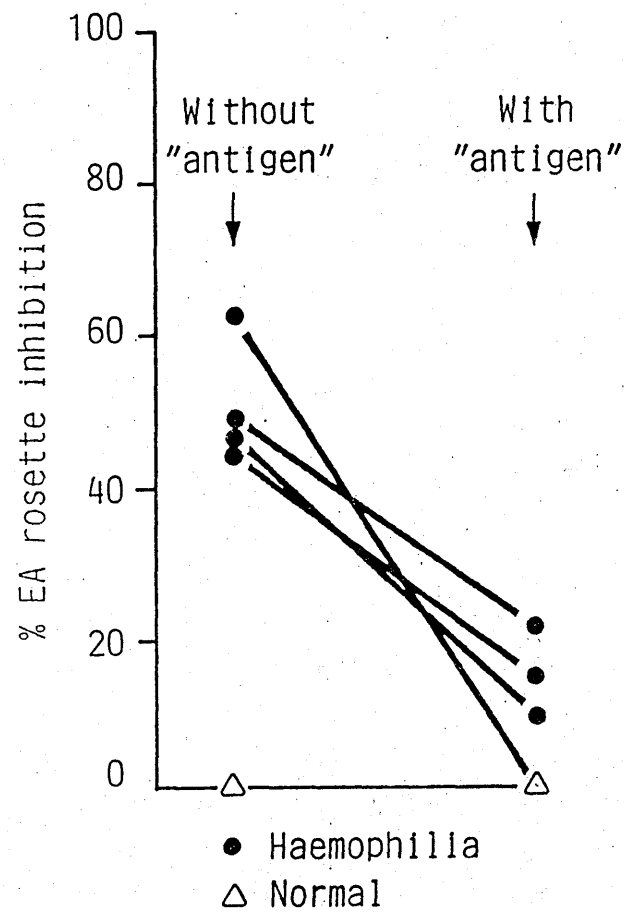


FIGURE 43 : Removal of Fcγ-receptor blocking activity from IgG from 4 haemophiliacs by incubation with autologous Hemofil-derived "antigen"

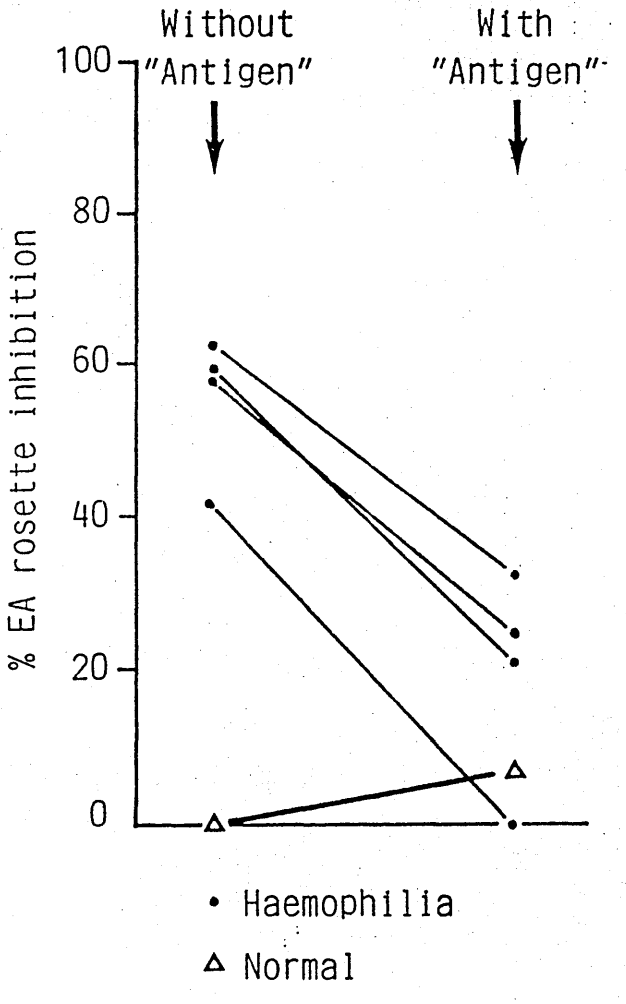


FIGURE 44: Removal of Fcγ-receptor blocking activity from IgG from 4 haemophiliacs by incubation with Hemofil-derived "antigen" from 1 subject

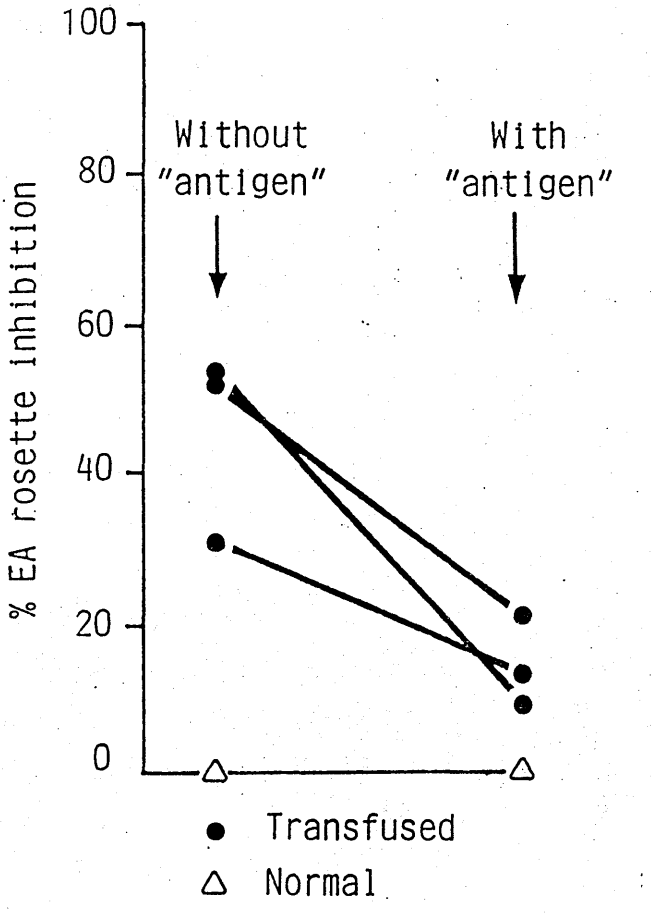


FIGURE 45 : Removal of Fcγ-receptor blocking activity from IgG from transfused dialysis patients by incubation with Hemofil-derived "antigen"

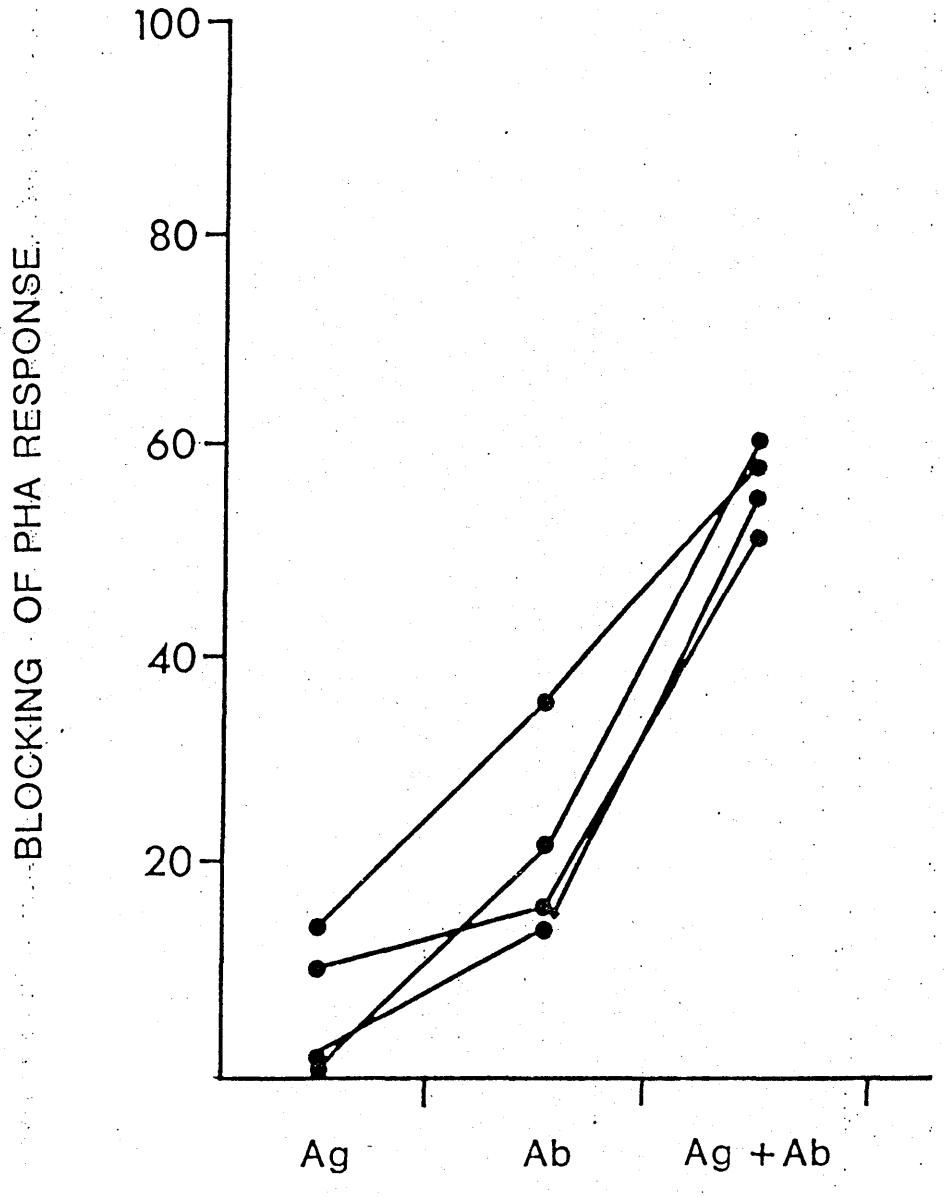


FIGURE 46: Inhibition of phytohaemagglutinin-induced blastogenesis of normal peripheral blood lymphocytes by:

i) "antigen" derived from Hemofil (Ag)

ii) IgG preparations from 4 haemophiliacs (Ab)

iii) "antigen" and IgG incubated together (Ag + Ab)

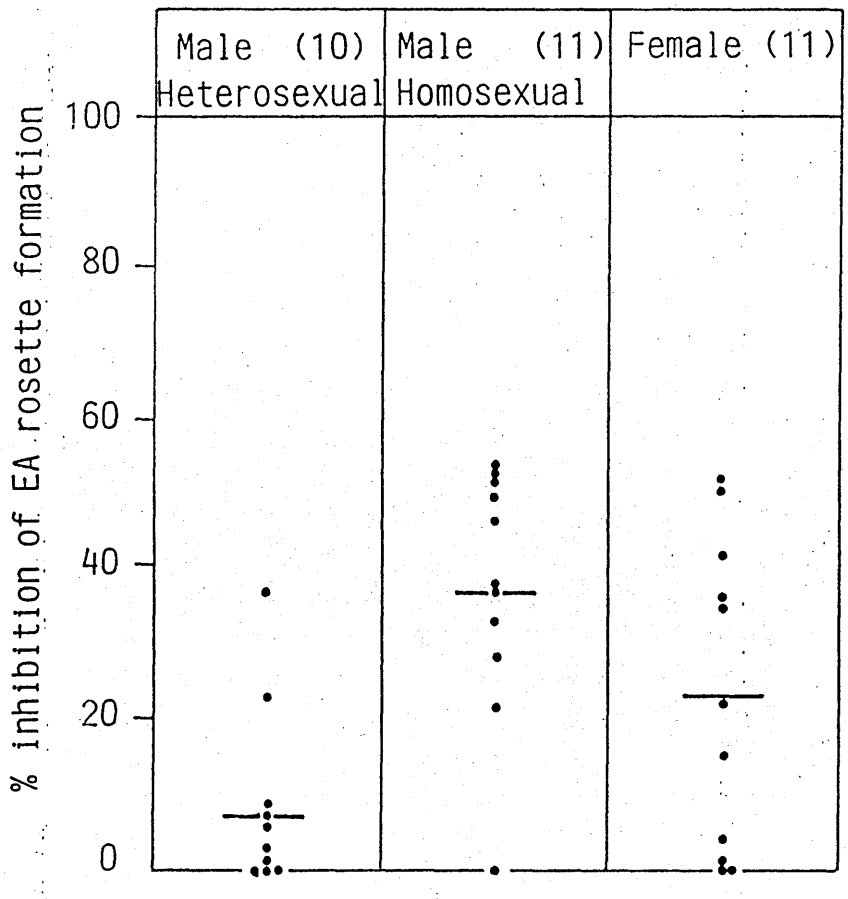


FIGURE 47 : Inhibition of EA rosette formation (37°C) of peripheral blood lymphocytes from a normal subject by serum from patients attending a Gerintourinary Clinic

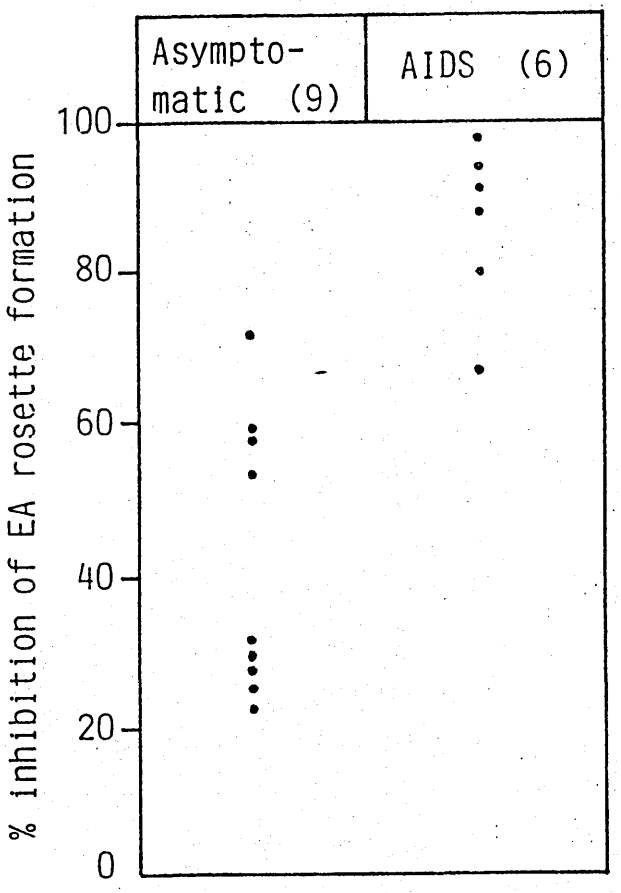


FIGURE 48: Inhibition of EA rosette formation by IgG preparations from 15 homosexual subjects, 6 with clinically defined AIDS

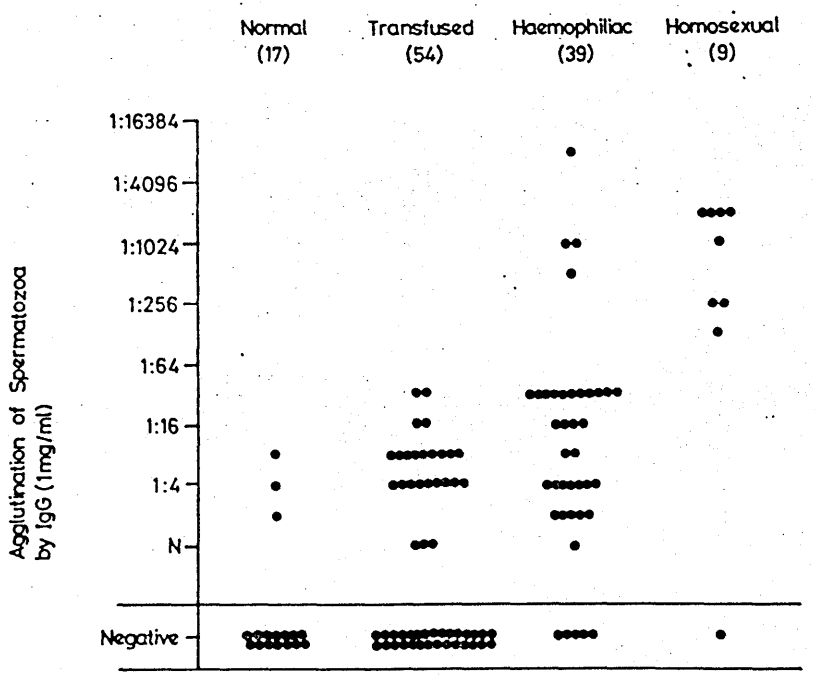
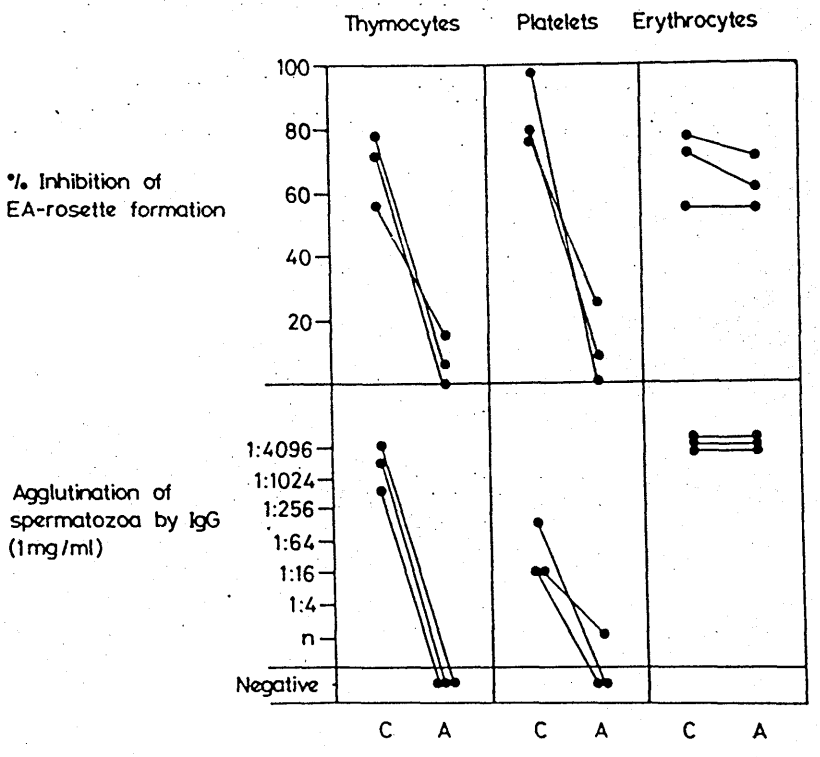


FIGURE 49 : Agglutination of spermatozoa by IgG preparations from 119 subjects



c = control

a = absorbed

FIGURE 50 : Removal by absorption of anti-sperm and anti-lymphocyte activity from 3 IgG preparations

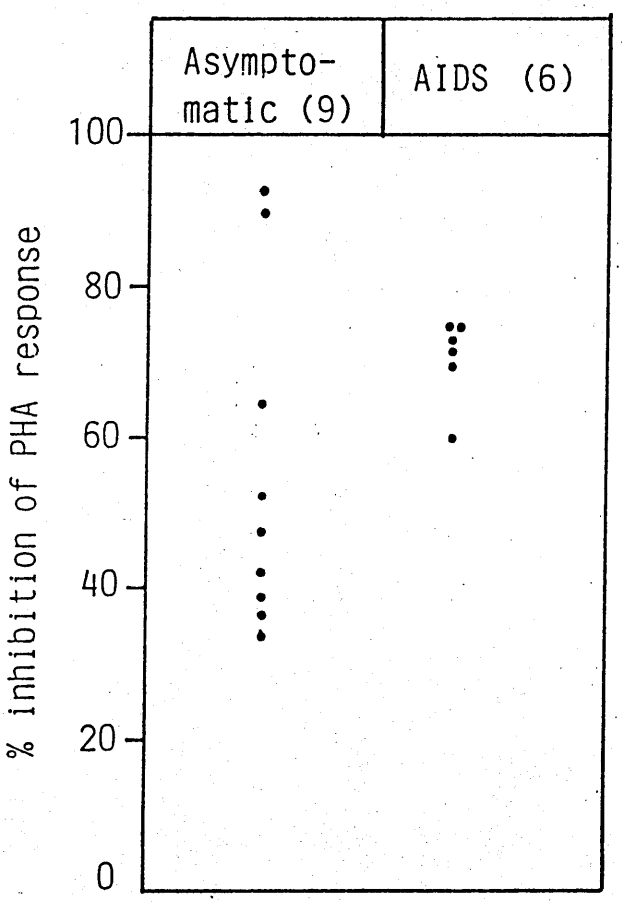


FIGURE 51: Inhibition of the phytohaemagglutinin response of normal peripheral blood lymphocytes by IgG preparations from 15 homosexual men, 6 with clinically defined AIDS

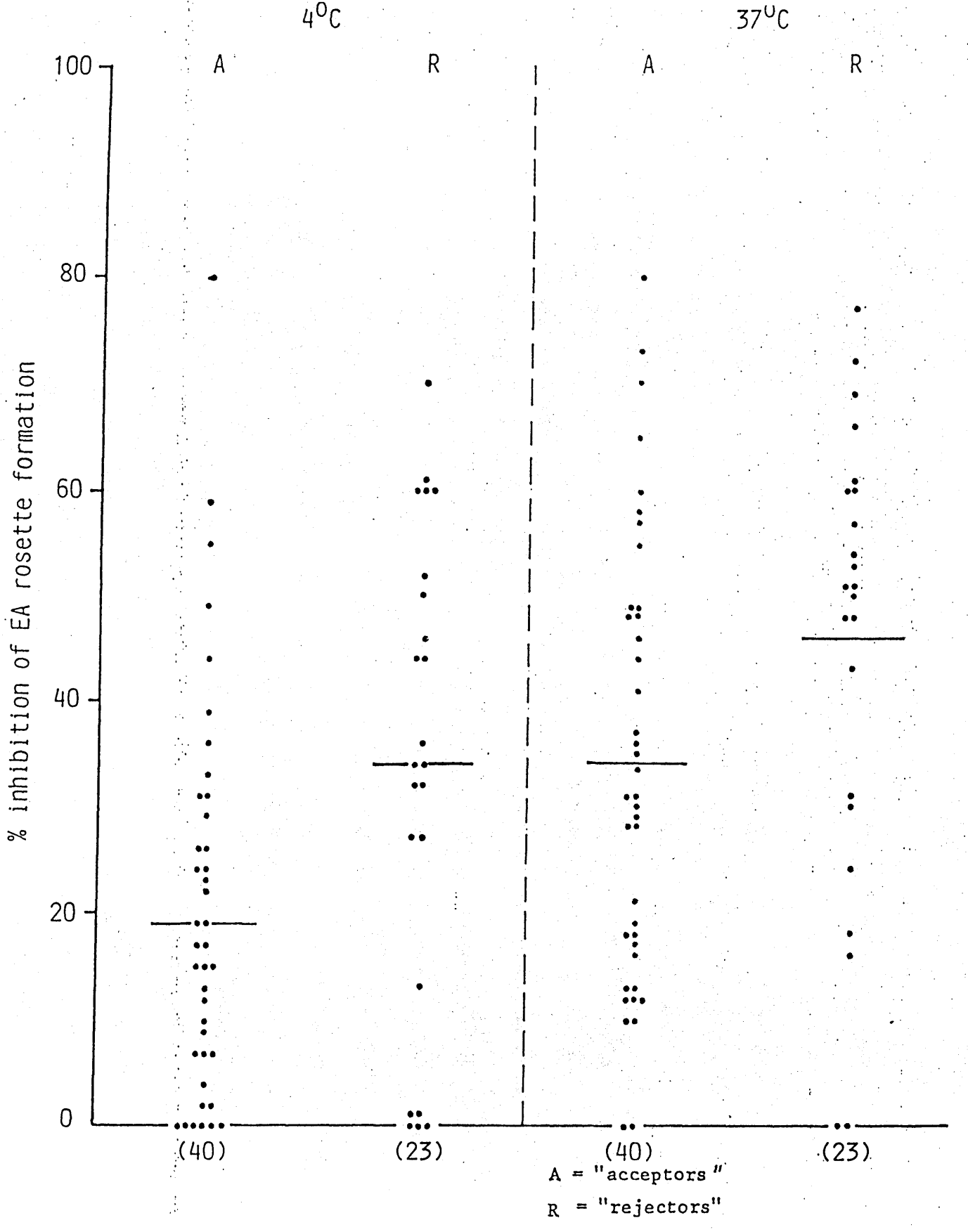


FIGURE 52 : Comparison between one-year renal allograft survival and pretransplant IgG Fcγ-receptor blocking activity against normal peripheral blood lymphocytes

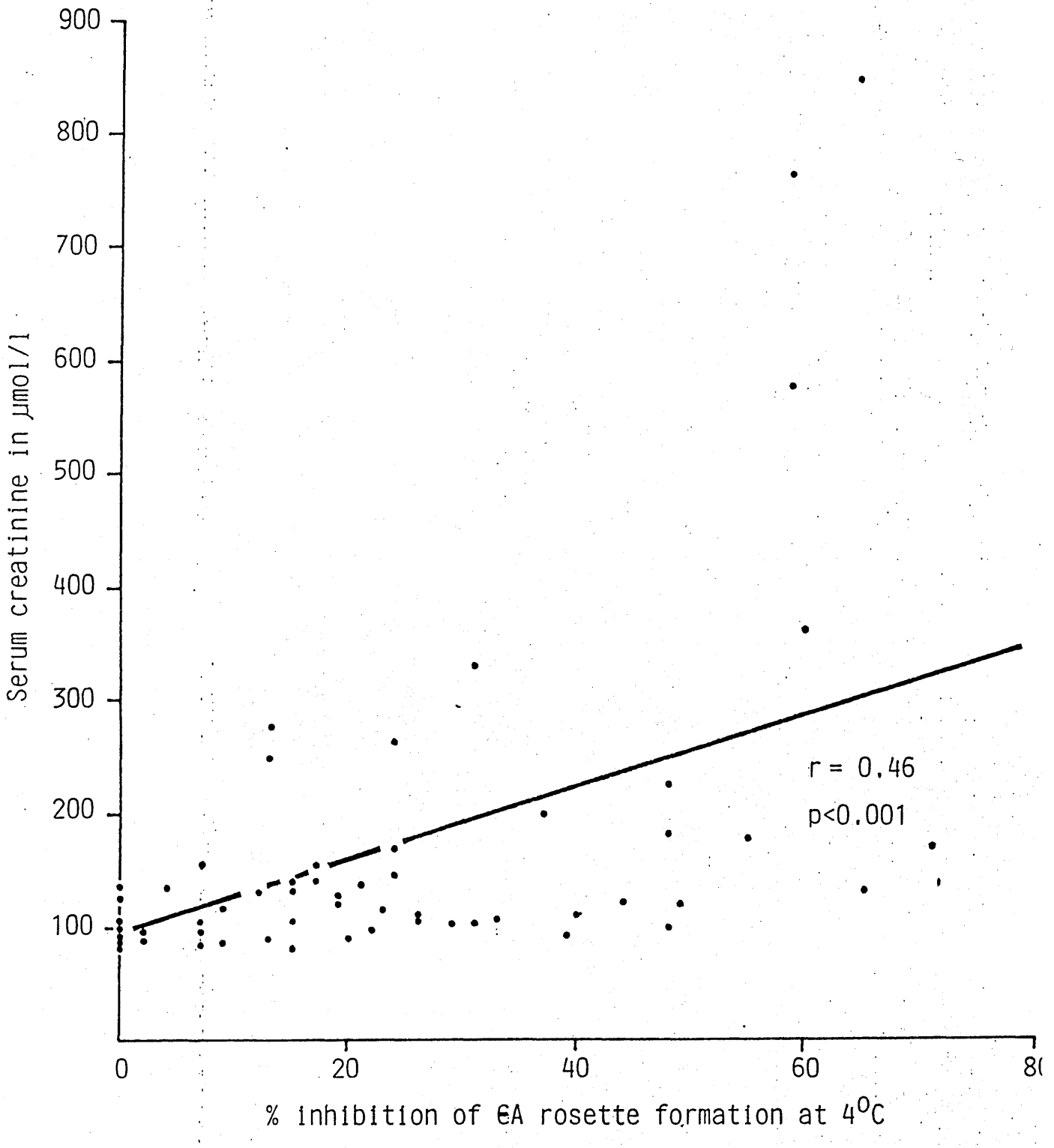


FIGURE 53 : Comparison between serum creatinine one year after renal transplantation and pretransplant IgG Fcγ-receptor blocking activity at 4°C against normal peripheral blood lymphocytes

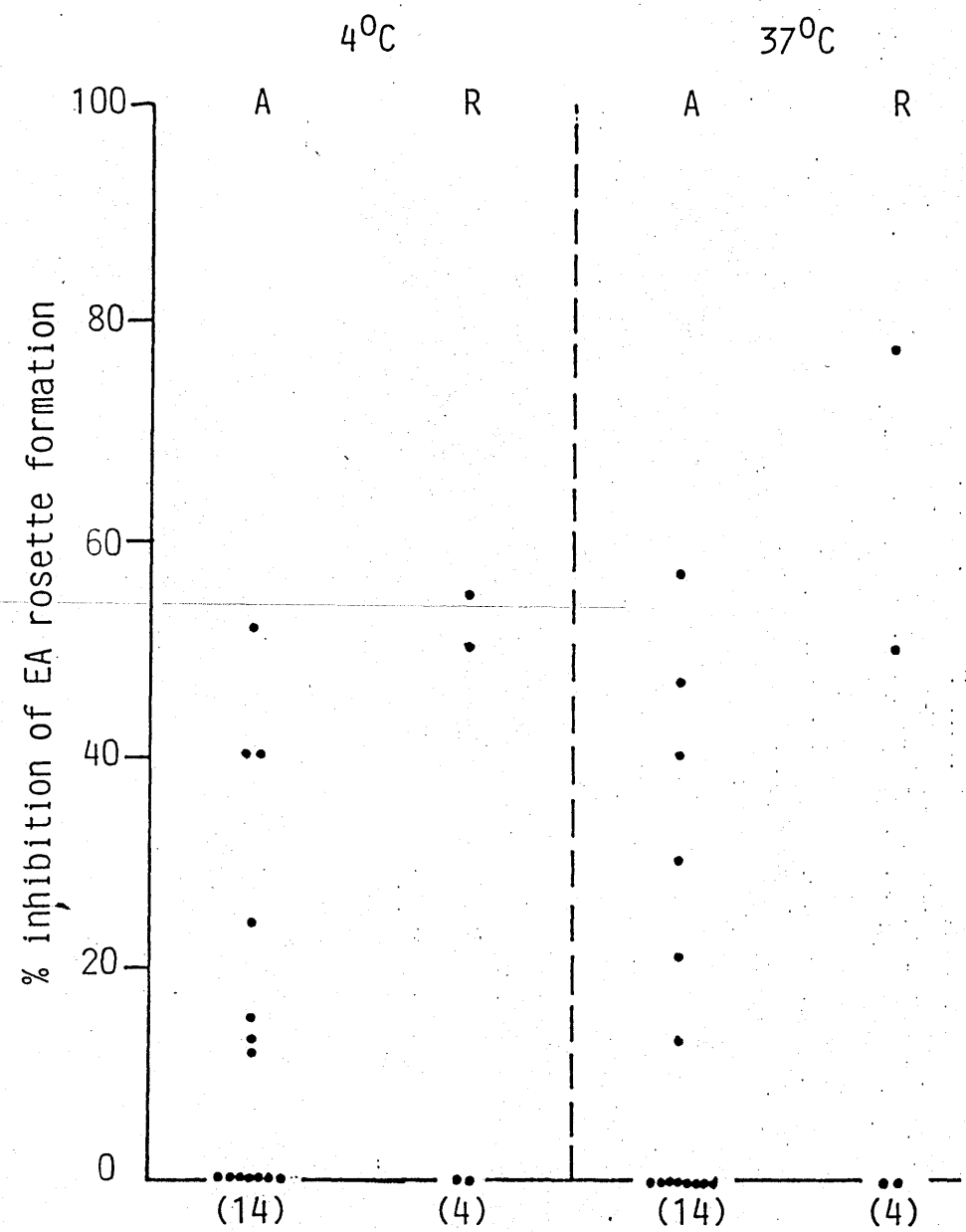


FIGURE 54: Comparison between one-year renal allograft survival and inhibition of EA rosette formation of donor peripheral blood lymphocytes by pretransplant IgG preparations from 18 patients

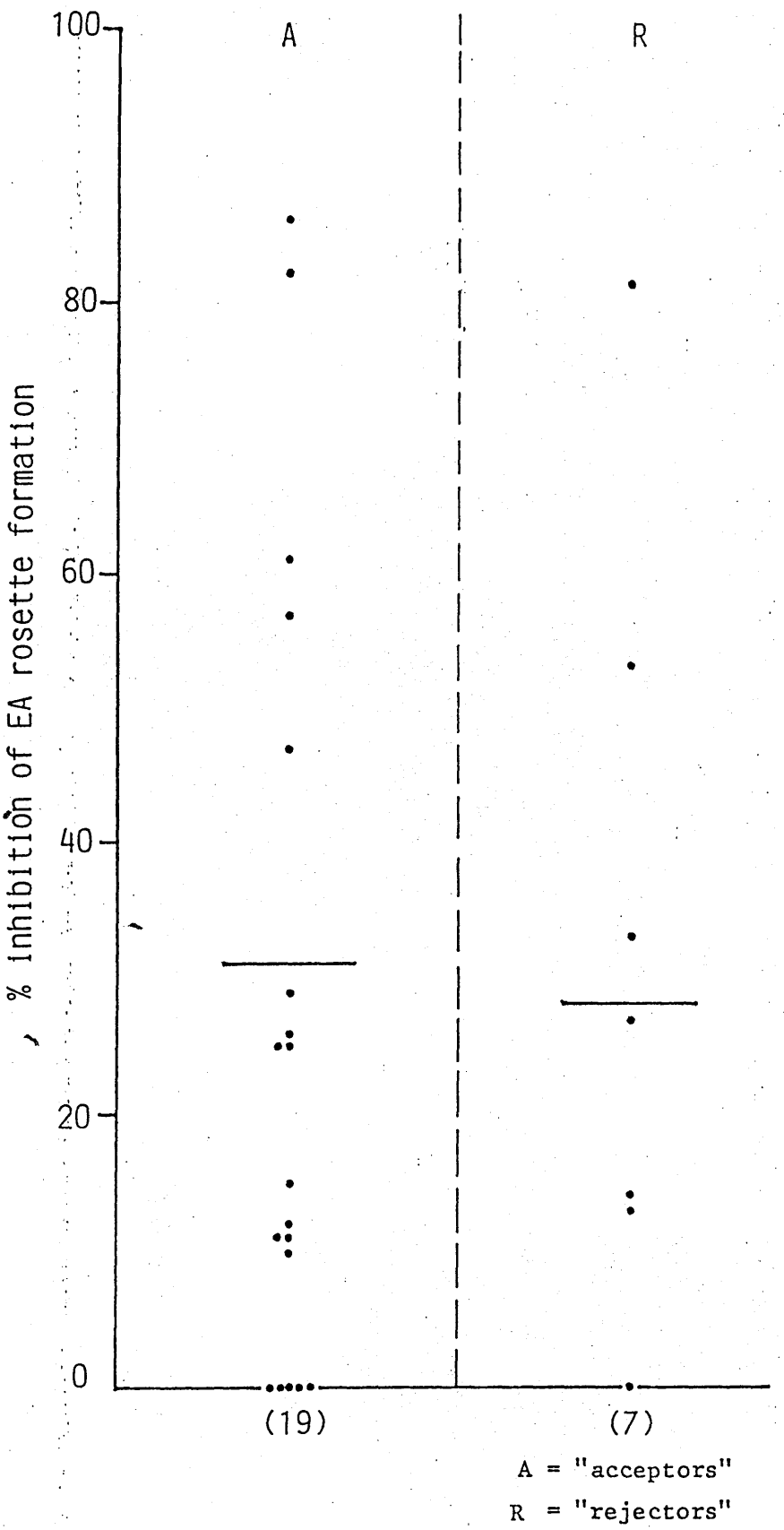


FIGURE 55 : Comparison between one-year renal allograft survival and pretransplant IgG Fcγ-receptor blocking activity at 4°C against normal B lymphocytes

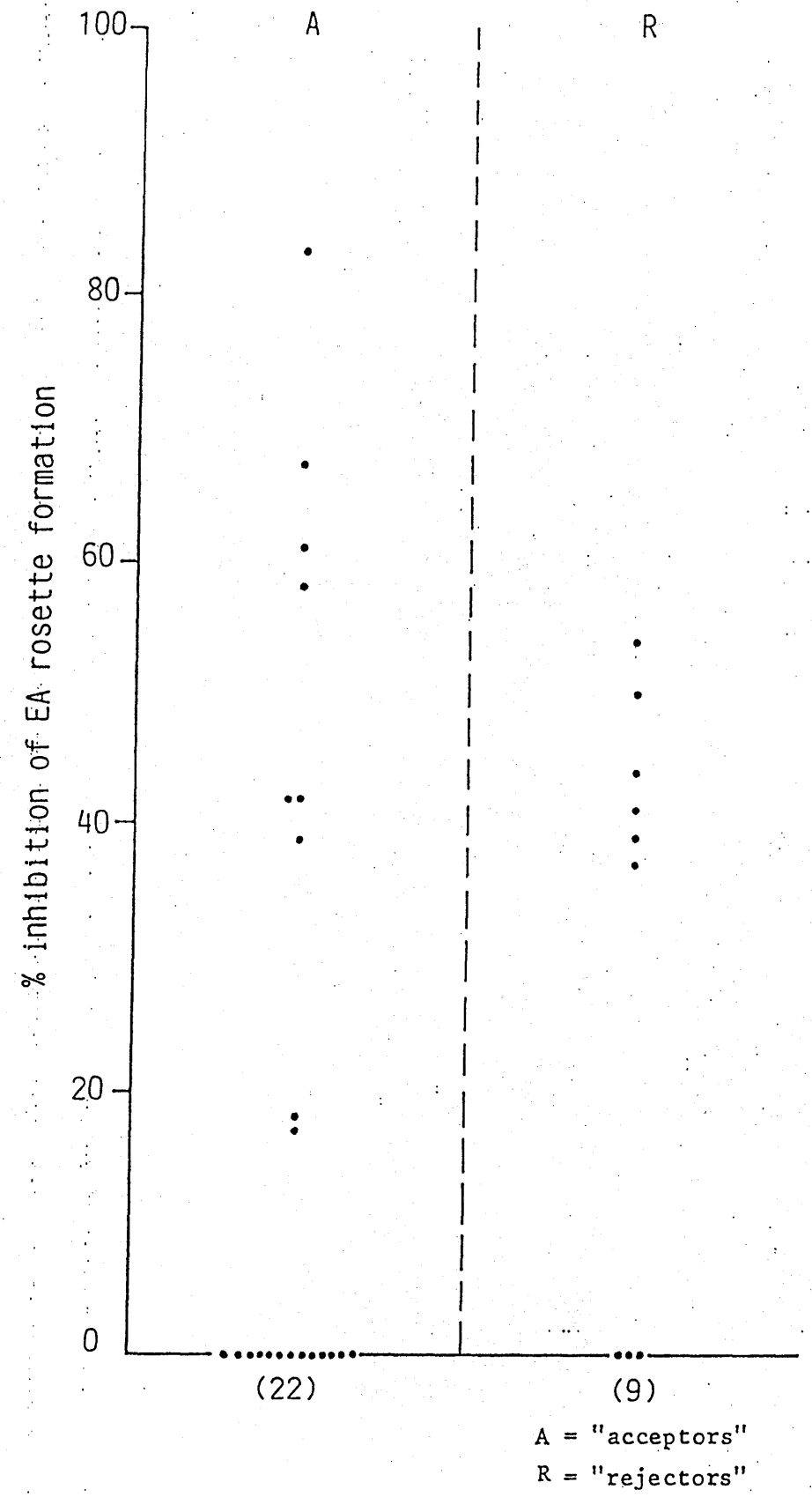


FIGURE 56 : Comparison between one-year renal allograft survival and pretransplant IgG Fcγ-receptor blocking activity at 4°C against donor splenic B lymphocytes

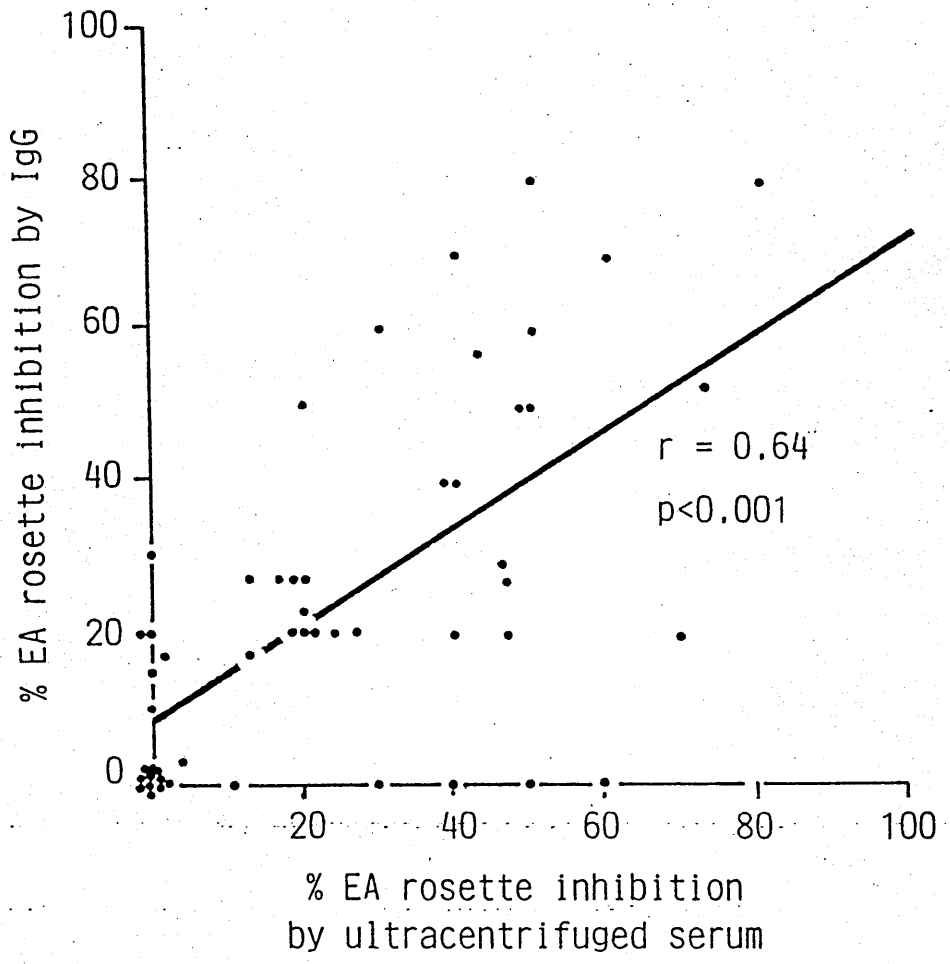


FIGURE 57 : Comparison of Fcγ-receptor blocking activity against normal peripheral blood lymphocytes between IgG and ultracentrifuged serum

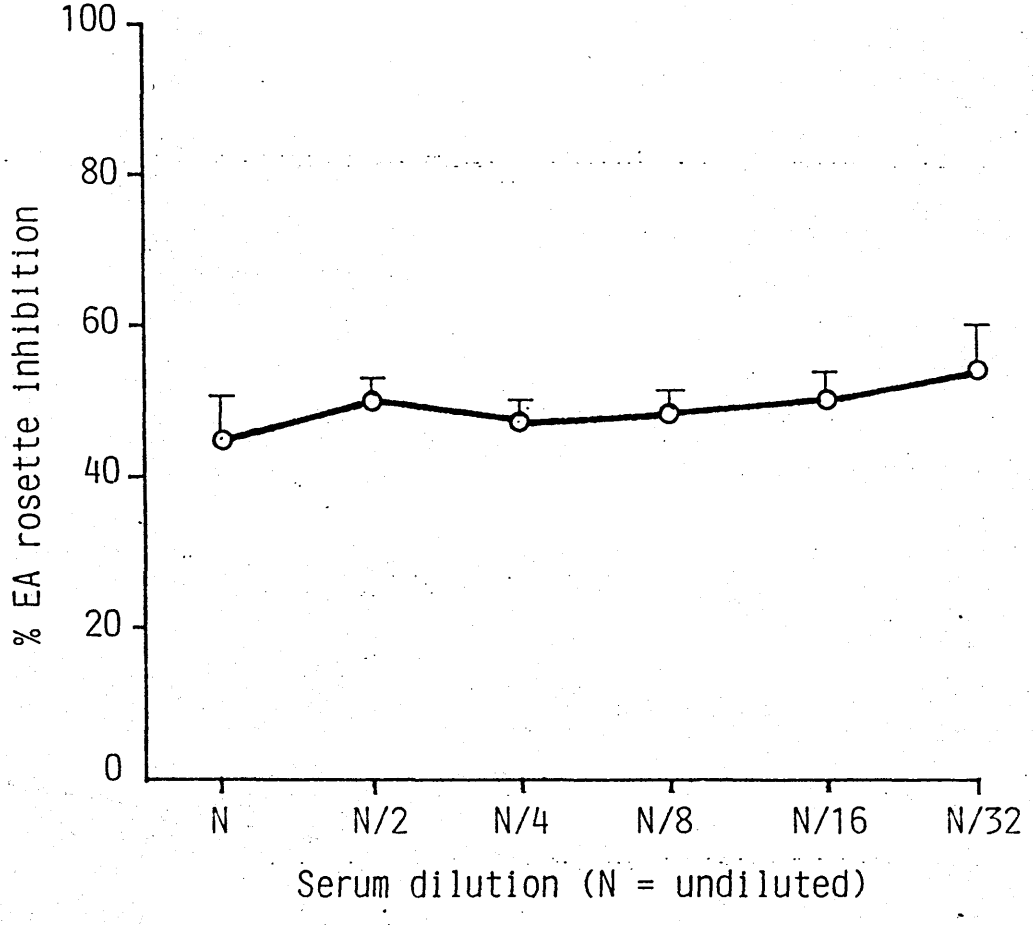


FIGURE 58 : Fcγ-receptor blocking activity (mean + standard deviation) against normal peripheral blood lymphocytes at 37°C in serial dilutions of sera from 6 transfused patients on renal replacement therapy

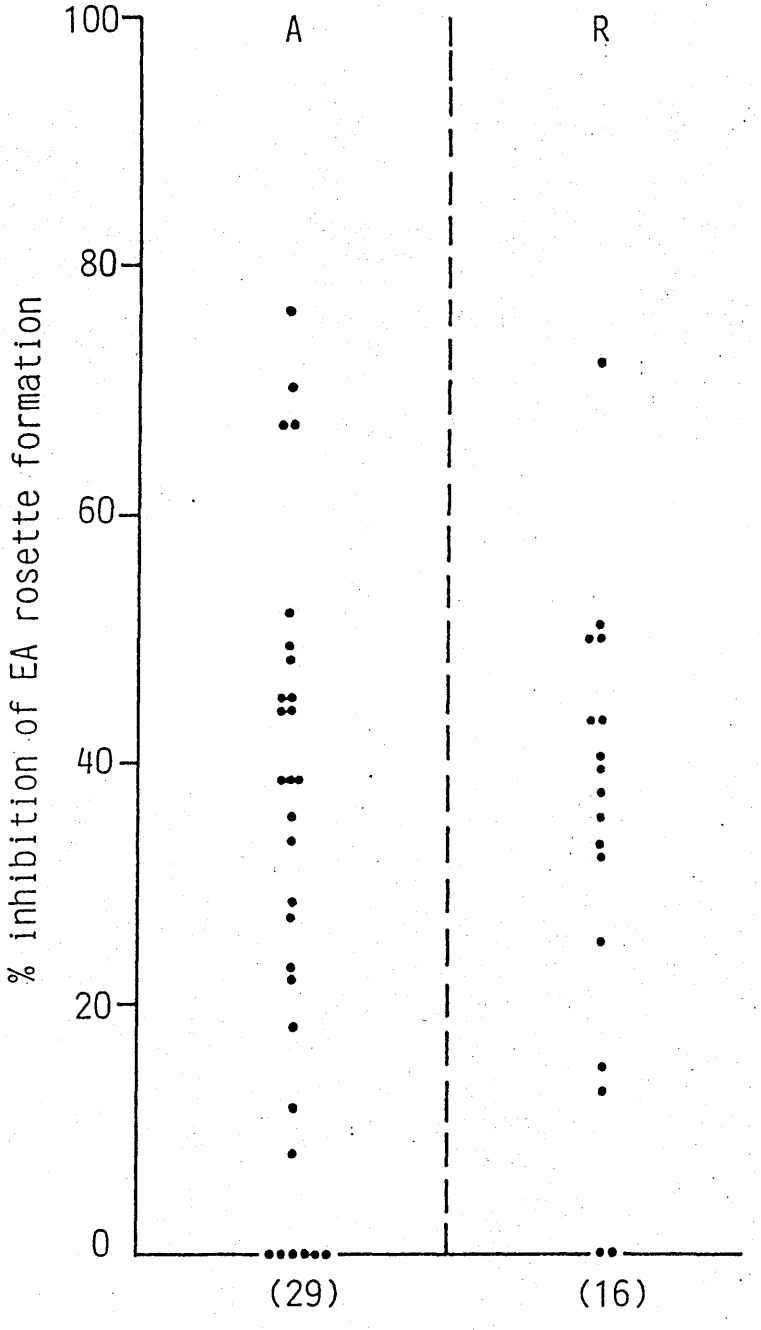


FIGURE 59: Comparison between one-year renal allograft survival and inhibition at 4°C of EA rosette formation of normal peripheral blood lymphocytes by pretransplant ultracentrifuged serum from 45 patients

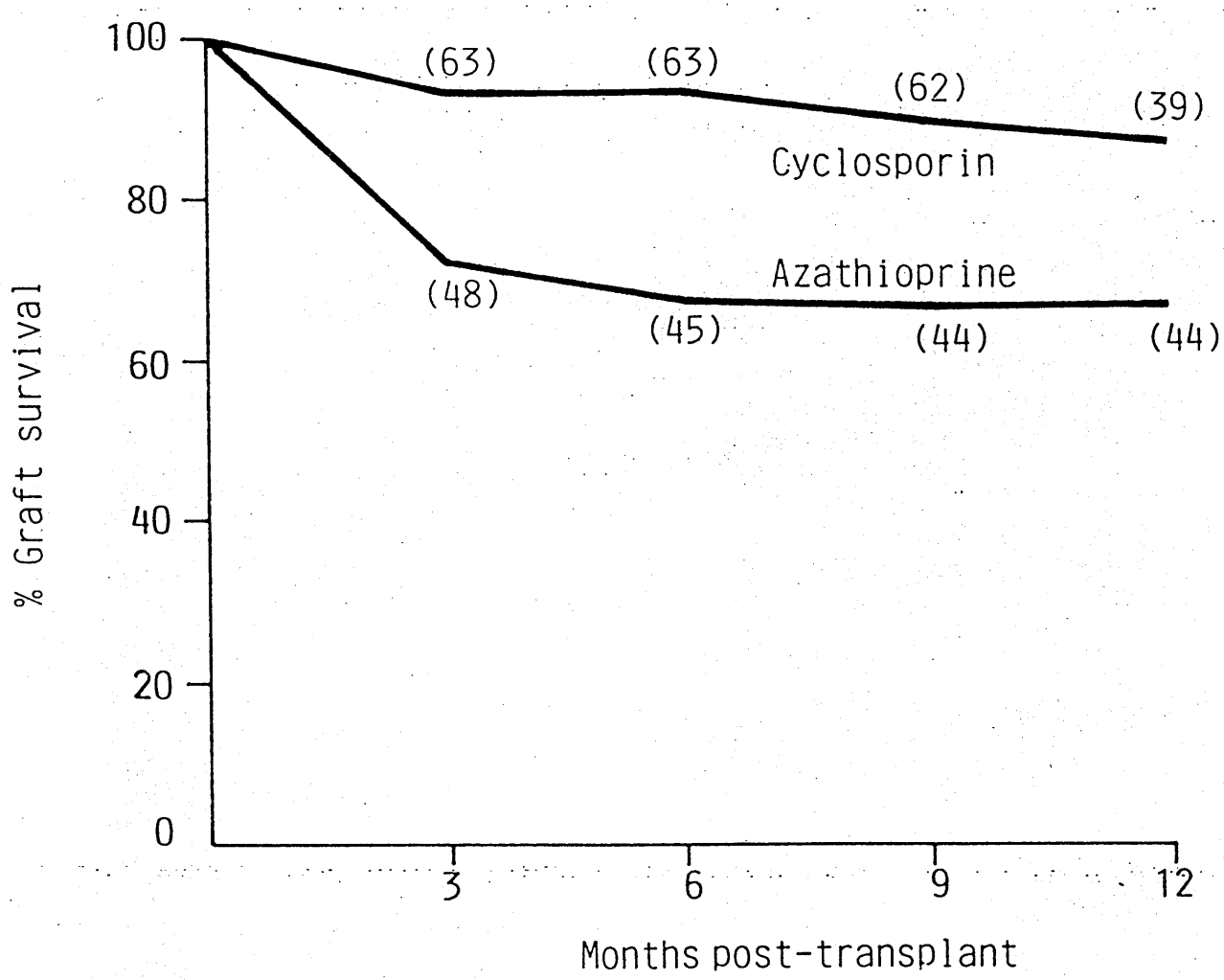


FIGURE 60 : Comparison between Cyclosporin and Azathioprine: Cummulative renal allograft survival (Glasgow 1983 - 1984)

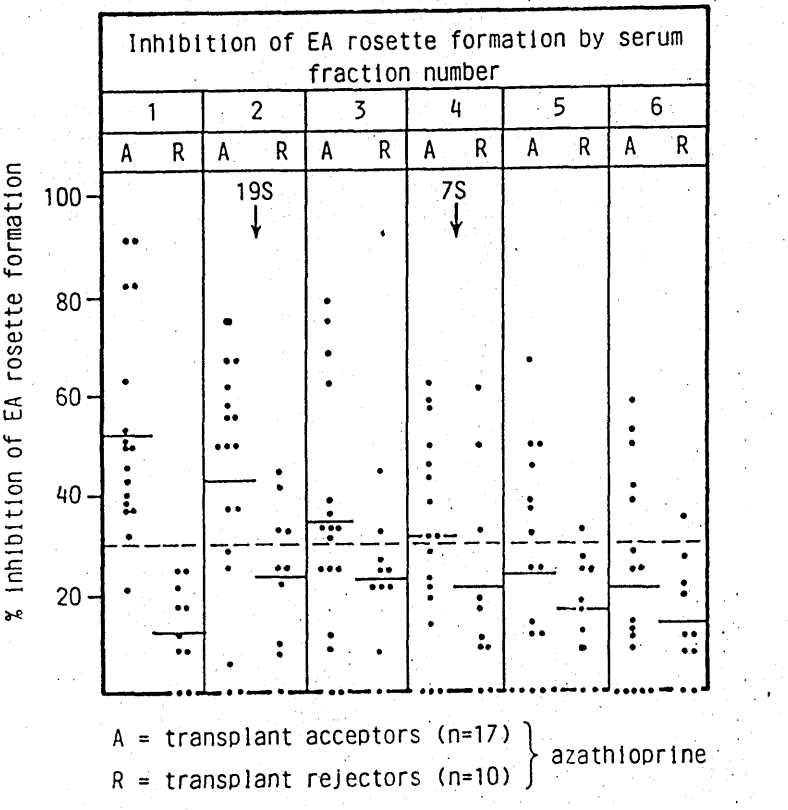


FIGURE 61 : Comparison in 27 Azathioprine-treated patients between one-year renal allograft survival and pretransplant serum fraction Fcγ-receptor blocking activity at 37°C against normal peripheral blood lymphocytes

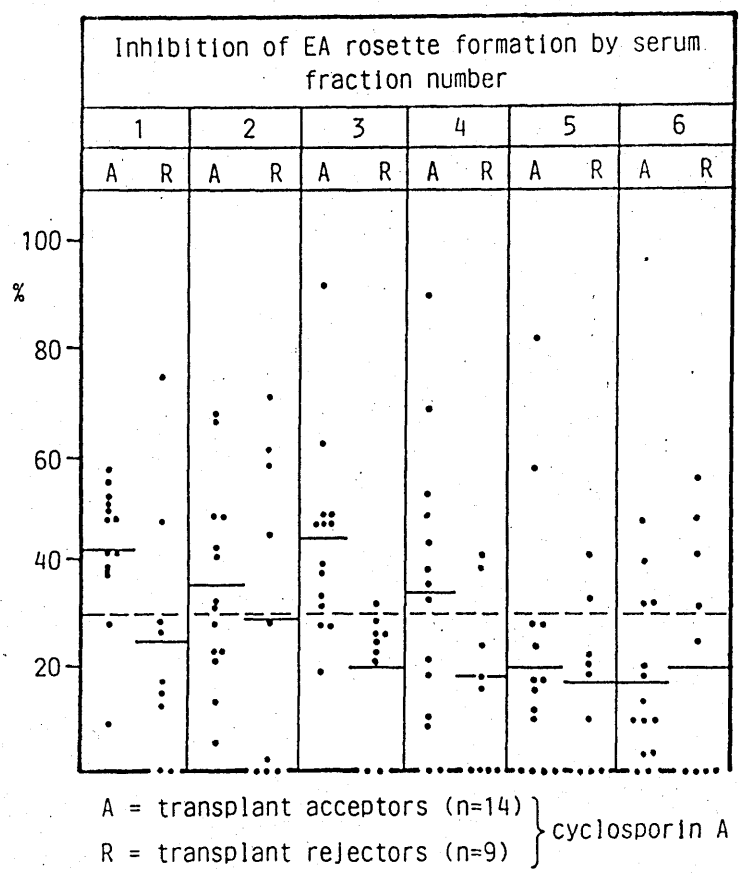


FIGURE 62 : Comparison in 23 Cyclosporin-treated patients between one-year renal allograft survival and pretransplant serum fraction Fcδ-receptor blocking activity at 37°C against normal peripheral blood lymphocytes

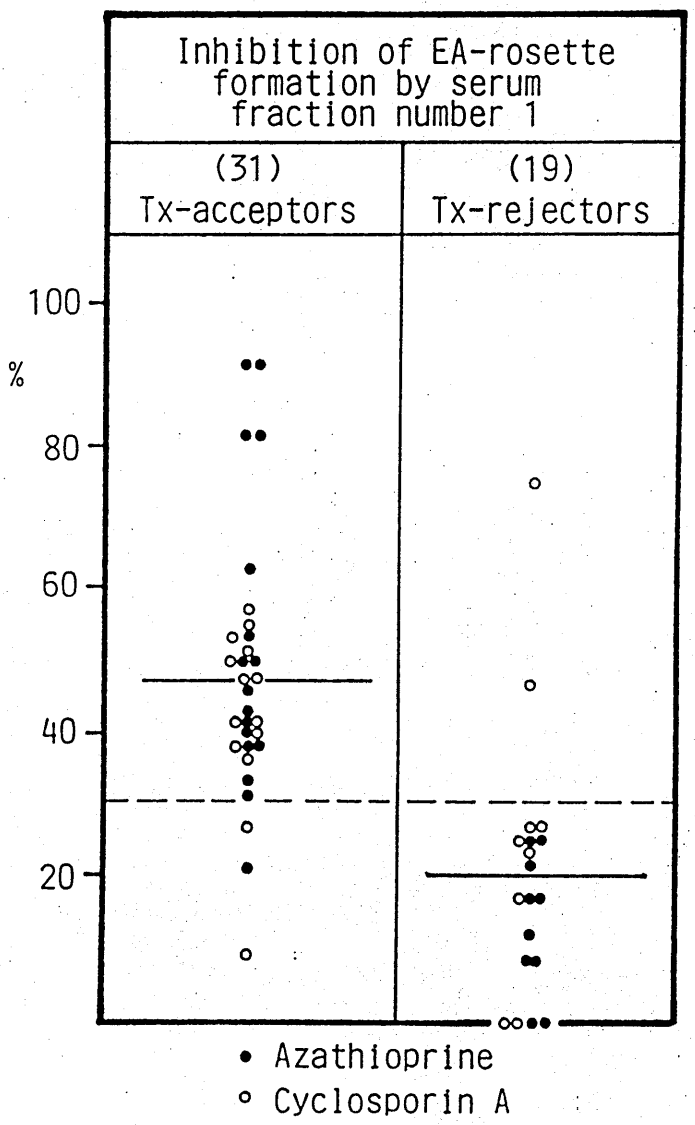


FIGURE 63 : Comparison in 50 patients between one-year renal allograft survival and pretransplant serum fraction 1 Fcγ-receptor blocking activity at 37°C against normal peripheral blood lymphocytes (T_x = transplant)

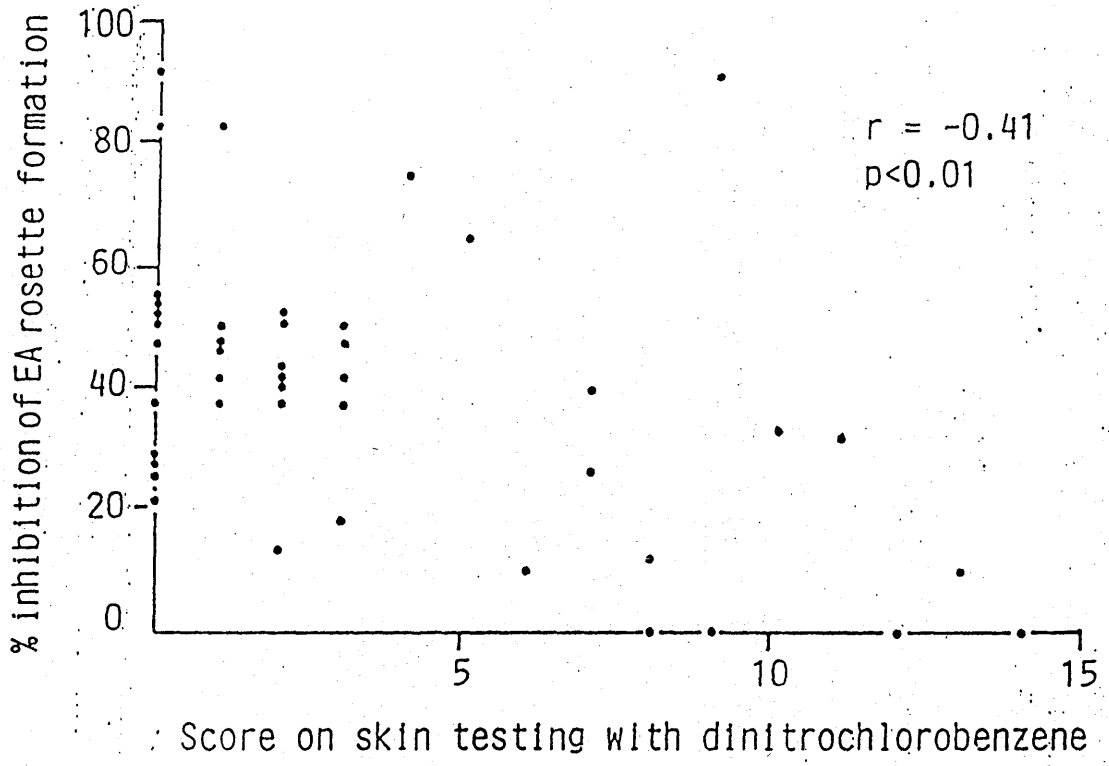


FIGURE 64: Correlation in 44 patients between Dinitrochlorobenzene skin test score and inhibition of EA rosette formation of normal peripheral blood lymphocytes by pretransplant serum fraction 1

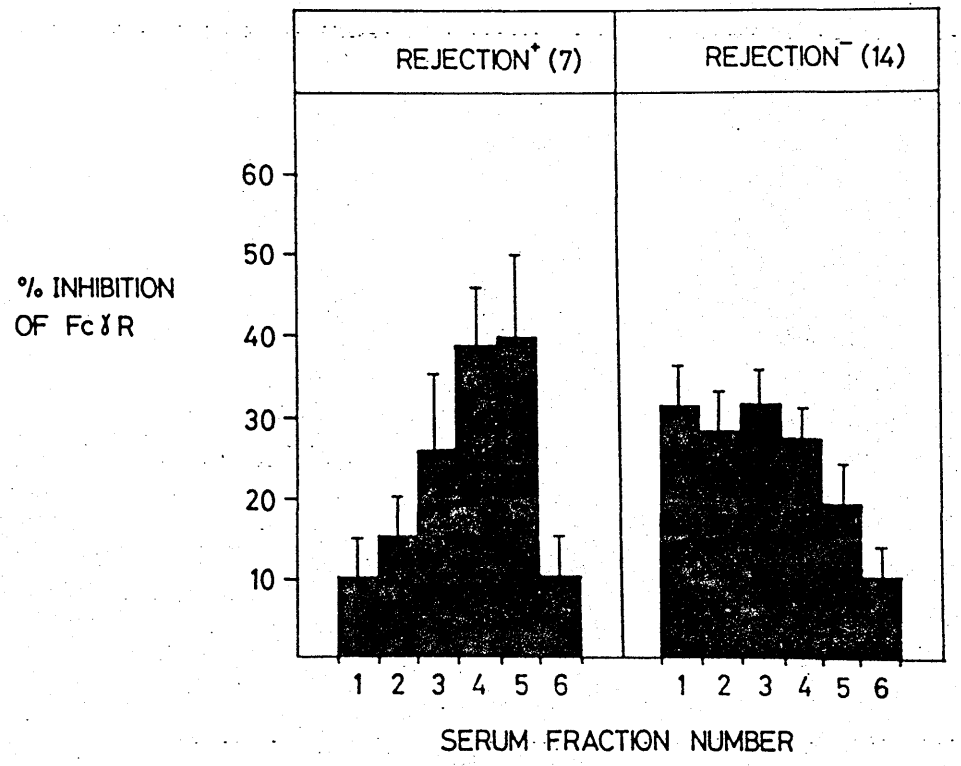


FIGURE 65: Fcγ-receptor (FcγR) blocking activity of serum fractions from 21 renal allograft recipients
(Rejection + : graft lost through rejection)
(Rejection - : functioning kidney 8 years post-transplant)

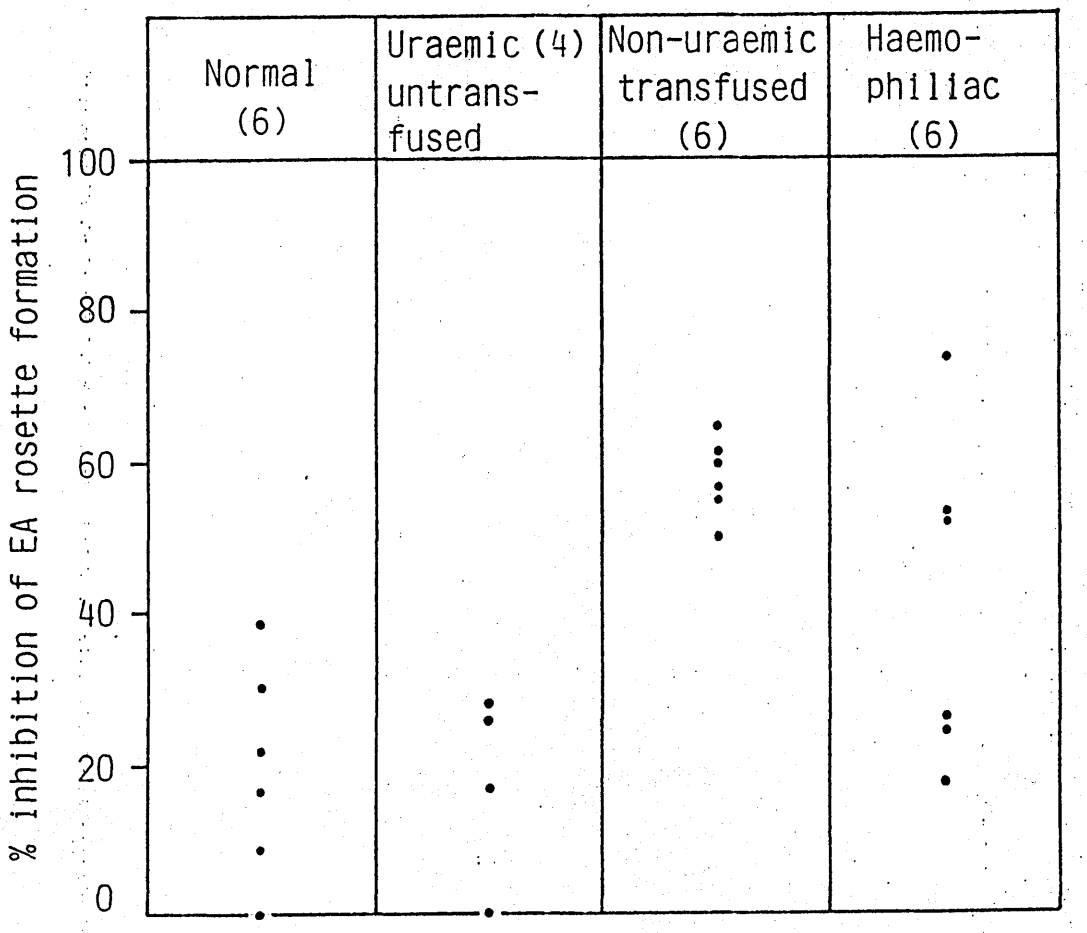


FIGURE 66: Inhibition of EA rosette formation of normal peripheral blood lymphocytes by serum fraction 1 from 22 untransfused and transfused subjects

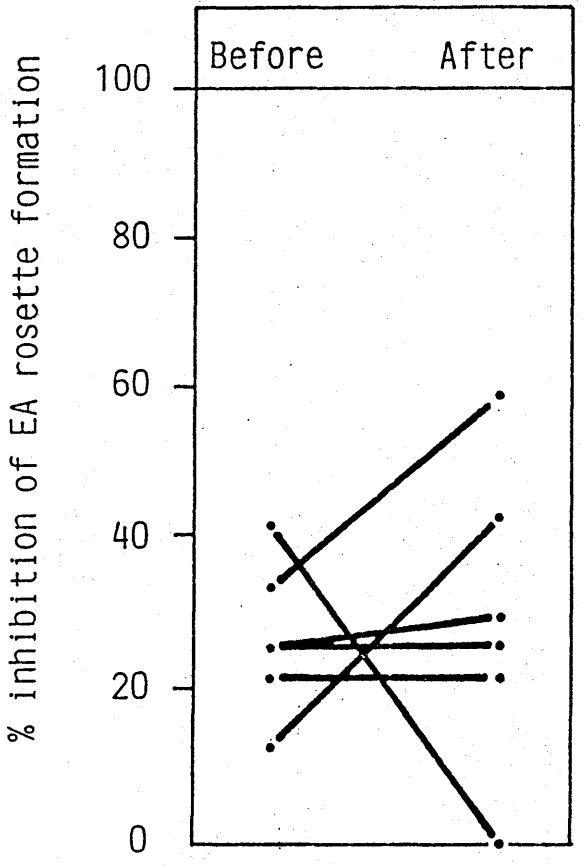


FIGURE 67: Inhibition of EA rosette formation of normal peripheral blood lymphocytes by serum fraction 1 from 6 renal dialysis patients before and after elective blood transfusion

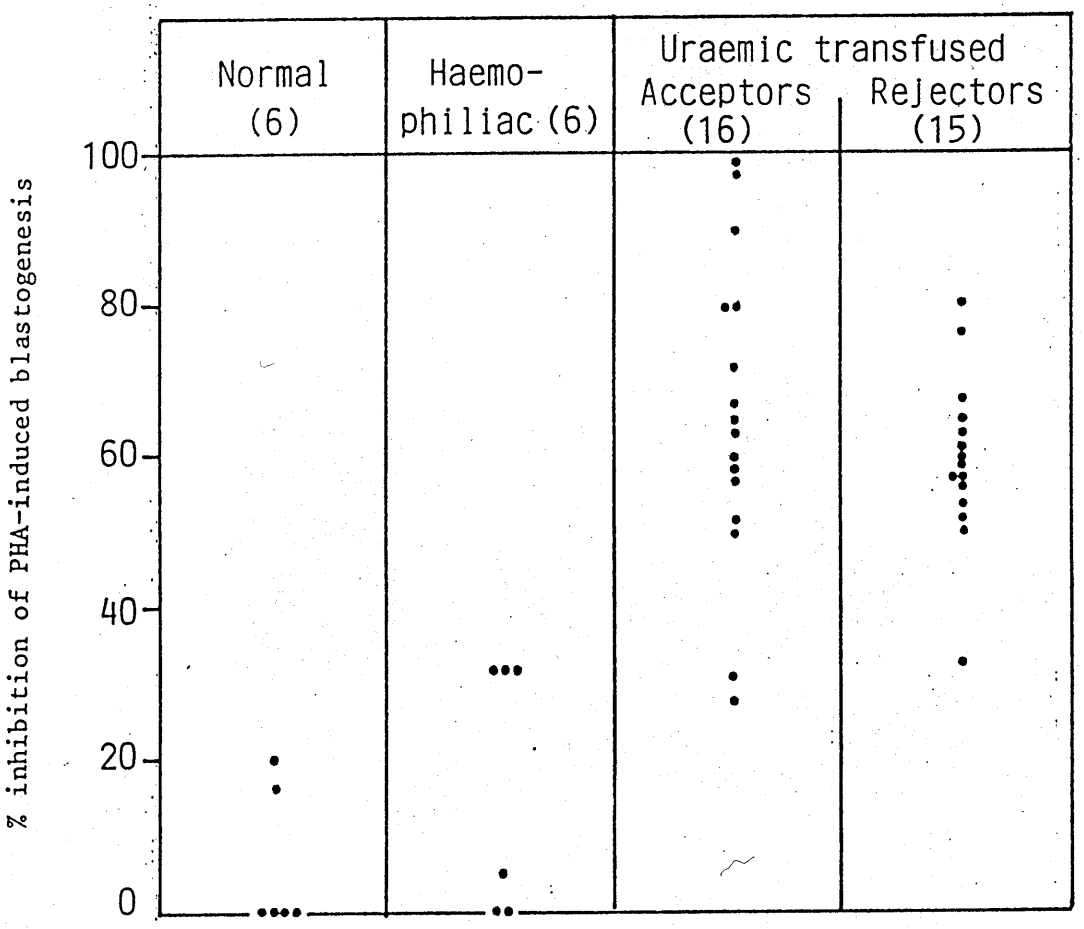


FIGURE 68: Inhibition of the phytohaemagglutinin response of normal peripheral blood lymphocytes by serum fraction 1 from 43 untransfused and transfused subjects

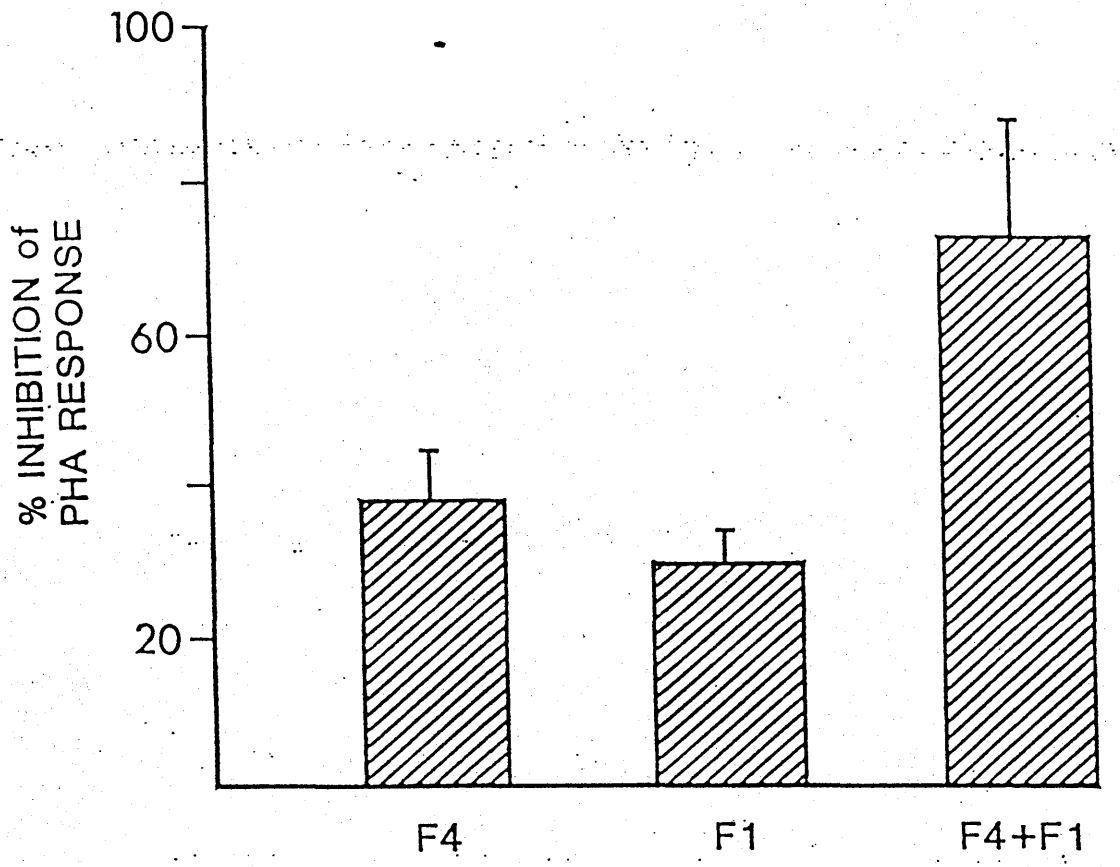


FIGURE 69: Inhibition (mean + standard deviation) of the phytohaemagglutinin response of normal peripheral blood lymphocytes by serum fractions 1 and 4, alone and in combination, from 3 transfused uraemic subjects

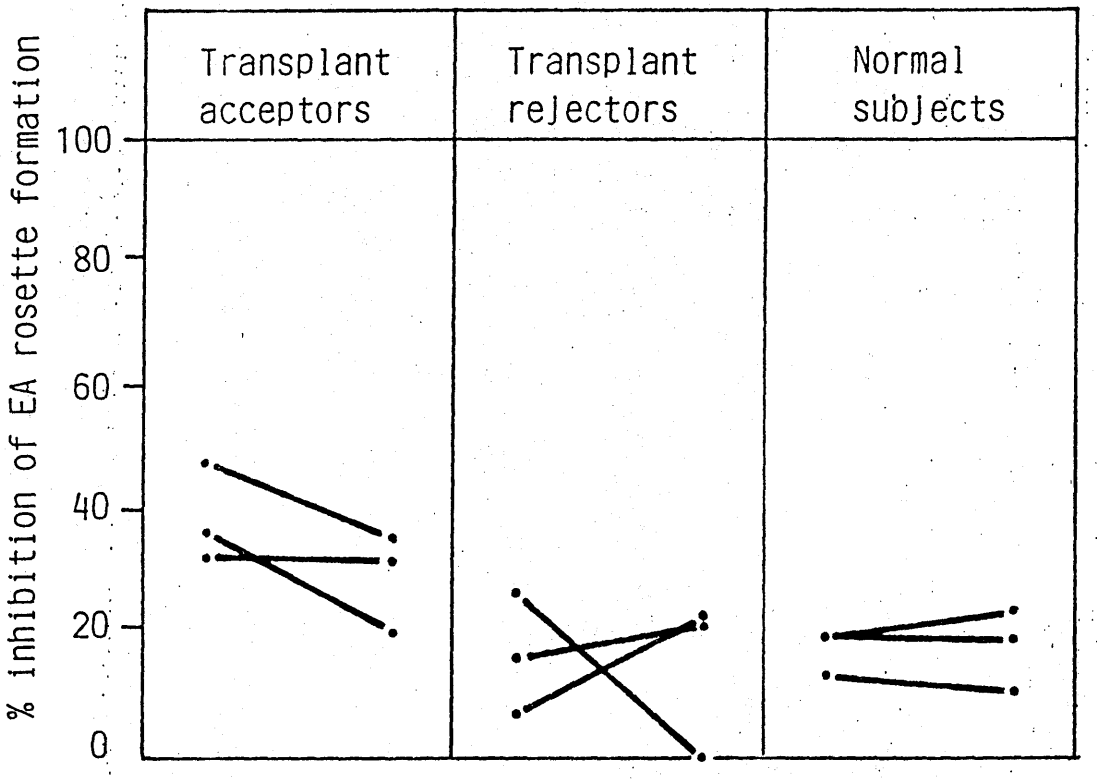


FIGURE 70: Inhibition of EA rosette formation of normal peripheral blood lymphocytes by serum fraction 1 before and after absorption with Protein A

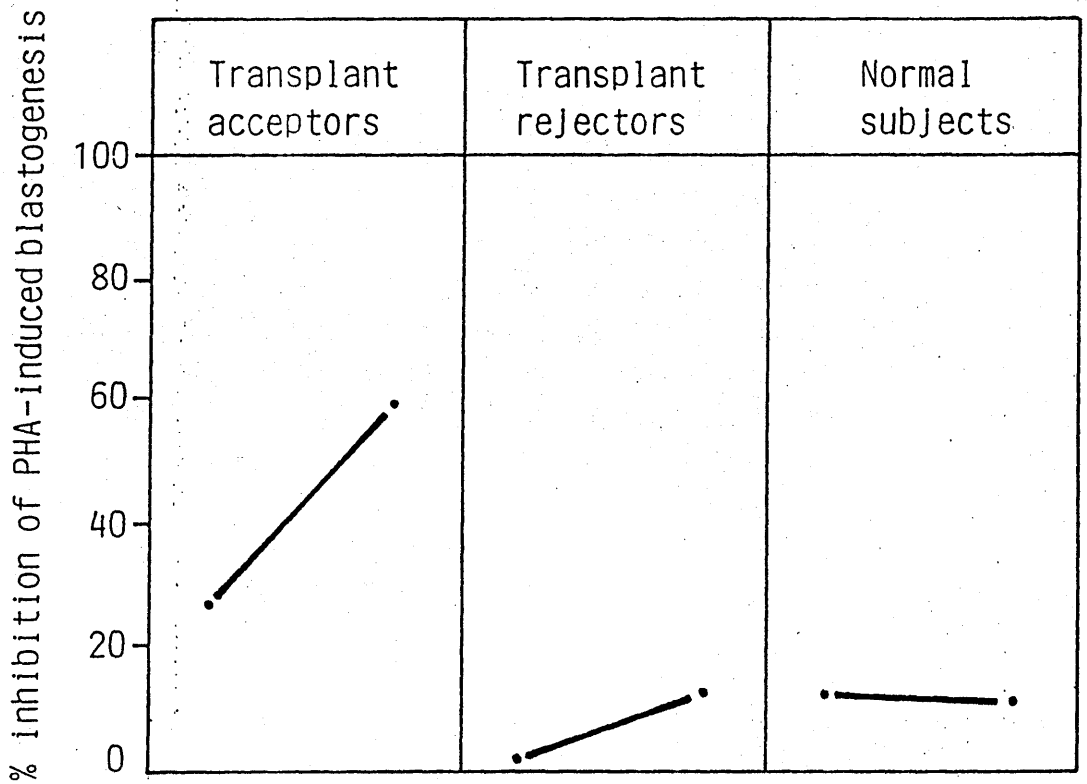


FIGURE 71: Inhibition of the phytohaemagglutinin response of normal peripheral blood lymphocytes by serum fraction 1 before and after absorption with Protein A