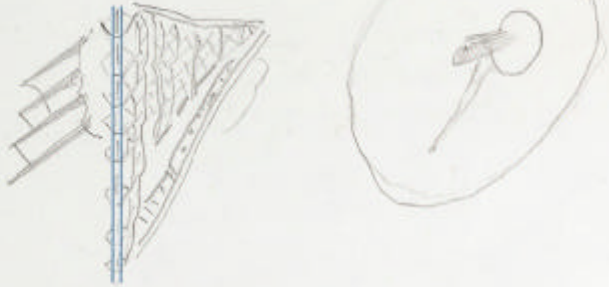
WOOD, PRINTER SOUTHWELL, NOTTS.

British Transplantation Society



1978 SPRING MEETING

ROYAL FREE HOSPITAL

Wednesday 29th March, 1978

TIMETABLE

10.00 a.m.	PAPERS 1 — 4 (Transplantation Biology) Chairman: J. Sachs
11.00	Coffee
11.30	PAPERS 5 — 10 (Clinical Transplantation) Chairman: J. R. Salaman
1.00 p.m.	Sandwich Lunch
2.00	Business Meeting
2.45	PAPERS 11 — 14 (Detection of Rejection) Chairman: R. W. Blamey
3.45	Tea
4.00	PAPERS 15 — 18 (Immunosuppression) Chairman: J. Castro
5.00	Society Reception and Buffet

1. PROLONGED SURVIVAL OF VEIN ALLOGRAFTS IN THE RAT

F. J. Prendergast, J. K. McGeachie, J.W. Fabre, C. G. Winearls and P. J. Morris

Nuffield Department of Surgery, Radcliffe Infirmary, Oxford

Segments of ilio-lumbar veins (0.5 - 0.75 cm in length) were interposed in defects created in the left common iliac artery, using micro-surgical techniques. Graft patency was assessed by palpation in all cases, and by direct inspection in selected cases. Vein allografts were performed in the Lewis ⟶ DA and DA ⟶ Lewis combinations with Lewis ⟶ Lewis isografts serving as controls.

Between 10 and 15 grafts have been performed in each of three groups, and the grafts have been followed for a minimum of ten weeks. Apart from technical failures, all the grafts have remained patent throughout the period of observation. The grafts were macroscopically normal on inspection, without aneurysm formation.

Antibody responses to the grafts over the first three weeks have been studied in the DA ⟶ Lewis combination using a Cr^{51} release lymphocytotoxicity assay. These show a strong lymphocytotoxic response at weeks two and three.

Histological examination of the vein allografts is in progress. Groups of 5 are being sacrificed at weeks 1, 2, 4, 10, 18 for light microscopy and ultrastructural studies. More detailed studies of the antibody response to the vein allografts are in progress and localisation of antibody in the graft is to be examined by immunofluorescent techniques.

However, it does appear that gross damage to a vein allograft does not occur, even across a strong histocompatibility barrier, despite a vigorous humoral response to the graft.

2. A QUANTITATIVE COMPARISON OF WHOLE ANTIBODY AND F (ab')$_2$ IN KIDNEY ALLOGRAFT ENHANCEMENT

C. G. Winearls, P. R. Millard, J. W. Fabre and P. J. Morris

Nuffield Department of Surgery, Radcliffe Infirmary, Oxford.
Department of Pathology, Radcliffe Infirmary, Oxford.

The use of F(ab')$_2$ instead of whole antibody has been suggested as a method of avoiding hyperacute damage to renal allographs during the induction of passive enhancement. We have compared the efficacy of F(ab')$_2$ and whole antibody in the Lewis ⟶ DA renal allograph enhancement model. A F(ab')$_2$ preparation which was >99% pure was shown to be ineffective at doses 10x greater than whole antibody, making its clinical use impractical. However F(ab')$_2$ did not block the induction of enhancement by whole antibody.

Treatment	No. of Rats	Median blood urea (mg/100ml) ± SD at day 10	Median survival (days)
Nil	5	760 ± 62	11
0.38 mg IgG	5	121 ± 75	> 30
3.0 mg F(ab')$_2$	6	632 ± 83	10.5
3.0 mg F(ab')$_2$ *plus* 0.38 mg IgG	4	95 ± 29	< 30

3. "Ia LIKE" ANTIGEN ON RAT KIDNEY

D. N. J. Hart and J. W. Fabre
Department of Pathology, Radcliffe Infirmary Oxford

Ia antigens show a restricted tissue distribution in the mouse, being found on B cells, macrophages, epidermal cells, sperm cells and some T lymphocytes. The presence on non-lymphoid organs has not been adequately studied and they have not yet been demonstrated on kidney tissue.

We examined the tissue distribution of Ia antigens in the DA rat strain using an exhaustively red cell absorbed Lewis (H-1f) anti DA (H-1a) serum. In backcross analysis with an indirect radioactive binding assay this serum was shown to detect the products of a single locus. Quantitative absorption analysis with the binding assay, using DA lymph node lymphocyte targets were used. These showed, that liver, heart and brain had little Ia antigen whereas kidney tissue had 10 - 20% the amount of Ia compared with lymph node lymphocytes. The kidney tissue completely removed the Ia activity, showing that all the Ia specificities assayed for were present on kidney.

The significance of these findings in the context of renal allografts rejection and enhancement will be discussed.

4. EXPERIMENTAL SKIN TRANSPLANTATIONS IN IMMUNIZED RECIPIENTS:

M. Jonker, C. Koch, A. Blussevan Oud Alblas and J. J. van Rood.
Department of Immunohaematology, University Hospital, Leyden

A study of the influence on skin graft survival of previous immunization and multiple grafts on the recipent:

1) The data of 36 immunized recipients were compared with those of 37 non-immunized recipients. Recipients were immunized by bloodtransfusions, leukocyte injections or pregnancies. The table shows the data of transplants exchanged between MLC positive individuals. Previous immunization caused a shortening in survival time if the immunizing and skin donors shared an HLA-A or -B antigen. The variance in the mean survival times was significantly larger in all three groups of immunized recipients as compared with the non-immunized recipients. This larger variance could be attributed to: a) Sensitization for HLA-A and -B. b) Sensitization for HLA-D or -Dr. This could be demonstrated in the group of recipients that received HLA-A and -B identical skin grafts. A longer survival (mst 13.2 ± 5.8) was observed when B-cell specific antibodies against donor cells were present in pre-transplant serum of the recipients than when no such anti-bodies were demonstrable (mst 11.3 ± 3.8). c) Sensitization for other, non HLA associated antigens may have caused either rapid graft rejection or prolongation of graft survival.

HLA compatibility donor/recipient	HLA-A or -B shared by immunizing and skin donor	mean survival times in days $\pm$ S.D. (n) immunized	non-immunized
HLA-A,B =	yes	7.2 ± 5.3 (12)	10.2 ± 0.9 (18)
	no	12.0 ± 3.4 (16)	
HLA-A,B =		12.3 ± 4.8 (9)	11.7 ± 0.6 (19)

2) The influence of multiple skin grafting. When an MLC positive skin was transplanted simultaneously with an MLC negative skin, the survival of the MLC negative skin grafts was significantly shorter than the survival of MLC negative skin grafts transplanted alone. This indicates that an MLC positive graft could provide help for a more rapid rejection of an MLC negative graft.

5. TRASYLOL (APROTININ) AND KIDNEY PRESERVATION

A M. Godfrey & J. R. Salaman
K.R.U.F. Institute of Renal Disease, Cardiff

In vitro experiments have shown that Trasylol can protect the metabolism of kidney slices from the harmful effects of ischaemia (1). We have administered Trasylol in vivo to rats and sheep to see whether it could prevent acute tubular necrosis from occuring after similar periods of ischaemia. For the rat experiments Trasylol (2.500 KiU/Kg) was given intravenously to anaesthetised rats prior to clamping of the right renal pedicle. Control animals were injected with saline and in all experiments a contralateral nephrectomy was carried out after the clamp was removed. Clamping for one hour produced no ill effects in either group but two hours caused 100% mortality in both groups. After one and a half hours 40% of the Trasylol group died which was significantly less than the control group. (80% $p > 0.02$). In the sheep experiments 100,000 KiU of Trasylol or Saline was given intravenously prior to removal of the right kidney. This was placed in a water bath (39.5°C) for 45 minutes and then implanted into the neck of the same sheep. Renal function was determined by collecting the excreted urine. The six kidneys obtained from Trasylol treated sheep all functioned after re-implantation although clearance values were reduced. However only two of seven saline treated kidneys produced urine subsequently. Before replantation both groups of kidneys were flushed with Collins solution containing ^{131}I-albium. Ultrafiltrate was collected from the ureter and it was found that in the control group there was a 33% leakage of albumin into the ureter. In the Trasylol group this leakage was only 5.7% ($p < 0.0025$) and this would confirm other work which has shown that Trasylol helps to preserve normal capillary permeability.

1. Truss, F. (1972)
New Aspects of Trasylol Therapy. 5, 285.

6. PERFUSION PRESERVATION OF HUMAN CADAVERIC KIDNEYS

F. K. Merkel, S. K. Seim, K. E. Maisel, and P. J. Volek
Department of Surgery, Section of Transplantation, Rush Presbyterian-St. Luke's Medical Center, Chicago

With increasing numbers of patients on dialysis and with the beginnings of world-wide kidney sharing, it is critical to analyze the factors thought to be important in selecting cadaveric kidneys.

277 cadaver kidneys were procurred at 45 hospitals from heart-beating brain-dead donors by a single team, preserved at a single center and distributed to 37 hospitals. Fourteen kidneys were not suitable for transplantation. Donor homeostasis was achieved with 25% salt-poor albumin, crystalloid and vasopressor. Pretreatment was 30 mg/kg methylprednisolone, heparin 20,000 units, diuretics, and phenoxybenzamine. The kidneys were perfused in-situ with iced Collins' solution and immediately preserved en-bloc by pulsatile perfusion using a special cryoprecipitated plasma.

These factors were evaluated regarding short and long term success:

1. **Preservation Time** — Kidneys preserved 4-23 hours, 24-35 hours and 36-37 hours all exhibited a one-month function of 70% and one-year of 40%. Patients receiving kidneys preserved 36-67 hours required more dialysis post transplant; however, function rates were unchanged.
2. **Creatinine** — Kidneys removed from donors with serum creatinines of 0.1 to 1.5 mg/dL, 1.6 to 3.0 mg/dL or greater than 3.0 mg/dL all worked equally well.
3. **Vasopressors** — There were no differences whether or not donors received vasopressors of any type.
4. **Perfusion Characteristics** — Average flow was 230 ml/min/kidney (range 55-500). Four kidneys (1.4%) were discarded for poor flows. Ten percent of the kidneys exhibited a rise ≥ 5 mm Hg during preservation; 35% of those patients required dialysis and 6% of those kidneys failed because of non-immunological reasons.

Cadaveric kidneys treated by these methods survived equally well despite differences in preservation time (≤ 67 hours), use of vasopressors, serum creatinine, or profusion characteristics. Such kidneys should provide a basis for confident world-wide sharing programs.

7. AUTOLOGOUS AND HOMOLOGOUS LYMPHOCYTE SUPPRESSION BY PLASMA FOLLOWING MULTIPLE TRANSFUSIONS

Proud, G., Shenton, B. K., Smith, B. M., Taylor, R. M. R.
Department of Surgery, Royal Victoria Infirmary, Newcastle

This centre, among others, has reported improved renal graft survival in patients to whom previous blood transfusions had been given (1). Murray, et al. noted a possible benefical effect of preformed cytotoxic antibodies in these patients (2).

This study investigated the in vitro suppressive effect of plasma from multiple transfused (untransplanted) renal failure patients on the response of autologous and normal homologous lymphocytes to antigen, PPD, (Purified protein Derivative of mycochacterium tuberculosis). The tanned sheep erythrocyte electrophoretic mobility (TEEM) test was used in calculating plasma inhibitory activity (3). This was standardised by measuring the plasma concentration at which 50% inhibition of the lymphocyte response to PPD occurred. Non-transfused (untransplanted) renal failure patients acted as controls.

	50% lymphocyte inhibition by plasma			
Renal failure – no transf.	*Conc. (x10–3)*	*Dil.*	*S.D.±(x10-3)*	Number
a) Homologons lymphocytes	8.4	1/119	1.39	12
Renal failure – multiple transf.				
c) Autologous lymphocytes	0.68	1/1470	0.2	11
b) Homologous lymphocytes	1.8	1/556	0.3	7*
t test : a x b $p<0.001$				
b x c $p<0.001$				
a x v $p<0.001$				

*Homologous and autologous lymphocyte testing on transfused patients used identical plasma sample. Autologous lymphocyte testing was done at a later date and the plasma stored. Four patients received transplants in this time and were excluded.

Plasma from transfused patients suppressed autologous lymphocytes to a lesser degree than homologous lymphocytes. All the transfused patients had preformed cytotoxic antibodies, but not to the test lymphocytes, on microcytotoxicity testing. Cytotoxic antibodies may account for the greater inhibitory effect on homologous lymphocytes. This may be due to a lymphocyte blocking — rather than a lymphocytotoxic — property of immunoglobulins, and may explain Murray's earlier findings.

1) Uldall, P.R., et al. Lancet. 1977. *ii*: 316.
2) Murray, S., et al. Tissue Antigens. 1974. *4*: 548.
3) Shenton, B.K., et al. J. of Immunological Methods. *14*: 123.

8. MATCHING FOR B-CELL ANTIGENS OF THE HLA-DR (D-RELATED) SERIES IN CADAVERIC RENAL TRANSPLANTATION

A. T. Tine & P. J. Morris
Nuffield Department of Surgery, Radcliffe Infirmary, Oxford

The influence of HLA-D matching on the outcome of renal allographs is not fully known. So far HLA-D compatibility has only been measured indirectly by the degree of reactivity in the mixed lymphocyte culture (MLC) test between donor and recipient. Even if the MLC test does have prognostic value (and the reports are conflicting) it cannot be used prospectively with cadaveric donors because of the 5-7 days needed for completion of the test.

The serologically detected HLA-DR antigens which have recently been defined are either identical or closely related to the HLA-D determinants. Typing for these DR antigens may give the relevant direct information on the role of HLA-D in renal transplantation.

We have retrospectively typed 84 donors and recipients for 7 HLA-DR antigens. Only 4 recipients shared 2 DR antigens with their donor and all transplants are functioning from 5 to 19 months after operation. The group of recipients who shared 1 antigen with their donor have both a higher rate of success and a better quality of function (as judged by serum creatinine levels) than the group with 0 antigens shared. Although the differences do not reach statistical significance we feel that a continuing prospective study is justified with particular emphasis on performing transplants where the donor and recipient share 2 HLA-DR antigens.

9. PROGNOSIS OF CADAVER KIDNEY GRAFTS WITH A REJECTION EPISODE OCCURRING WITHIN TWO WEEKS OF TRANSPLANTATION

M. Cochran, G. Swarez & M. Fox

The Transport Unit, Royal Hospital, Sheffield

Rejection following renal transplantation may occur at any time but most commonly within three months. Those with very early rejection appear to do worse. In an attempt to clarify this we examined the outcome of grafts in patients who developed rejection within two weeks of first cadaver transplantation.

The course over one year of 23 patients with early rejection was compared with 89 patients whose rejection episode, if any, occurred later than two weeks. Management including immunosuppressive regimen was identical.

Age and sex distribution was similar in both groups. The time of onset of the first rejection in the early rejecting patients occurred as early as 5 days post-operatively, but within that group the timing of the first episode had no relation to the eventual outcome. However there were more rejection episodes over one year in patients with early rejection than the other group. Renal function at 6 and 12 month was compared. Although some kidneys in the early rejection group did well, prognosis of the graft was substantially worse, with a successful outcome of the graft in 30% of patients compared with 60% in the late rejection group.

There was no difference between the incidence of HLA antigens in common in either group group nor did the number of mismatches bear any relation to whether rejection occurred early or late, or to the eventual outcome. None of the patients in either group had detectable cytotoxic antibodies. Presence of absence of acute tubular necrosis did not effect the result either.

Graft survival is not as good in patients with rejection occurring two weeks after operation as in those whoser rejection is later. None the less since good function may be achieved in the former group one should treat rejection whether early or late with the same energy.

10. HYPERLIPIDAEMIA FOLLOWING RENAL TRANSPLANTION

R. Gokal, J. Mann, P: J. Morris, D. O. Oliver

Renal Unit, Churchill Hospital, Oxford and Nuffield Department of Surgery Oxford

Disorders of lipid metabolism have been described in transplant patients and these may contribute to morbidity and mortality. Plasma cholesterol and triglyceride levels were measured in 60 transplant patients in 60 age and sex matched controls. The patients, all with good graft function and on stable maintenance prednisolone dosage (5-22.5mg daily: mean 11.8mg) were studied 8-28 months (mean 14.6 months) following transplantation. Sixty per cent of the patients had lipoprotein abnormalities: type 11B 42%, type 11A 8%, type 1V 10%. As a comparison, the most prevalent lipoprotein phenotype in 131 chronic dialysis patients was type 1V (40%) Cholesterol levels were significantly higher in the patients than in controls (mean 7.24 $\pm$ SEM 0.25 mmol/l v 5.09 $\pm$ 0.16 mmol/l; $p < 0.001$) as were the triglyceride values (2.29 $\pm$ 0.18 mmol/l v 1.25 $\pm$ 0.007 mmol/l; $p < 0.001$). Thirty-five patients had lipid estimations performed before and sequentially after transplantation for periods of 6-20 months to study the "natural history" of hyperlipidaemia. Lipid levels were elevated in the first 6-8 months at a time when prednisolone dosage was being reduced . Once on maintenance steroids (5.0-22.5 mg daily), the lipid patterns appeared to revert to normal and remain stable.

There is a high prevalence of hyperlipidaemia in transplant patients. It appears that during the early period following transplantation lipid levels are variable and changing and should not form the basis for any assessment or therapy. No specific aetiological factors emerged to explain the hyperlipidaemia.

11. CYCLIC NUCLEOTIDE LEVELS IN MIXED LYMPHOCYTE CULTURE

The Department of Surgery, The University of Leicester.

R. F. M. Wood, R. L. Stacey, N. Mistry

Standard Mixed Lymphocyte Culture (MLC) requires an incubation period of several days, limiting its value in cadaver transplantation. This study explores a possible means of achieving an earlier result by measuring levels of cyclic nucleotides. These are known to act as "second messengers" in the immune system and rapid increase in lymphocyte Guanosine 3' : 5' — Cyclic Monophosphate (cGMP) have been demonstrated following exposure to mitogens. Smaller alterations in Adenosine 3' : 5' — Cyclic Monophosphate (cAMP) have also been reported.

MLC experiments were performed using lymphocytes from inbred rate. Studying the combinations:— August/Wistar and August/AS. Standard MLC reactivity was measured after 24 and 48 hours of culture by ^{14}C Thymidine uptake. cAMP was measured in a competitive protein binding assay and cGMP by radio-immunoassay. In assays performed after ¼, 1, 2½ and 5 hours of mixed culture there was no increase in levels of either cGMP or cAMP. In both the August/Wistar and August/AS combinations there was a signicant increase in cGMP or cAMP . In both the August/Wistar and August/AS combinations there was a significant increase in cGMP compared to autologous controls. The stimulation index achieved was comparable to that of a standard two way MLC measured at 48 hours. Isolated peaks of cAMP activity were recorded after 12 hours in the August/AS combination and after 18 hours in the August/Wistar combination. The results indicate that measurement of lymphocyte cGMP may provide a rapid determination of MLC reactivity.

12. THE VALUE OF MONITORING DNA AND RNA IN PERIPHERAL BLOOD LEUKOCYTES AS AN INDICATOR OF REJECTION

A. M. de Araugo, A. G. White, J. L. Anderton

Nuffield Transplant Unit, Edinburgh

DNA and RNA synthesis by peripheral blood leukocytes from renal allogragraft recipients were measured by microplate method.

The effects of rejection, infection and changes in immunosuppression were examined, as well as, the possibility of monitoring the level of immunosuppression by the level of DNA and RNA synthesis.

Of 52 patients studied, fourteen suffered nineteen episodes of rejection. Fifteen of these episodes were associated with significant increase in DNA synthesis, which in the majority of cases occurred before biochemical evidence of rejection.

High DNA peaks within thirty-five days of transplantation were invariably associated with irreversible rejection. Peaks occurring later than this usually indicated that the rejection episode could be reversed.

Thirty-four patients with stable graft function experienced 78 episodes of clinical infection. On only three occasions was the DNA and RNA synthesis significantly increased. The levels of DNA and RNA synthesis might provide a parameter for monitoring the level of immunosupression but it is apparent that whatever the level of immunosuppression in some patients, graft destruction will still occur, possibly as a result of presensitisation or genetic influences.

13. IMMUNOGLOBULINURIA IN HUMAN RENAL TRANSPLANT RECIPIENTS

R. A. Sells, M. Chapman, I. Papatheofanis, D. Swirsky

Renal Tranplant Unit, Liverpool

The purpose of this study was to determine the levels of immunoglobulin in the urine (UIgG) of patients who received renal transplants, and to confirm or refute recent assertions connecting rejection with increased UIgG.

23 transplants were studied (21 patients)), sequential aliquots of urine were stored free of preservative, and IgG assayed in batches using immunoprecipitation fluoronephelometry.

All patients excreted IgG for the initial four days. Thereafter four patients with normal transplant function excreted no sifinificant quantities of IgG (<20 mg/L).

In nine patients with reversible rejection episodes, a secondary rise in UIgG (mean 182.6 mg/ ± 71.2 SE) was found 0-6 days prior to rejection. In ten patients with irreversible rejection, there was a previous rise UIgG 0-2 days prior to rejection (mean 212.8 mg/L ± 42.2 SE). In these 19 cases there were 7 later episodes of increased UIgG unaccompanied by rejection and there was one case of rejection without an increase in UIgG There were two cass ATN, one perinephric abscess, one urinary leak and one severe hyperglycaemia. In all cases UIgG rose significantly.

In two cases the activity of urinary IgG against donor lymphocytes was assessed using the Sandwich FAHG technique. Binding of the UIgG to donor white cells where rejection was not occuring

Urinary IgG levels are raised immediately after successful transplantation, fall when normal renal function develops, and rise when rejection and other complications occur. In preliminary studies of rejection the antibody appears to donor-specific.

14. PROGNOSTIC IMPLICATIONS OF IMMUNOGLOBULINURIA IN HUMAN RENAL ALLOGRAPHS

K. B. Kwun, J. Bramis, M. Haimov, L. Burrows

The Mount Sinai Hospital and The Mount Street School of Medicine, New York

To evaluate the extent of injury in short and long term renal allografts, the urinary excretion of immunoglobulins was studied in eight cadaveric transplants, ten long term transplants, and in normal controls.

Aliquots of urine were concentrated and immunoglobulins quantiated. In patients without rejection, (Group I) immunoglobulins remained at trace to absent levels. In patients with a rejection crisis, immunoglobulins rose to high levels without parallel serum changes. With a reversal of rejection (Group II), immunoglobulinuria diminished to baseline levels. If rejection was irreversible (Group III), urinary immunoglobulins remained high until anuria. Long term stable allografts recipients had low levels except those with unstable function who had higher levels, but never approximating the acute situation.

Immunoglobulin excretion including IgA, IgG and IgM correlates with rejection; with a reversible levels fall to normal. If not spillage continues signalling irreversibility. A good prognosis in long term allografts is shown by low levels of immunoglobulinuria: unstable function leads to higher levels. Immunoglobulinuria can be used as an additional test to evaluate the reversibility of rejection, and also has significance in the long term situation.

15. THE USE OF PLASMAPHERESIS IN RENAL TRANSPLANTATION

R. B. Naik, H. A. Lee, Ruth Ashlin, Caroline Wilson and M. Slapak

Transplant Unit, St. Mary's Hospital, Portsmouth

In a previous study it has been shown that plasmapheresis had an abrogating effect on the hyperacute rejection, presumably antibody mediated, of heterologous kidneys (1). In the study the effect of plasmapheresis in nine patients is reported. In group A six patients were plasmapheresed six times on alternate days immediately following transplantation. Two-two and a half litres of plasma were removed and exchanged synchronously for the equivalent volume of Purified Plasma Fraction (PPF). In group B, three patients who were rejecting their kidneys in the first three months after transplantation and in whom (a) the normal method (methyl prednisolone pulse therapy ± Heparin) has been unsuccessful or was contra-indicated, (b) the biopsy showed histological changes of rejection with blood vessel damage, were plasmapheresed on six consecutive days. Three to three and a half litres of plasma were removed and exchanged for PPF.

In group A there was no evidence that the patients had either beneficial or derimental effect. There were no complications of the treatment. In group B all three patients showed an objective response. The first patient is alive but has lost her graft. Two patients reversed their creatinine rise and are alive with functioning kidney grafts. All patients showed a significant fall in their complement levels.

Plasmapheresis has a part to play in the treatment of a rejection episode uncontrollable by conventional means. There does not appear to be any obvious complication of the method.

Kashii A., Lafortune L., Derry. C. D., Misra M. K., P'Eng F. K., Sayhoun A. and Slapak M (1972). European Surgical Research 3, 262.

16. INDUCTION OF UNRESPONSIVENESS TO SKIN ALLOGRAPHS WITH ANTIGEN, ALS AND PROCARBAZINE HYDROCHLORIDE (PHC)

L. Brent and S. C. Opara

Department of Immunology, St. Mary's Hospital London.

It has been shown previously that the life of H-2 incompatible skin allografts can be substantially and specifically prolonged by pretreatment of the recipient with a single dose of donor strain liver or spleen extract and a single dose of B. pertussis vaccine, followed postoperatively by 3 doses of ALS ("old regimen"). In this way 30-50% of grafts were accepted on a long-term (generally life-long) basis. The unresponsiveness was shown to be mediated by suppressor T cells and this gives it a special clinical interest. There are, however, two main problems to be overcome: a) improvement of the survival figures to clinically acceptable levels, and b) the necessity for pretreatment. This contribution is concerned with the former.

The use of several chemical immunosuppressive agents in combination with ALS was studied systematically, using three alternating doses of ALS and the drug in the first post-operative week and, as before, giving donor strain extract 16 days before transplantation. Drug doses were made to be non-toxic and comparable by using one-quarter of the LD50. Of the 5 drugs investigated, PHC alone acted in a markedly synergistic manner, the proportion of long-surviving grafts being 70-85% compared with 29-35% using the old regimen concurrently. B. Pertussis inoculation could now be dispensed with.) Additional immunosuppression with drugs did not prove useful and, in some cases, even counter-productive.

The specificity and nature of the unresponsiveness thus induced was closely similar to that induced with the old regimen: whilst unresponsiveness could be transferred to ALS-treated recipients with purified T cells, no evidence favouring the participation of antibody or complexes could be found.

17. SUPPRESSION OF THE MIXED LYMPHOCYTE REACTION BY NIRIDAZOLE METABOLITES ACTION BY NIRIDAZOLE METABOLITES

J. Miller, Michelle Bird, B. Jones, P. Massey D. Millar, Suzanne Reeves & J. R. Salaman

K.R.U.F. Institute of Renal Disease, Cardiff

We have previously shown that Niridazole will prolong the survival of heterotopic heart allografts in rats, particularly when combined with Azathioprine and Prednisolone, and that urine dialysates from rats or humans treated with Niridazole will inhibit the MLR. Our present aim has been to isolate and identify the active Niridazole metabolites from human urine.

Control and test urines were collected from a normal volunteer before and after taking 6 x 250 mg Niridazole over a 48 hour period. Control urine dialysate (CUD) and Niridazole urine dialysate (NUD) were prepared and fractionated on a DEAE Sepharose column, and in each case 80 x 5 ml fractions were eluted. CUD and NUD fractions were added to the one-way MLR at a range of concentration. While CUD fractions failed to inhibit ^{3}H-thymidine incorporation in stimulated cultures, dose-related MLR inhibition was observed with NUD fractions 22-28, 38-43 and 61-65. These fractions corresponded to the elution profile of the 3 radioactive metabolites isolated from rat urine following treatment with 3H-labelled Niridazole.

18. IN VITRO EFFECTS OF THE IMMUNOSUPPRESSIVE AGENT CYCLOSPORIN A

D. J. G. White, R. Y. Calne, A. Plumb and G. Pawelec

Department of Surgery University of Cambridge

The fungal metabolite Cyclosporin A has been shown to be an extremely potent, non-toxic suppressor of graft rejection in rats, pigs and dogs (1), Borel (2) has suggested that rather than being cytostatic or anti-lymphocytic Cyclosporin A affects an early stage of triggering of the immunocompetent lymphoid cell.

We have confirmed the inhibitory effect of Cyclosporin A on mitogenic responses and demonstrated that a concentration of 1 ug/ml inhibits both mixed lymphocyte cultures and the primed lymphocyte test. Approximately 50 times more Cyclosporin A was required to inhibit transformation of B cell mitogens than was needed to inhibit PHA stimulated lymphocytes. No significant inhibition of the destruction of target cells by preformed cytotoxic cells was observed.

The growth of a mouse "T cell like" thyoma was inhibited by addition of between 1 and 10 ug/ml of Cyclosporin A. The growth of a mouse "B cell like" myeloma and normal pig kidney monolayers were not inhibited by these doses. The addition of Cylosporin A to mitogen stimulated lymphocyte cultures at various times during the culture period demonstrated that it is effective prior to the incorporation of DNA precursors into the dividing cell.

From these results we conclude that Cyclosporin A exerts its immunosuppressive activity by inhibiting lymphocyte division, with a higher affinity for T cells than B cells. The inhibition of the thymoma growth would suggest that this inhibitory effect is not produced by prevention of antigen recognition.

1. Kostakis, A. J., White, D.J.G. and Calne, R.Y. (1977) IRCS Med.Sci. 5, 280.
2. Borel, J. F., Feurer, C., Gubler, H. U. and Staähelin, H. (1976) Agents and Actions, vol. 6/4, pp. 468-475. Birkhäuser Verlag, Basel.