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ABSTRACTS

ANTI-H-2 ANTIBODIES INDUCED BY SYNGENEIC IMMUNIZATION

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The technique of "syngeneic immunization" which we have used was the same as "routine" anti-H-2 alloimmunization: i.e. longterm i.p. injections of a suspension of l.n. + spleen + thymus cells (without adjuvant, in protein free medium) into syngeneic recipients.
In the first experiment, 16 out of 41 BALB/c (39 %) mice produced anti-H-2 cytotoxic antibodies. All positive sera contained anti-K^k antibodies but many sera were more or less polyspecific.
In the second experiment, 29 out of 38 C57Bl/6 (76 %) mice produced anti-H-2 cytotoxic antibodies. The positive sera reacted mostly with the H-2 d, s, k haplotypes but some sera were more polyspecific. However, at variance with the first experiment, some of the syngeneic-immune B6 sera behaved as monospecific anti-d, s or k reagents, respectively.
The H-2 specificity of syngeneic-immune sera was verified by extensive panel studies, testing of F_2 hybrids and immunoprecipitation.
Further experiments are in progress at present to elucidate the mechanism of "triggering" and antibody production during syngeneic immunization.
We have no conclusive evidence neither about the "triggering" mechanism which induces the production of these anti-H-2 antibodies nor about the control of their production. We definitely disregard the possibility, that the anti-H-2 antibodies were induced due to hereditary genetical differences among the donor/and/recipient individuals of the inbred strains (e.g. the "residual heterozygosity" of inbred strains or rough technical-breeding-mishaps). We consider as possible a great array of different epigenetical differences among the individuals tested, including their virus status, inaparent cancerous cells or somatic mutations (the causes of altered self). Alternatively, a disturbed regulation of the immunological network, induced by the large dosis of the injected lymphoid cells, might have been the primum movens for proliferation of dominant or individually preferent clones.
Both these aspects might well operate in combination, presuming that altered self can trigger a disturbed immunological network.

Attempts to establish a model of passive enhancement of kidney allografts in dogs, and possible theoretical reasons for the high efficacy of passive enhancement in rats.

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The success of passive enhancement for suppressing kidney graft rejection in rats raised considerable hopes that it might be a safe and useful approach to donor specific immunosuppression in clinical transplantation. In this study, we report attempts to induce passive enhancement of kidney grafts in dogs. 5 pools, each of several litres, of putative enhancing sera were raised by repeated intra-dermal and intravenous injections of allogeneic lymph node lymphocytes over a period of 2-10 months. Seven dogs were immunised, each with the lymphocytes of a different dog, the same donor being used for all immunisations of any one animal. The 5 dogs which gave good lymphocytotoxin titres were selected, plasmapheresed several times and then exsanguinated. All blood from any one animal was pooled, sterilised by millipore filtration, divided into aliquots and frozen. Experiment I: The 5 dogs used as the immunising donors of the sera were used as kidney donors to 9 unrelated recipients (one of the donor kidneys had a vascular abnormality and could not be used). These recipients were screened with lymphocytotoxicity assays and binding assays with ^{125}I rabbit F(ab')$_2$ anti dog F(ab')$_2$ (RAD) on blood lymphocytes to check that the enhancing sera did not react with the recipients, to avoid absorption of the antibody by host tissues. Nine recipients were treated with 5ml/kg of serum on the day of grafting and on day 1. Two rejected their grafts hyperacutely, and the other 7 survived for 8,9,9,10,11,14 and 14 days. Untreated controls survived for 7,8,9,9,9,10 and 12 days. Experiment II: The 5 batches of enhancing sera were tested by lymphocytotoxicity and binding assays with ^{125}I RAD on blood lymphocytes of unrelated dogs. Donor-recipient combinations were chosen on the basis of strong reactivity against the donor, and negative reactivity against the recipient. Of 8 transplants performed, 5 dogs appeared to reject their grafts hyperacutely, and the other 3 survived for 7,8 and 10 days. These results are much inferior to those one would have anticipated from studies in many different rat strain combinations. A hypothesis pointing out a possible fundamental reason why passive enhancement might be ineffective in man and other animals and highly effective in the rat will be presente

THE INDUCTION OF SPECIFIC TRANSPLANTATION TOLERANCE BY SPLENIC ALLOGRAFTS

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Spleen allografts were exchanged between inbred strain 2 and strain 13 guinea pigs whose major histocompatibility complex differs only at the I-region and who rapidly reject reciprocal skin allografts.The spleen grafts were heterotopic and auxilary since they were joined to the infrarenal aorta and vena cava without removal of the hosts' spleens.No immunosuppression was given. 480 transplants were analyzed with the following results :

90% of the spleen grafts 13 to 2 survived spontaneously throughout the lifetime of the hosts whereas the spleen grafts 2 to 13 were rejected within 3 weeks.Serial mixed lymphocyte reaction (MLR) studies on lymphocytes from lymphnodes of strain 2 recipients with surviving spleen transplants revealed an early,markedly suppressed host-anti-donor MLR,and concomitantly a signifi cant host-anti-"self" MLR.The latter could be ascribed to chimeric donor cell No serological changes were noted.These in vitro findings were paralleled clinically with a high mortality of graft-versus-host disease (GVHD).Animals that survived this initial GVH onslaught continued to be permanently MLR suppressed against the donor strain ,yet with an absence of the above descril reactivity against "self" .Such animals accepted donor-type skin allografts indefinitely and rejected third-party skin allografts like controls.This type of specific transplantation tolerance was adoptively transferable to normal, non-irradiated recipient strain guinea pigs with intravenously administered lymphocytes .Sera were ineffective.Studies are under way to characterize the cells responsible for the induction and maintenance of tolerance in this species.

Demonstration of intensely Ia positive dendritic cells in the interstitial connective tissues: probable identification of the passenger leucocyte.

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"Passenger leucocytes" have frequently been proposed as an important component of the immunogenic stimulus offered by a graft, but experiments and clinical studies aimed at eveluating their importance have been severely handicapped by a lack of any precise definition of the nature of the passenger leucocytes and of any means of visualising them. In this study, we have used a mouse monoclonal antibody to rat Ia antigens to demonstrate by immunofluorescence on frozen sections an intensely Ia positive dendritic cell in the interstitial connective tissues of every tissue we have studied (heart, kidney, liver, pancreas, thyroid, skin, skeletal muscle, ureter and bladder) with the striking exception of brain. The anti Ia antibody demonstrates these cells in a most striking fashion as isolated cells with a profusion of cell processes. The interstitial dendritic cell in the heart was studied in detail, and was shown not to have the characteristics of macrophages, in that it was negative for acid phosphatase, B glucuronidase, and ATP-ase, and at least most and probably all were negative for non-specific esterase. The cell was also poorly phagocytic for colloidal carbon. Further studies showed that the cells were negative for surface immunoglobulin and the W/13 antigen (found on granulocytes and T lymphocytes in the rat) but positive for the leucocyte common antigen and SD antigens of the MHC. The cell was shown to be sensitive to irradiation and cyclophosphamide, and to be of bone marrow origin by reconstitution studies. Of particular interest was that following a lethal dose of irradiation, the number of interstitial dendritic cells was undiminished at 24 hours following irradiation, and that it was not until the 3rd to 5th days following irradiation that the heart was completely depleted of the cells. Recent studies with long surviving allografts suggest that the interstitial dendritic cell is an important component of the immunogenic stimulus offered by a graft, and that it is probably the major target in passive enhancement. These and other studies suggest that the interstitial dendritic cell is of particular importance in transplantation, and almost certainly represents the "passenger leucocyte".

As d present I have been supported gp's after ims rather than previously advanced.

ANTIGENIC ANATOMY OF HUMAN KIDNEYS AND RENAL GRAFT REJECTION

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We have investigated the "antigenic anatomy" of the kidney in order to relate different patterns of graft rejection to immune reactions against different classes of antigens. Indirect immunofluorescence stains of normal human kidneys demonstrated ABO blood group antigens on arterial, glomerular, peritubular capillary (PTC) and venular endothelium. Monoclonal antibodies to determinants common to all HLA-A, B, C antigens (W6/32HL and MAS 017b) and to β_2-microglobulin stain arteries and glomeruli more strongly than PTC. Individual HLA-ABC antigen determinants were more difficult to demonstrate with either monoclonal reagents (PA 2.1 anti A-2 and BB 7.1 anti B-7, kindly provide by Prof.W.Bodmer) or allogeneic tissue typing sera. Monoclonal antibodies to DR (DA2, MAS-020, NEI-011) stain glomeruli strongly and PTC moderately, but arteries are usually negative. Both monoclonal (MAS-044b from Sera-lab) and allogeneic (LB typing sera) antibodies to antigens of the possible second B cell locus stain PTC and glomeruli of some kidneys strongly. Sera from transplant recipients or typing allosera that contain antibodies to tissue specific endothelial-monocyte antigens stain PTC intensely, glomeruli moderately and arteries weakly or undetectably.

Thus, those antibodies which are associated with hyperacute rejection, react with antigens (ABO and HLA-A,B,C) that are expressed on arterial and glomerular endothelium. In contrast those antibodies which are frequently associated with acute rejection, react with antigens (E-M and HLA-DR) that are primarily expressed on venous and PTC endothelium.

THE LOCALISATION OF HLA-ABC AND DR ANTIGENS IN HUMAN KIDNEY

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The precise localisation and estimate of the amount of HLA-ABC and DR antigens within the human kidney may explain why HLA-DR matching improves graft survival, and why some B-cell positive transplants fail immediately while others are successful. We have used monoclonal antibodies to monomorphic HLA-ABC and DR antigens in immunofluorescence and immunoperoxidase to localise these antigens within the kidney.

The results are summarised:

Tissue	HLA-DR	HLA-ABC
Endothelium - capillaries	+++	+++
large vessels	±	+++
Glomeruli (incl. mesangium)	++	+++
Tubules	±/++	+
Interstitial cells (dendritic cells)	+++	+

The finding of a large number of DR positive dendritic cells may explain why DR matching is perhaps more effective than ABC matching in improving graft survival, if these cells provide an important component of the immunogenic stimulus for allograft rejection, as has been suggested in the rat model (Hart and Fabre, Transplant. Proc. in press).

Our finding of DR on endothelium, particularly the large amount on capillary endothelium is perhaps inconsistent with the published reports that B-cell positive crossmatch transplants are as successful as those with a negative crossmatch, However, there have now been some recent reports of immediate graft loss in the presence of a B-cell positive crossmatch, and we would suggest that in these cases the antibody is directed at DR (or ABC). In the successful B-cell positive crossmatch transplants (which make up the majority) the antibody is perhaps directed at non-DR antigens which are not found on endothelium.

Prolongation of canine segmental pancreatic allografts with cyclosporin A.

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This study was undertaken to assess the effectiveness of cyclosporin A (CyA) alone in prolonging normoglycaemia and graft survival in dogs given segmental pancreatic allografts with the duct system occluded with prolamine (Ethibloc), a new alcoholic amino acid solution. 70 unrelated dogs were divided into 6 groups. I. control dogs (25) provided the normal range of blood glucose levels, glucose tolerance test, and glucose degradation values. II. total pancreatectomy without a segmental graft (10). III. total pancreatectomy and segmental Ethibloc occluded pancreatic autotransplantation (12); in all segmental transplants the duct was filled with 0.2 ml of Ethibloc, and in addition an arteriovenous fistula was performed between the distal splenic artery and vein to avoid primary vascular thrombosis. IV, total pancreatectomy, segmental pancreatic allotransplantation, no immunosuppression (5). V. total pancreatectomy, segmental pancreatic allotransplantation-cyclosporin A 25mg/kg/body weight (oral solution) given indefinitely, commencing on the day of the transplant (10). VI total pancreatectomy, segmental pancreatic allotransplantation - cyclosporin A 40mg/kg/body weight (oral solution) given indefinitely as in V (8).

Results. Totally pancreatectomised dogs died at a mean of 6.3 days (range 3-12 days) and were never normoglycaemic.

	Group	Treatment	Graft Survival in days
III	Segmental pancreatic autotransplantation (12)	None	90,>100,>100,>100,>100,>100,>100 >100,>100,>100,>100,>100
IV	Segmental pancreatic allotransplantation no immunosuppressive treatment (5)	None	8,9,9,9,10
V	Segmental pancreatic allotransplantation (10)	CyA 25mg/kg	7,8,13,15,17,17,18,18,22,40
VI	Segmental pancreatic allotransplantation (8)	CyA 40mg/kg	6,8,9,>90,>100,>100,>100,>100

These results indicate that CyA alone can significantly prolong the survival of canine pancreatic segmental allografts, but in doses considerably greater than found to be effective in prolonging renal allograft survival in the dog (Homan et al. 1980).

Homan WP, French ME, Millard PR, Denton T, Fabre JW, Morris P.
Studies on the effect of cyclosporin A upon renal allograft rejection in the dog.
Surgery 1980: 88, 168-73.

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CYCLOSPORIN A CAN ABROGATE THE SENSITIZING EFFECT OF BLOOD TRANSFUSION IN RATS WITHOUT INTERFERING WITH ITS BENEFICIAL INFLUENCE.

In the BN/Rij to Wag/Rij Rat model, prolonged survival of organ allografts can be easily obtained by a single transfusion of donor-type blood. After optimal conditioning (more than 7 days before transplantation), BN/Rij heart transplants survive indefinitely, whereas kidney grafts show a marked prolonged survival time. In the Wag/Rij to BN/Rij donor-host combination, pretreatment with donor blood always results in accelerated graft rejection, even if the interval between transfusion and grafting is short (2-3 days). The present study was undertaken to investigate the influence of Cyclosporine A (Cy-A) on the blood transfusion effect in both combinations. Male rats of the inbred Wag/Rij $(RTI)^y$ and BN/Rij $(RTI)^n$ strains were used. Recipients were injected i.v. with 2 ml of citrated whole donor blood 7 days before heart transplantation. Cy-A was dissolved in olive oil and administered by i.m. injection. Recipients were given 15 mg.kg $^{-1}$.day $^{-1}$ Cy-A (in 0.5 ml olive oil) for 7 days, starting on the day of transplantation. Control rats were given 0.5 ml olive oil for the same period of time. It was found that administration of Cy-A did not interfere with the beneficial effect of a donor-specific blood transfusion in the BN/Rij to Wag/Rij combination. Indefinite graft survival was obtained whether or not Cy-A was given. In the reverse donor-host combination Cy-A was able to overcome the sensitizing effect of transfusion. A donor-specific transfusion given 7 days before transplantation led to accelerated rejection of heart allografts in the Wag/Rij to BN/Rij combination. However, if Cy-A was given in addition from the day of transplantation, indefinite graft survival was obtained. The remarkable immunosuppressive properties of Cy-A have been extensively reported. Although the prolonging effect of Cy-A on graft survival in nonconditioned recipient is well established there are conflicting data on its effect in primed hosts. Our results obtained in the Wag/Rij to BN/Rij combination favour the opinion that Cy-A can be very effective in abrogating sensitization.

BENEFICIAL INFLUENCE OF CYCLOSPORINE-A ON KIDNEY GRAFT SURVIVAL IN RHESUS MONKEYS, WITHOUT CHANGING THE POSITIVE "BLOOD TRANSFUSION EFFECT".

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In clinical and experimental transplantations, Cyclosporine-A (Cy-A) has been shown to be a very powerful immunosuppressive agent. However, as there was also evidence of side effects, a preclinical study in rhesus monkeys was started to determine the influence of different dosages of Cy-A on kidney graft survival if given as the only drug or in combination with conventional immunosuppression (i.s.: 2 $mg.kg^{-1}$ azathioprine and 1 $mg.kg^{-1}$ prednisolone, daily). It seemed further of interest to investigate what effect Cy-A would have on the beneficial influence of pretransplant blood transfusions. Heterotopic kidney transplantations were performed in unrelated rhesus monkeys which were matched with their donors for 1 DR antigen. The animals received three pretransplant blood transfusions. Administration of Cy-A and other i.s. was performed daily for only 3 weeks after grafting. Results clearly showed that there was no difference in graft outcome between animals treated with 10 or 25 $mg.kg^{-1}$ Cy-A. With both dosages, a perfect immunosuppressed state was achieved even 1-2 weeks after cessation of treatment, without signs of side effects. The administration of conventional i.s. in combination with Cy-A had an additive favourable influence: recipients treated with the combined therapy survived significantly longer than animals which received Cy-A only ($p < 0.01$). In addition, it was found that the administration of Cy-A to transfused recipients led to survival times which were in the same range or sometimes even better than was achieved previously in transfused monkeys without Cy-A.

Definately NOT signif

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Are pretransplant blood transfusions beneficial to all renal allograft recipients?

Third party pretransplant blood transfusions (PBT) have a profound influence on renal allograft survival. Although a beneficial effect of unknown mechanism generally prevails, unwanted side effects may occur, such as sensitization of the prospective recipient against the potential kidney donor. Preëxistent antibody reactivity against the donor can be circumvented by careful cross-matching; the cellular immune reactivity against the donor is more difficult to assess. As, in spite of PBT, many recipients still reject their grafts, while some other untransfused recipients do not, it is questionable whether PBT are beneficial for all patients. We found that PBT significantly prolong renal allograft survival in immunosuppressed dogs; this model is used in our laboratory in an attempt to define an optimal transfusion protocol. In this study renal allografting was performed in DLA 2 haplotype different (2 DLA), DLA 1 haplotype different (1 DLA) and DLA identical (0 DLA) littermate (lit) and non-littermate (non-lit) donor-recipient pairs (DRP's). Tissue typing was done using routine serological methods and mixed lymphocyte reactions (MLR's). Transfusion of 100 ml of whole blood from third party donors were given 4, 3 and 2 weeks prior to transplantation. Kidney grafting was performed irrespective of the outcome of the cross-match test. Azathioprine, 2 mg/kg bw/day, and prednisolon, 1 mg/kg bw/day, was given postoperatively during 100 days, and gradually withdrawn thereafter in 50 days. The results are summarized in the table.

mismatch	NOT TRANSFUSED N	median survival time (days)	% rejection*	TRANSFUSED N	median survival time (days)	% rejection*	EFFECT OF PBT ON GRAFT SURVIVAL
2 DLA lit	7	13	100%	5	168	>60%	prolongation ($p<0.05$)
1 DLA lit	7	16	70%	10	108	60%	prolongation (NS)
0 DLA lit	6	>300	17%	10	300	50%	shortening ($p<0.05$)
0 DLA non-lit	7	>300	12%	9	184	78%	shortening ($p<0.05$)

* cut-off point arbitrarily chosen at 300 days.

Conclusions: The beneficial effect of PBT is dependent on the matching grade. After drug withdrawal, transfused recipients reject a DLA identical kidney more often than untransfused recipients. Cross-match tests, nor MLR's, are predictive in this respect. Immunization against minor histocompatibility antigens of the kidney donor, caused by third party PBT, must be responsible for this effect. Thus, PBT can be harmful. Deliberate PBT to prospective recipients of matched living related donors, and to patients with common phenotypes, waiting for HLA-DR identical cadaveric kidneys, may therefore not contribute to better long term renal transplant survival statistics.

A dose effect of pretransplant blood transfusions on renal allograft survival immunosuppressed dogs.

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Pretransplant transfusions of third party blood have a beneficial effect on renal allograft survival in man and experimental animals. In some clinical studies a dose effect of blood transfusions has been reported; the effect of a single transfusion is limited or even absent. However, others report a strong beneficial effect of one single transfusion. A reversed correlation between transfusion free interval and transfusion effect has been suggested by some authors. Even one single peroperative transfusion, can have a beneficial effect. Limitation of the number of third party blood transfusion will reduce the chance of sensitization against a prospective donor. In this study DLA mismatched non related kidneys are transplanted in dogs. Azathioprine 2 mg/kg bw/day and prednisolone 1 mg/kg bw/day were given postoperatively until day 60 and diminished gradually until discontuniation at day 110. Three transfusions of 100 ml of fresh blood from three different third party donors were given 4, 3 and 2 weeks prior transplantation (group IV). One third party blood transfusion of 100 ml was given 2 weeks prior transplantation (group III), and peroperatively (group II). Nontransfused dogs were used as controls (group I). Positive crossmatch tests before operation did not exclude dogs from transplantation. Median survival times of the transplanted dogs are given in the table.

Groups	Transfusion	N	Median Survival Time (days)	Positive crossmach test (number of dogs)
I	No	25	19	-
II	1 perop.	5	12	-
III	1 preop.	10	14.5	3
IV	3 preop.	10	57	4
			($p < 0.001$)	

An effect of blood transfusions was only seen when three blood transfusions were administered. One transfusion given either pre-or peroperatively was not effective. The degree of sensitization, as expressed by positive crossmatch tests was not different after one or three transfusions. These data indicate that a certain number of blood transfusions is required to obtain a beneficial effect in immunosuppressed dogs. Timing seems to be important as well; this is presently under study.

Title: The effect of HLA-A and -B compatible blood transfusions on kidney graft survival

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The observation that pretransplant blood transfusions given to kidney patients improve kidney graft survival, is well accepted. However, no concensus has been reached on the optimal number of transfusions. Opelz found that the more transfusions the better the kidney graft survival. To avoid the problem of developing lymphocytotoxic antibodies, 20 dialysis patients were prospectively transfused with 2 or 3 units of HLA-A and -B compatible blood. Two control groups are available. The first consists of patients who were transplanted before 1977 in the same center and did not receive any blood transfusion before transplantation. The second consists of patients who were transplanted before and after a patient from the protocol group. All these patients had received random blood transfusions prior to transplantation. Fourteen out of the 20 protocol patients were transplanted and have a 9 months follow up period. Graft survival is 94% and differs significantly from graft survival in the non-transfused control group ($p < 0.001$). Compared to the second control group there is a slightly better graft survival in the protocol group, although not statistically significant. No differences in the mean number of rejection periods nor in patient survival were observed in these two groups.

On the basis of these preliminary data, one could speculate about the mechanism responsible for the "transfusion effect". From our data it can be concluded that non-HLA-A and -B antigens present on the leucocytes, seem to play an important role in the favourable effect of blood transfusions on cadaveric kidney graft survival.

THE EFFECT OF BLOOD TRANSFUSION AND HLA-DR MATCHING ON KIDNEY TRANSPLANT SURVIVAL ... . WESSEX REGION.

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The effect of blood transfusion and the HLA DR matching on kidney graft survival has received considerable attention in recent years. The beneficial effects claimed by some workers for pre-transplant blood transfusion are disputed by others. The effect of pre-graft blood transfusion on the first 150 cadaver donor transplants performed in the region has been analysed after exclusion of 13 non-immunological/technical failures. 82 of the later transplants in the series, matched originally on the basis of the HLA A & B locus only, are the subject of the DR analysis. Blood transfusion was given in the form of whole blood or packed cells. No attempt was made to give leucocyte free blood and frozen blood was not used. DR typing was performed on separated donor spleen B lymphocytes, whilst the recipient DR type was determined either before or at the time of transplant. The DR technique was that recommended for the 7th Histocompatibility workshop. Separation of the B cells was by T rossetting with neuraminidase treated sheep red cells. Antisera were available to measure DR1 - DRW8.

Our data shows no beneficial effect of pre-transplant blood transfusion in our region:-

Graft survival:-	3 months	6 months	12 months	
Transfused (103 cases)	64%	59%	53%	p = not significant
Non-transfused (34 cases)	70%	62%	47%	

A separate analysis which includes per-operative blood transfusion does not alter these figures.

Those patients receiving a DR-well-matched kidney had significantly better graft survival than those who received donor incompatibilities although the DR well matched transplants tended to have less HLA incompatibilities (Average 1.2) than those with DR incompatibilities. (Average 1.6)

Graft survivals were as follows:-

No. of DR incompatibilities	3 months	6 months	12 months
0	94% (18)	94% (17)	84% (13)
1	59% (39)	55% (34)	37% (24)
2	55% (25)	41% (22)	37% (18) (patients at risk)

p = 0.0005 at all points

The effect of DR incompatibilities on the survival of second and subsequent grafts (24 patients) is shown below.

No. incompatibilities (DR)	3 months	6 months	12 months
0	8 (8)	8 (8)	6 (6)
1	2 (8)	2 (8)	1 (6)
2	3 (8)	1 (7)	1 (6)

The number of grafts surviving is shown and grafts at risk are included. Although the number of cases in each group is small our findings are clearly in agreement with data from the 8th Workshop, in that patients receiving a second or subsequent transplant had superior graft

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B-lymphocyte antibodies and human kidney survival

After immunisation by blood transfusions, lymphocytotoxic antibodies are detectable in the serum of approximately 50% of patients on chronic haemodialysis. These antibodies may differ in reactivity with subpopulations of lymphocytes (T- or B-lymphocytes). If the antibodies are not lymphocytotoxic for donor T-cells but only for donor B-cells, kidney transplantation might be a safe procedure in many instances.
The antibody frequencies in haemodialysis patients before first transplantation are given in Table I:

	Females	Males
T-lymphocyte antibodies	3/30 (10%)	0/42 (0%)
B-lymphocyte antibodies	3/30 (10%)	12/42 (28%)
T-B-lymphocyte antibodies	13/30 (43%)	4/42 (10%)

Before first transplantation, 53% of female and 38% of male patients had B-lymphocytotoxic antibodies in the serum, in some cases in combination with HLA-ABC-antibodies against T-cells.
After transplantectomy (24 patients), the prevalence of polyvalent non-specific B-cell antibodies was 100%.
For 34 patients with B-cell antibodies before transplantation, crossmatches with separated T-B lymphocytes of the kidney donor were performed. In 7 patients, the B-cell crossmatch was negative, in 27 patients (80%) positive. In 2 patients, lymphocytotoxic auto-antibodies were also detectable.

Transplant-survival and positive donor-B cell crossmatch

Follow up (months)	Controls Number	Controls Survival(%)	Positive B-cell "X-match" Number	Positive B-cell "X-match" Survival(%)
3	226	82	21	80
6	206	79	16	78
12	174	76	9	78
24	130	73	3	78

The results demontrate that transplantation across a B cell-positive, T cell-negative crossmatch is a safe procedure. Only four of 27 patients with a positive B-cell crossmatch rejected their kidney, none of them in a hyperacute fashion. In 16 of these 27 patients, the standard NIH-crossmatch on unseparated lymphocytes was also positive. This implies that for patients with B-cell antibodies, Organ Exchange Programs that use standard NIH-crosmaatches for donor-recipient selection are disadvantageous. Many of these patients could have been transplanted safely if the proper crossmatches had been performed.

TRANSFUSION INDUCED PLASMA SUPPRESSIVE ACTIVITY - EFFECT ON GRAFT SURVIVAL.

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It is well established that pre transplant blood transfusions benefit kidney graft survival. The mechanism of this graft protective effect is poorly understood.

In chronic renal failure patients we have associated transfusion with an elevation of non specific plasma immunosuppressive activity. Immunospecific affinity chromatography studies on plasma from transfused dialysis patients has linked plasma suppressive activity (PSA) with the plasma protein α_2 macroglobulin (α_2M).[1]

In a prospective study on 87 unselected renal transplant patients we have investigated the relationship between pre transplant blood transfusions, PSA measured at the time of transplantation and subsequent graft survival. All patients except 2 had been transfused preoperatively with between 1 and 52 units of whole blood or packed cells. PSA was measured using an electrophoretic technique and results expressed as the mean number of µl of recipient plasma required to suppress by 50% the response of a panel of allogeneic lymphocytes to the antigen PPD. Actuarial graft survival curves (minimum follow up 3 months) were compared for patients exhibiting a high PSA (<5µl) and a low PSA (>=5µl).

RESULTS.

Graft survival (excluding 10 non immunological failures)

	n.	3 months	6 months	12 months	
High PSA	47	100%	95%	92%	
Low PSA	30	70%	70%	62%	
		$p < .01$	$p < .05$	$p < .05$	(analysis according to Barnes)

In a separate study the PSA of 10 non transplanted and non transfused dialysis patients were measured. This was done before and at intervals after the transfusion of 3 units of whole blood. All patients developed a significant elevation of PSA after transfusion. Immunospecific absorption of α_2M from the plasma of these 10 patients and from the 87 transplanted patients removed the majority of the suppressive activity. In common with other works[2] we conclude that α_2M is immunosuppressive. Plasma suppressive activity rises after transfusion and is associated with a significant improvement in early graft survival.

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Title : Cell Mediated Lympholysis (CML): an in vitro model of the renal allograft reaction.

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Cell mediated lympholysis (CML) non-reactivity in recipients of related and unrelated donor kidneys has been described by several investigators. We have studied CML-activity in more than 80 unrelated kidney donor - recipient combinations. Thirty-six of them were studied longitudinally: i.e. CML-activity of the recipient lymphocytes from different pre- as well as posttransplantation times was measured against the splenocytes of the specific kidney donor.

The development of CML non-reactivity occurred in more than 70% of the kidney recipients and was significantly correlated with good graft survival. Contrarily, patients who rejected their renal allograft were CML-reactive.

Several influences (like: immunosuppressive drugs, the number of pre-operative blood transfusions, involvement of suppressor cells) has been investigated which may be responsible for the occurrence of the CML non-reactivity. Variables such as HLA-A, -B, -DR match, MB match, sex, the degree of presensitization have been analyzed. Our results indicate that the development of donor specific CML non-reactivity seems to correlate with the clinical situation of the patient. Furthermore, variables which predispose significantly for the development of CML non-reactivity are compatibility for the HLA-B antigens between donor and recipient and sex. Furthermore, no cytolytic capacity of the lymphocytes from 7 recipients after pool stimulation could be observed against the specific kidney donor splenocytes, although there was a normal reactivity against the control and pool cells. However, absence of cell lysis after pool stimulation could also be demonstrated in those effector-target combinations which were HLA-B locus compatible.

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POSTTRANSPLANT MONITORING OF CELL MEDIATED CYTOTOXICITY TOWARDS DONOR SPECIFIC KIDNEY CELLS.

Kidney graft survival, even between HLA identical siblings, depends on continuous immunosuppression and even then rejection still occurs occasionally. Moreover definite graft survival has been reported to occur in recipients of HLA mismatched kidneys. These observations indicate that also non-HLA antigens are involved in kidney transplantation. In the clinical situation non-HLA antigens have been demonstrated on endothelial cells with serological methods.

We developed a cell mediated cytotoxicity test directed towards non-DLA antigens present on dog kidney cells. Kidney cells were obtained by a wedge biopsy during transplantation. In vitro cultured kidney cells has been used as ^{51}Cr labeled target cells for in vitro as well as in vivo generated effector cells. Adsorption studies using different types of monolayers consisting of leukocytes, PHA blasts and kidney cells, revealed antigens, which were not detectable with the normal cell mediated cytotoxicity assays, using PHA blasts as targets. With this new assay we could clearly demonstrate precursor CTL as well as a direct cell mediated cytotoxicity after transplantation directed towards donor kidney cells. The relevance of these tissue specific CTL detected after transplantation and their relation with rejection will be discussed.

NATURAL KILLER CELL ACTIVITY IN HUMAN RENAL ALLOGRAFT RECIPIENTS

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Natural killer (NK) cells are Fc-receptor positive lymphocytes (1) which may be closely related with the cells mediating antibody-dependent cellular cytotoxicity (2) and may also play a role in immunological surveillance against malignant disease. Human NK activity may be diminished in the first 50 days after transplantation (3) but there are no reports on the NK cell activity of long term renal allograft recipients. We have examined NK activity against the myeloid cell line K-562 in patients with stable renal function between 4 months and 9 years after transplantation

Mean % specific ^{51}Cr release from ^{51}Chromium labelled K562

		Effector:target cell ratio			
		100:1	50:1	25:1	12:1
Control subjects (n = 22) (mean age = 32.9 years)	Mean ± S.D.	63.8 ± 8.8	53.2 ± 9.7	42.9 ± 11.4	32.7 ± 13.2
Dialysis patients (n = 15) (mean age = 34.5 years)	Mean ± S.D.	59.1 ± 9.9	49.1 ± 10.7	40.1 ± 11.5	29.6 ± 13.2
Transplant patients (n = 22) (mean age = 40.4 years)	Mean ± S.D.	19.6 ± 21.0	15.4 ± 17.3	11.3 ± 14.1	7.2 ± 10.8

By non-parametric analysis the NK cell activity of the transplant patients was significantly lower than that of both the control subjects and the dialysis patients ($p<0.002$). However some transplant patients had normal NK activity and it was found that the NK activity of the 13 patients who were less than 48 months post-transplant was significantly lower than that of the 9 patients who had been transplanted for longer than 48 months ($R = 69.5$, $p< 0.05$).

These results suggest that the impaired NK cell activity seen in human renal allograft recipients may, in some cases, be a reversible phenomenon in the long-term.

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TREATMENT OF ACUTE RENAL ALLOGRAFT REJECTION WITH RABBIT ANTI-HUMAN THYMOCYTE GLOBULIN (RATG)

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In a prospective therapeutic trial 20 patients with acute rejection were treated with RATG (2-5 mg/kg) and the results were compared to those in a group of 20 patients who received high, oral doses of prednisone for their rejection crisis, and to those in a group of 28 patients who never experienced a rejection. In the RATG-group the prednisone dose was not increased and a dose-by-rosette protocol was used to keep T cell levels between 50 and 150 per mm^3. In this group 15 of the 20 patients responded to the treatment. In five patients rejection was irreversible despite a consequent course of high-dose prednisone orally. One patient lost her kidney due to a technical failure. In the Prednisone-group 13 patients showed a good response, but three of them only after a subsequent course of RATG. The remaining seven patients in the Prednisone-group underwent nephrectomy for irreversible rejection, before a course of RATG could be given. One patient in this group died due to sepsis. In either group there were six second rejection-episodes, but they developed later in the RATG group. Renal function after three and six months was similar in all three groups studied. Less infections were observed in the RATG-group.

Prior to rejection, both treatment groups did not have higher concentrations of circulating T cells than the 28 patients who never experienced a rejection. Circulating lymphocytes and T cells did not decrease during prednisone treatment. In the patients who responded to RATG, the T cells dropped to the desired levels during the treatment course with an immediate rise after discontinuation of RATG. The results demonstrate that treatment of acute rejection with RATG is a safe procedure and that it is effective in most cases. Rejections that did not respond to RATG were also resistant to high doses of prednisone. Conversely, some patients still responded to RATG after a course of high prednisone had failed. Furthermore, RATG treatment has the advantage of being steroid-sparing.

RENAL GRAFT RUPTURE. REPORT OF SEVEN CASES

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In a series of 350 consecutive kidney transplantations (KT), graft rupture (GR) occurred in 7 female recipients of cadaveric kidneys, aged 26-50 years. In spite of a previous graft (3 patients), of 1-7 pregnancies (6 patients) and/or of multiple blood transfusions (6 patients), only 1 recipient had significant titer of cytotoxic antibodies. All 7 ruptures occurred 3-7 days following KT, during an acute rejection episode with severe oliguria, the first symptoms supervening during or shortly after a hemodialysis session in 5 cases. One patient with gross hyperacute rejection underwent immediate nephrectomy. In the 6 other cases, the kidney was sutured, the hematoma drained, and corticotherapy increased. Postoperative complications consisted of local abscess in 3 patients, responsible for staphylococcal endocarditis in 2. In 1 patient, irreversible rejection led to graft removal 44 days after GR. Despite good kidney function, late infections led to graft removal 39 days postoperatively in 1 patient (rupture of mycotic aneurysm at arterial anastomosis), and to death in 1 patient (pulmonary mycosis) 107 days postoperatively. In the 3 remaining cases, evaluated 180, 732 and 1877 days postoperatively, serum creatinine ranged from 0.8 to 1.7 mg/dl. In conclusion, GR complicating early acute rejection may be successfully treated by conservative surgical management and increased corticotherapy, with an acceptable risk of infectious complications.

KIDNEYS FOR TRANSPLANTATION FROM PERIPHERAL HOSPITALS

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In an attempt to facilitate the retrieval of kidneys for transplantation, Surgical Research Fellows were appointed in Nottingham and Leicester in October 1979. Their chief duty was to attempt to increase the number of kidneys from peripheral hospitals, that is those not closely associated with the transplant units.

A marked increase in the number of kidneys obtained from peripheral hospitals is seen while in the number of kidneys from the central hospitals, associated with the transplant programme, is maintained:-

	NUMBER OF KIDNEYS			
	Central Hospitals	Peripheral Hospitals	Total	National Total
October 1978 - September 1979 Before appointment of Research Fellows	24	11	35	879
October 1979 - September 1980 After appointment of Research Fellows	29	27	56	974

Since the appointment of the two surgical Research Fellows, the number of kidneys retrieved in the area has increased by 60%, compared to a national increase of 10.8%. In addition, fewer kidneys have been wasted and multiple organ donation has been facilitated.

	Corneas	Hearts	Livers
October 1978 - September 1979	0	0	0
October 1979 - September 1980	28	2	2

The B.B.C. Panorama documentary, "Are the donors really dead?" was broadcast in October 1980. From that time there has been a marked change in the attitude of relatives to organ donation. In the 12 months prior to October 1980, the relatives of 2 out of 30 potential donors withheld consent to the removal of organs. In 3 months since October 1980, the relatives of 5 out of 8 potential donors have refused consent.

RENAL TRANSPLANTATION (TP) IN PATIENTS ON CONTINUOUS AMBULATORY PERITONEAL DIALYSIS (CAPD)

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There is reluctance in some units to transplant patients on CAPD because of the fear of infection, unknown graft survival and increase in peritoneal vascularity. Since January 1979, 72 patients have been managed by CAPD in this unit. 12 of these patients aged 10 to 57, received first grafts (2 living related; 10 cadaver). They were transplanted (mean 5.25) 1.5 to 18 months after being on CAPD. 8 patients are well with good graft function (mean creatinine 134 umol/L ± 53), 10.5 months (5-18) months after TP. One recipient died of a pulmonary embolus 5 months after TP with a functioning graft, while 3 recipients lost their grafts from rejection 2-12 weeks post TP and reverted to CAPD successfully. Over the same period of time there were 55 predominantly haemodialysis (HD) patients who received first grafts. In this group there were 23 graft failures (15 rejection; 9 non-immunological). There was no significant difference in the actuarial graft survival in the two groups.

Intraperitoneal catheters were removed in 10 patients 2-12 weeks after TP. They were used for dialysis in 2 patients immediately after TP. There were no episodes of peritonitis post TP. No major technical problems were encountered at surgery.

CAPD is not a contraindication to TP. There have been no problems with the catheter or peritonitis and graft survival has been comparable to HD patients receiving TP.