



Tuesday 2.00 p.m. in the Derwent Room

CHAIRMAN: Mr. Miles Fox, Sheffield  
Professor Peter Morris, Oxford

INVITED LECTURES

- 2.00 - 2.45 Prof. M. Dubernard, Lyons, France  
Pancreatic Transplantation - An Overview and Personal Experience
- 2.45 - 3.00 Mr. P. McMaster, Birmingham  
Combined Kidney and Pancreas Grafting in Man
- 3.00 - 3.15 Mr. T.J. Duffy (Prof. R.V. Calne), Cambridge  
Clinical Pancreatic Transplantation in Cambridge
- 3.15 - 3.30 M.S. Nolan, N.J. Lindsay, N.P. Ingram, A. Herold, D.N. Slater,  
S. Beck, M. Fox, Sheffield  
Preservation of the Rat Pancreas with a View to Islet Function  
Following Transplantation
- 3.30 - 3.45 N.J. Lindsay, J.A. Royds, M.A. Parsons, M.S. Nolan, A. Herold,  
P. Little, N.P. Ingram, P.F. Boyle, S. Beck, D.N. Slater, C.B. Taylor,  
W.R. Timerly, M. Fox, Sheffield  
Plasma Gamma Enolase: A Marker of Islet Damage?
- 3.45 - 4.00 N.P. Ingram, M.S. Nolan, N.J. Lindsey, P.F. Boyle, S. Beck,  
D.N. Slater, M. Fox, Sheffield  
Advantages of Roux-Loop Jejunal Drainage of Exocrine Secretion  
Following Pancreatic Transplantation - An Experimental Study
- 4.00 - 4.30 TEA
- CHAIRMAN: Professor M. Dubernard, Lyons, France  
Mr. P. McMaster, Birmingham
- 4.30 - 4.45 M.J. Taylor, T.J. Duffy, Cambridge  
Cryopreservation of Isolated Pancreatic Islets: The Pattern of  
Insulin Secretion After Slow Cooling and Warming in the Presence  
of Either Dimethylsulphoxide or Glycerol
- 4.45 - 5.00 H.G. Gooszen, R. van Schiljguarde, F.T. Bosman, M. Froklich,  
M.P.M. van der Burg, Leiden, Holland  
A Comparison Between the Effect of Reduction of Islet Mass and  
Ductobliteration on Endocrine Function
- 5.00 - 5.15 D. Alderson, J.R. Farndon, Newcastle-upon-Tyne  
Effects of Transplant Mass on Insulin Release by Collagenase-  
Dispersed Pancreatic Fragments in the Diabetic Dog
- 5.15 - 5.30 D.W.R. Gray, B. Fairbrother, P. McShane, H. Reece-Smith, P.J. Morris,  
Oxford. The Survival of Isolated Pancreatic Islets in Rats Rendered  
Immunologically Unresponsive to Renal Allografts
- 5.30 - 5.45 M.R. Newton, H. Reece-Smith, P. McShane, P.J. Morris, Oxford  
The Distribution of MHC Antigens in the Rat Pancrease and Isolated Islets
- 5.45 - 6.00 D. Alderson, J.R. Farndon, Newcastle-upon-Tyne  
The Metabolic Effects of Islet Transplantation in the Diabetic  
Dog

COMBINED KIDNEY AND PANCREAS GRAFTING IN MAN

P. McMaster. Queen Elizabeth Hospital, Birmingham

Pancreatic graft failure after transplantation has been a frequent event and the cause of this has often been unclear. The pancreatic segmental graft seems particularly prone to primary vascular thrombosis and rejection and possibly also to progressive sclerosis and graft failure. We have therefore assessed in our current programme of combined kidney and pancreas transplantation the frequency and pattern of rejection in combined grafting and made an assessment of glucose homeostasis at one year.

In the last eighteen months, eight patients have undergone combined kidney and pancreas transplantation from CAPD with ductal injection with Polyisoprene latex. One of these patients died of myocardial infarction with both kidney and pancreas function at 35 days. Of the remaining seven patients, six kidneys are functioning and five pancreases are functioning with four of these pancreatic grafts now over one year.

Detailed Indium labelled platelet studies have shown that pancreatic rejection has been an uncommon feature although rejection of the kidney has been seen and our carbohydrate serial studies have shown no evidence of progressive deterioration as yet due to graft sclerosis.

Ductal injection of the pancreas associated with a kidney graft appears a simple and safe technique and clearly warrants a further review.

## CLINICAL PANCREATIC TRANSPLANTATION IN CAMBRIDGE

T.J. Duffy, R.Y. Calne. Department of Surgery, Cambridge University, Addenbrooke's Hospital, Cambridge.

Since August, 1979 15 pancreas grafts have been performed in 14 patients with severe diabetic micro-angiopathy at Addenbrooke's Hospital, Cambridge; 10 combined pancreas and renal transplants, 1 combined pancreas and liver transplant and 4 pancreas transplants alone. In cases of two-organ grafts, both were transplanted from the same cadaver donor. In cases 1-11 pancreatic exocrine secretion was inhibited by intraduct latex, while in cases 12-15 the duct was drained into a defunctioned loop of small intestine. Five patients have died between 3 days and 11 months post-operatively, two with functioning pancreas grafts. Three grafts were lost in the early post-operative period (<48 hours) due to vascular thrombosis. Animal studies showed that a distal arteriovenous fistula improves flow through the vessels, leading to the adoption of this technique. Of the surviving patients, one has excellent pancreatic function at 3½ years and another has partial function, a duct injection case with no a.v. fistula. Late graft loss occurred between 3 and 80 weeks after transplantation: it has been difficult to define the relative contributions of rejection, progressive fibrosis and late thrombosis in this group of patients.

## PRESERVATION OF THE RAT PANCREAS WITH A VIEW TO ISLET FUNCTION FOLLOWING TRANSPLANTATION

M.S. Nolan, N.J. Lindsay, N.P. Ingram, A. Herold, D.N. Slater, S. Beck, M. Fox. Transplantation Laboratories, Royal Hallamshire Hospital, Sheffield.

Three solutions, hyperosmolar citrate (HOC), modified Collins (MC<sub>2</sub>) and Sacks 11 solutions were evaluated as media for cold storage preservation (24, 30 and 36 hours) of pancreatic endocrine function. In addition, the effect of a normothermic ischaemic interval (30 or 60 minutes at 37°C) prior to 24 hour cold storage was investigated using MC<sub>2</sub> and HOC. Preservation of endocrine function was assessed, in vivo by pancreatic isotransplantation with restoration of normoglycaemia in the streptozotocin-induced diabetic rat, and in vitro by a normothermic pancreatic perfusion assay. Twenty four hour preservation was equally effective using MC<sub>2</sub> and HOC, but not Sacks 11. Thirty hours preservation was consistently successful using MC<sub>2</sub> only, but the cold-ischaemic interval could not be extended to 36 hours with success. Additionally, MC<sub>2</sub> - cold storage proved more effective than HOC for grafts with 30 or 60 minutes prior normothermic ischaemia. Hypoglycaemia and hyperinsulinaemia occurred in all recipients of Sacks 11 cold-stored grafts, but not using MC<sub>2</sub> or HOC. IVGTT K values at three months were lower with HOC cold-storage grafts than MC<sub>2</sub> stored grafts and insulin responses were significantly reduced. Normothermic perfusion corroborated the findings in vivo with Sacks 11 stored grafts showing 'insulin washout' at the commencement of perfusion. Although amelioration of the diabetic state was achieved with a 30 or 60 minute normothermic ischaemic interval, no insulin response to glucose stimulation was observed during perfusion.

## PLASMA GAMMA ENOLASE : A MARKER OF ISLET DAMAGE?

N.J. Lindsay, J.A. Royds, M.A. Parsons, M.S. Nolan, A. Herold, P. Little, N.P. Ingram, P.F. Boyle, S. Beck, D.N. Slater, C.B. Taylor, W.R. Timerley, M. Fox. Transplantation Laboratories, Royal Hallamshire Hospital, Sheffield.

The enzyme enolase exists as 3 isoenzymes of which one, gamma, is confined to neurones and cells of the diffuse neuroendocrinological system. In the pancreas only the islets of Langerhans contain gamma enolase and consequently liberation into the bloodstream may provide a marker of islet cell damage. We have measured plasma levels during ischaemia, trauma and transplantation in the rat; situations which could be expected to damage the pancreas.

Ischaemic damage produced an increase of plasma gamma enolase within 24 hours. Trauma also resulted in a similar but more transient rise. Pancreas grafts were performed between WAG strain donors and AGUS strain recipients, previously being made diabetic with Streptozotocin, exocrine drainage being via either a Roux-en-Y loop or the ureter. Blood samples were taken for gamma enolase, glucose, alpha amylase, and insulin levels. In the duct to ureter group urine was taken for alpha amylase determinations. Allografts were sacrificed on the day following the recurrence of hypoglycaemia, and control isografts on day 12 post transplantation. All grafts were examined histologically.

In 7 out of 8 allografts there was an elevation of gamma enolase post transplant which preceded by at least 24 hours significant and consistent change in any other parameter. Of the 4 isografts there were variable rises in gamma enolase levels but only one which showed a substantial elevation in the period 8 - 12 days post transplantation. This correlated with histological evidence of pancreas damage due to duct obstruction.

We concluded that gamma enolase appears to be a marker of islet cell damage and may prove useful in the diagnosis of islet rejection.

## ADVANTAGES OF ROUX-LOOP JEJUNAL DRAINAGE OF EXOCRINE SECRETION FOLLOWING PANCREATIC TRANSPLANTATION - AN EXPERIMENTAL STUDY

N.P. Ingram, M.S. Nolan, N.J. Lindsey, P.F. Boyle, S. Beck, D.N. Slater, M. Fox. Urology/Transplantation Laboratories, Royal Hallamshire Hospital, Sheffield.

Apart from rejection, the most difficult problem in clinical and experimental pancreatic transplantation is the management of the exocrine secretions. Various techniques such as duct ligation and ductal obliteration have been attempted, but experimental work has shown a gradual long term decline in endocrine function (1). The most physiological technique is to drain the secretions into the bowel, but previous clinical and experimental experience has been fraught with leakage and technical failure (2).

A new technique has been developed in the rat avoiding a sutured anastomosis. A Roux-en-Y Loop of jejunum is constructed at a preliminary operation. Four weeks later pancreatic isotransplantation is performed. Microvascular anastomoses are carried out using a sutureless cuff technique (3). A short polyethylene stent is tied into the pancreatic duct and the free end is inserted into the Roux Loop via a needle stab incision and secured by a seromuscular layer suture. Animals previously made diabetic with Streptozotocin have uniformly become normoglycaemic within 24 hours of transplantation and have followed so far to six months. Unlike duct ligated, obliterated, (latex and prolamine), and free peritoneal draining controls, histology of the pancreas has remained normal. Normoglycaemia has been maintained and serum insulins and oral and intravenous glucose tolerance tests have remained normal. The technique has proved to be simple and reliable and provided preservation of normal endocrine function.

### References:

1. Nolan, M.S., Lindsey, N.J., Savas, C.P., Herold, A., Slater, D.N., Beck, S., Fox, M. Pancreatic transplantation in the Rat; Long Term Study following different methods of management of exocrine drainage. Transplantation (1982) In Press.
2. Sutherland, D.E.R. Pancreas and Islet Transplantation. Diabetologia (1981) 20: 161 - 185.
3. Nolan, M.S., Lindsey, N.J., Savas, C.P., Slater, D.N., Fox, M. Pancreatic Transplantation in the Rat: a simplified method using aortic interposition and cuff technique. Transplantation (1982), 33. 327-329.

CRYOPRESERVATION OF ISOLATED PANCREATIC ISLETS: THE PATTERN OF INSULIN SECRETION AFTER SLOW COOLING AND WARMING IN THE PRESENCE OF EITHER DIMETHYLSULPHOXIDE OR GLYCEROL

M.J. Taylor, T.J. Duffy. MRC Medical Cryobiology Group, University Department of Surgery, Cambridge.

Islets of Langerhans prepared from the pancreases of WAG rats by collagenase digestion were stored at  $-196^{\circ}\text{C}$  after slow cooling ( $0.3^{\circ}\text{C}/\text{min}$ ) to  $-70^{\circ}\text{C}$  in the presence of multimolar concentrations of either  $\text{Me}_2\text{SO}$  or glycerol. Samples were rewarmed slowly ( $10^{\circ}\text{C}/\text{min}$ ) and dilution of the cryoprotectant was achieved using medium containing sucrose.

Function was assessed by determination of the time-course of the glucose-induced insulin release during in vitro perfusion at  $37^{\circ}\text{C}$  and also by isograft transplantation. Transplants were carried out by intraportal injection of a minimum of 1500 frozen and thawed islets into streptozotocin-induced diabetic recipients and tissue function was assessed by monitoring blood glucose levels and body weight changes.

Islets frozen and thawed in the presence of glycerol failed to reduce high serum glucose levels of recipient rats and in vitro measurements showed that islets frozen with glycerol were unable to demonstrate a glucose-sensitive insulin release pattern.

Reversal of the diabetic condition was achieved in 2/5 animals receiving isologous islets which had been frozen and thawed with  $2\text{M Me}_2\text{SO}$ ; and in 1/3 animals using islets cryopreserved with  $3\text{M Me}_2\text{SO}$ . Perfusion studies showed that islets frozen with 2 and  $3\text{M Me}_2\text{SO}$  were able to secrete insulin in response to a glucose challenge but that the pattern of release was atypical compared with the dynamic-release curves obtained for untreated control islets.

A COMPARISON BETWEEN THE EFFECT OF REDUCTION OF ISLET MASS AND DUCTOBLITERATION ON ENDOCRINE FUNCTION

H.G. Gooszen, R. van Schilfgaarde, F.T. Bosman, M. Frohlich, M.P.M. van der Burg. Department of Surgery & Chemical Pathology, University Hospital, Leiden.

Ductobliteration for the ablation of exocrine secretion in segmental pancreatic transplantation is safe. A decrease in endocrine function however, is always seen when in situ ductobliteration of the left pancreatic segment is performed in combination with a right hemipancreatectomy in beagles (9-15 kg). Intravenous glucose tolerance tests (mean K-value at 1 month  $1.4 \pm 0.4$ , at 15 months  $1.2 \pm 0.4$ ,  $n=8$ ; mean K-value unmodified controls  $2.9 \pm 0.8$ ,  $n=60$ ) and peripheral insulin response curves showed that this decrease is not progressive (mean insulin peak-value before operation  $61 \pm 12$  uU/ml plasma,  $n=20$ ; at 3 months  $23 \pm 6$  and at 9 months  $26 \pm 7$  uU/ml plasma).

Two groups of dogs were studied and compared to unmodified controls, in order to investigate whether ductobliteration as such or the reduction of islet mass is the main cause of this decrease in endocrine function.

In group I, the left lobe of the pancreas was removed, leaving the right lobe in situ, freely draining into the duodenum (4 dogs). At 3 months after operation normal K-values were found (mean  $3.0 \pm 0.6$ ) and peak-values of the insulin response curves (mean  $70 \pm 18$  uU/ml plasma) did not differ significantly from those observed before operation in the same group of dogs ( $76 \pm 14$  uU/ml plasma). At relaparotomy the right lobe showed a macroscopically normal aspect which was confirmed by normal architecture on histological examination.

In group II, ductobliteration of the right lobe was carried out after removal of the left lobe. At twelve months after operation the mean K-value was  $1.4 \pm 0.5$  and the mean insulin peak-value was  $26 \pm 13$  uU/ml plasma. At re-operation the right lobe was considerably shrunken in contrast to group I and showed the same qualitative changes on histological examinations as in left ductobliterated segments. Severe fibrosis and distortion of islet architecture with dispersion of hormone producing cells (immunoperoxidase techniques for  $\alpha$ ,  $\beta$ ,  $\delta$  and PP cells) were invariably seen.

We conclude that ductobliteration is the main factor responsible for the reduction of endocrine function.

We could not demonstrate a reduced endocrine function due to a hemipancreatectomy as such.

EFFECTS OF TRANSPLANT MASS ON INSULIN RELEASE BY COLLAGENASE-DISPERSED PANCREATIC FRAGMENTS IN THE DIABETIC DOG

D. Alderson, J.R. Farndon. Department of Surgery, University of Newcastle-upon-Tyne.

The abnormal glucose tolerance seen in experimental diabetic animals receiving pancreatic transplants has been assumed to be due to a reduced functional islet mass.

Collagenase-dispersed autografts were prepared following total pancreatectomy. Six dogs received 100%, and six received 50% of the tissue so formed. Twelve normal animals acted as controls.

Blood glucose, lactate, pyruvate, alanine, glycerol, 3-hydroxybutyrate, cholesterol, free fatty acids and insulin were determined in the fasting state and following an intravenous glucose load. Glucose clearance (K) was calculated.

Fasting euglycaemia was achieved and insulin levels were the same in both groups. Similar reductions in K, with elevated fasting levels of glycerol, cholesterol and 3-hydroxybutyrate, were present in both groups one month after transplantation ( $p < 0.01$  vs normals).

The results suggest that a simple relationship between transplant mass and insulin secretion does not exist. This merits further investigation, and has important implications on the suitability of free pancreatic transplants in the treatment of diabetes.

THE SURVIVAL OF ISOLATED PANCREATIC ISLETS IN RATS RENDERED IMMUNOLOGICALLY UNRESPONSIVE TO RENAL ALLOGRAFTS

D.W.R. Gray, B. Fairbrother, P. McShane, H. Reece-Smith, P.J. Morris. Nuffield Department of Surgery, John Radcliffe Hospital, Oxford.

Allografted rat islets are usually rejected rapidly and it has proved difficult to suppress rejection using methods which are successful for other organs. Tolerance of allogeneic islets might be achieved by first inducing tolerance of another organ allograft followed by transplantation of allogeneic islets of the same specificity, an approach with some clinical relevance.

Recently we have achieved this by transplanting LEWIS renal allografts into DA rats treated with Cyclosporin A for 14 days. After 75 days the rats were made diabetic with streptozotocin, then given LEWIS islet allografts under the transplant kidney capsule.

The above experiment has been repeated, using initially the same LEWIS to DA combination, with islets transplanted to both the kidney capsule and intraportally. The procedure was then repeated using a different strain combination (LEWIS to PVG). Finally the specificity of the effect was tested using islets of different strain to the original kidney transplant.

The results show that once a recipient rat has accepted a renal allograft under the influence of Cyclosporin A treatment, it will subsequently accept an islet allograft of the same strain as the kidney. This effect applied to both strain combinations tested, is not affected by the site of islet transplantation, and is specific for islets of the same strain as the renal allograft.

This approach is a most effective way of inducing tolerance of allogeneic islets, and suggests a future approach to the human diabetic with renal failure.

## THE DISTRIBUTION OF MHC ANTIGENS IN THE RAT PANCREAS AND ISOLATED ISLETS

M.R. Newton, H. Reece-Smith, P. McShane, P.J. Morris. The Nuffield Department of Surgery, University of Oxford, John Radcliffe Hospital, Oxford.

One approach to the treatment of diabetes has been to correct the deficit with transplanted pancreatic endocrine tissue. Initial optimism concerning the lack of immunogenicity of isolated islets has not been confirmed but the technical problems associated with exocrine drainage encourage the use of isolated islets despite their apparent increased vulnerability to rejection.

The distribution of MHC antigens in the DA rat pancreas and in isolated islets was investigated using monoclonal antibodies in conjunction with the immunoperoxidase technique. It was found that endocrine but not exocrine pancreas expressed Class I MHC antigens, Class II antigens being confined to cells with a dendritic morphology found throughout the pancreas.

The rejection of islet allografts can be more readily controlled if isolated islets are first placed under the capsule of a syngeneic kidney and then the composite organ is transplanted 7 days later as a vascularized allograft (1). The possibility that a change in MHC antigen expression or distribution on the islets could account for this was examined using the immunoperoxidase histological technique. These islets were found to contain large numbers of Class I and Class II positive mononuclear leukocytes, suggesting that reduced immunogenicity of the islets is not simply due to loss of Class II positive passenger cells. Alternative reasons will be discussed.

### Reference:

1. Reece-Smith, H., Homan, W.P., DuToit, D.F., McShane, P., Morris, P.J. Transplantation 1981, 31, 442-4.

## THE METABOLIC EFFECTS OF ISLET TRANSPLANTATION IN THE DIABETIC DOG

D. Alderson, J.R. Farndon. Department of Surgery, University of Newcastle-upon-Tyne.

Although euglycaemia following pancreatic transplantation has been accepted as indicative of adequate graft function, the development of angiopathic complications in diabetes has been linked to other metabolic derangements. This study examined the effect of free pancreatic transplants on these metabolic parameters in six dogs receiving intrasplenic islet autotransplantation and exocrine replacement following total pancreatectomy. Twelve normal dogs acted as controls.

Glucose, lactate, pyruvate, alanine, glycerol, 3-hydroxybutyrate, cholesterol, free fatty acids and insulin levels were measured in the fasting state and following an intravenous glucose load; at one, four and thirteen weeks post-transplantation. Fasting euglycaemia was achieved in animals by one week, but metabolic abnormalities persisted to one month with impaired glucose tolerance, elevated lipids and low alanine ( $p < 0.01$  vs normals). By three months, however, apart from reduced glucose clearance, all intermediary metabolites were normal. This improvement coincided with increased insulin output ( $p = 0.05$  vs one month).

We conclude that the function of free transplants improves with time (a feature not apparent in vascularized grafts whose function often deteriorates) and that the success of any pancreatic transplant can only be judged by a wide ranging metabolic assessment.

## A SINGLE CENTRE RANDOMISED STUDY OF CYCLOSPORIN A IN CADAVERIC RENAL TRANSPLANTS

I.G. Haynes, P. McMaster, J. Michael, D. Adu, J. Turney, S. Roger, T. Vlassis, S. Stock.

A standard regime has yet to be developed for the clinical use of Cyclosporin A in cadaveric renal transplantation and the need for continuing steroids is unclear. In this prospective, randomised study, comparison was made between patients receiving cadaveric grafts on conventional treatment Azo and Pred (Group 1) and patients receiving Cyclosporin A plus initial steroids for a fourteen day period only (Group 11). Cadaveric kidneys which produced an initial diuresis were included in this study.

Group 1 - Azothioprine 2.5 mgs/kg plus Prednisolone 20 mgs daily  
Group 11 - Cyclosporin A 15 mgs/kg plus Prednisolone 20 mgs. Steroids stopped at day 14

	<u>CyA + Initial Steroids</u>	<u>Azo + Pred</u>
No. of patients	35	33
Mean follow-up months	11.7	12.1
Current graft survival	82%	70%
Mean time of 1st rejection	13.3 days	6.4 days
6/12 % on Steroids	58%	100%
6/12 mean creatinine	223.6	137.6
1 year mean creatinine	227.4	121.0

Cyclosporin A and initial steroids only, are clearly an effective form of immunosuppression and results in delay in acute rejection episodes. Continuing steroids are not required in all patients but by six months only 42% of Cyclosporin patients were not receiving steroids. Renal function in Cyclosporin A patients is significantly inferior at six months and one year.

## THE USE OF CYCLOSPORIN A FOR TWO YEARS IN A SINGLE UNIT

M. Slapak, N. Digard, M. Searle, H.A. Lee. Wessex Regional Transplant Unit, St. Mary's Hospital, Portsmouth, Hants.

Beginning January 1st 1981, 129 patients having been transplanted of whom 74 received Cyclosporin A (CyA). The remaining patients received conventional immunosuppressive therapy (CIT) consisting of a baseline dose of 1.5 - 2 mg/kg body weight of Azathioprine and 0.3 mg/kg body weight of Prednisolone. In both groups rejection was treated by 3 x ½ gm pulses of Methylprednisolone up to a maximum of 6 gm over the 30 days. In the CyA group 12 patients received CyA only as baseline therapy, and the remaining patients receiving, in addition, 10 mg of Prednisolone daily. CyA serum levels were monitored by a radioimmune assay. Graft function was measured by the usual biochemical parameters as well as isotopic functional imaging.

Results: At 2 years, actuarial graft survival was 82% and 54% in the CyA and CIT groups respectively. The mortality was 7% (3 patients in CyA and 4 patients in CIT). The HLA, A and B compatibility had no correlation with graft survival in either group. 2 antigen DRW compatibility had a statistically beneficial effect on graft survival in the CIT group but had no effect on the CyA group. There was no deleterious effect, as reported by others, of DRW6.

The number of rejections in the first 3 months were not statistically different in the two groups. CyA nephrotoxicity was frequently observed and was seen to be briskly responsive to reduction of dosage. Serum levels of CyA did not correlate statistically with this nephrotoxicity. Early rejection did correlate with serum CyA levels of 300 ng in either the CyA or CIT groups. There was no correlation between warm ischaemic intervals, cold ischaemic or/and graft survival.

Conclusion: A new immunosuppressive agent, Cyclosporin A, has been used in a single unit for longer than 2 years, initial good results which were 30% better than we had ever previously reported had been maintained over the following year. We conclude that the advent of the new drug has demonstrated a marked improvement on our graft survival without any detriment to patient mortality and without any incidence, at present, of lymphoma.

CYCLOSPORIN A REDUCES THE SEVERITY BUT NOT THE INCIDENCE OF INITIAL RENAL ALLOGRAFT REJECTION EPISODES

R.M. Merion, D.J.G. White, R.Y. Calne. Department of Surgery, Addenbrooke's Hospital, Cambridge University, Hills Road, Cambridge.

In our experience, patients who are rejection-free during the first two months after renal transplantation seldom experience subsequent clinically evident acute rejection episodes. However, initial episodes of rejection occurring within eight weeks of transplantation are common and may contribute to early graft losses. We found that all initial rejection episodes occurred within eight weeks of grafting in 70 patients treated with Cyclosporin A (CyA) alone and 60 Azathioprine and steroid (Aza) treated patients. The incidence of rejection was remarkably similar in the two groups (65% v 69%; P=NS). Compared to Aza, the CyA group experienced less severe rejection episodes, resulting in fewer early graft losses (P < 0.02). Serum creatinine deteriorated more slowly in the CyA group (P < 0.001) and peak serum creatinine levels were lower (P < 0.001). CyA patients rarely (1/37) required haemodialysis support, while 11 of 24 Aza patients were dialysed (P < 0.001). Haemodialysis during a rejection episode was a significant risk factor for early graft loss (P < 0.001). Overall, among patients experiencing their first rejection episode, two-year actuarial graft survival was 72.9% in the CyA group and 60.8% in the Aza group. The addition of steroids to CyA during the first two months after transplantation might further reduce graft losses from early rejection episodes without the risks of long-term steroids. A randomised prospective clinical trial is planned to investigate this question.

CYTOMEGALOVIRUS (CMV) TITRES IN KIDNEY TRANSPLANT DONOR AND RECIPIENT:- INFLUENCE OF CYCLOSPORIN A (CyA)

K.R. Harris, A. Saeed, T.A. Geoghegan, N. Digard, H.A. Lee, M. Slapak. St. Mary's Hospital, Portsmouth, Hants.

262 patients were tested for serological evidence of CMV infection before transplantation with kidneys from donors who were CMV positive, negative, or unknown. Post transplant immunosuppression was with Cyclosporin A  $\pm$  minimal steroids or Azathioprine + steroids. The incidence of rising CMV titres in recipients indicative of primary infection or reinfection was recorded. Irrespective of donor titres there is a trend for a greater incidence of raised CMV titres in negative recipients 30.3%, than in positive recipients 24.7%, (P > 0.05). CMV positive recipients receiving kidneys from CMV positive donors have a 35% incidence of raised titres, whereas if their donor was CMV negative the rate is 27% (P > 0.05). However, if a CMV -ve recipient receives a CMV +ve kidney there is a 58% incidence of primary infection as opposed to 5% if he had received a negative graft (P < 0.005). There was no statistical difference in mortality or infection rates, regardless of donor recipient combination, between patients treated with CyA or with Azathioprine. In conclusion, an analysis of the data suggests that transplantation of a CMV negative recipient using a donor kidney which is CMV positive results in a significantly increased risk of a CMV infection in these recipients. The effect of CMV infection on graft failure rates will also be discussed.

INTERACTION BETWEEN CYCLOSPORIN A AND IMMUNE COMPLEXES IN SKIN ALLOGRAFT ENHANCEMENT IN MICE.

W.H. Barber, I.V. Hutchinson, P.J. Morris. Nuffield Department of Surgery, Oxford University, John Radcliffe Hospital, Oxford.

These experiments investigate the combined immunosuppressive effects of Cyclosporin A (CyA), donor strain alloantigens, and immune complexes formed with haptenated (TNP) alloantigens and anti-TNP antibody.

Tail skin from C57Bl-10 (H-2<sup>b</sup>) mice was grafted onto Balb-c X B10-A F<sub>1</sub> hybrids (H-2<sup>dxa</sup>). Recipients initially were given CyA orally at 75 mg/kg, a dose previously shown to prevent skin allograft rejection in mice<sup>1</sup>. After 14 days the dose was reduced to 25 mg/kg for 7 additional days and groups were given, on day 14, either no further treatment or donor strain antigens of various types. Other mice received low dose (25 mg/kg) CyA from the time of grafting to day 7. Animals whose CyA was stopped after 14 days rejected their grafts in 19.7 days. By continuing CyA at reduced a dose for 7 days, rejection was delayed to 25.5 days. CyA alone at 25 mg/kg X 7 days from day 0 only marginally prolonged survival (10.8 vs. 9.4 days in untreated controls). Donor blood and spleen cell infusion at day 14 did not affect graft survival. However, when immune complexes formed with haptenated (TNP) donor spleen cell membrane and anti-TNP antibody were given in addition to CyA, graft survival was enhanced to 29.5 days in the high dose (75 mg/kg X 14d + 25 mg/kg X 7d) group and to 14.3 days in the low dose (25 mg/kg X 7d) group.

Immune complexes have been shown to be effective alone in producing enhancement of skin allografts in mice and kidney allografts in rats<sup>2</sup>, and our results indicate that the immunosuppressive effect is augmented by CyA. The use of haptenated donor alloantigen and anti-hapten antibody to produce specific allograft enhancement may have clinical use in combination with CyA or standard immunosuppressive agents to augment their efficacy and to reduce doses required for adequate immunosuppression.

References:

1. S.P.M. Lems, P.J.A. Capel & R.A.P. Koene. Trans.Proc. 12: 283, 1980.
2. I.V. Hutchinson, W.H. Barber & L. Brent. Trans.Proc. in press.

THE EFFECT OF PRETREATMENT WITH DONOR ANTIGEN AND CYCLOSPORIN A ON RENAL ALLOGRAFT SURVIVAL, AND LYMPHOCYTOTOXIC ANTIBODIES, AND T CELL SUBSETS

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We have studied the effect of Cyclosporin A given with donor antigen (blood/skin graft) on renal allograft survival in a strongly incompatible rat model (DA/Lewis). Cyclosporin A was administered orally for 10 days prior to transplantation (15 mg/kg/day). Renal function, lymphocytotoxic antibodies and T cell subsets were assessed in the seven groups studied, i.e. 1) no treatment 2) Cyclosporin A alone 3) donor blood only 4) donor blood and Cyclosporin A 5) donor and Cyclosporin A followed by a 4 week interval before transplantation 6) donor skin graft, and 7) donor skin graft and Cyclosporin A.

Our studies show that renal allograft survival was better ( $p < 0.01$ ) in groups 2, 3, 4, 5, compared to controls. The most striking prolongation was seen with combined Cyclosporin A and donor blood pretreatment (controls  $6.88 \pm 0.6$  (9) days: treated  $20.5 \pm 5.4$  (7) days). This effect did not persist if a 4 week interval was allowed after Cyclosporin A and blood pretreatment.

The accelerated renal rejection seen in previously skin grafted animals (MST  $5.4 \pm 0.54$  days), was converted into enhancement by combining skin grafting with Cyclosporin A (MST  $11.8 \pm 0.4$  days). Lymphocytotoxic antibody formation was abolished in Cyclosporin A pretreated animals.

These data indicate that Cyclosporin A and donor antigen pretreatment is a potent method of achieving donor specific tolerance. T cell subset analysis indicated that the effect is probably T suppressor cell mediated. The relevance of these findings to human live donor transplantation is discussed.

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1. ...
2. ...

THE BLOOD TRANSFUSION EFFECT: THE ENHANCING AND DETRIMENTAL EFFECT OF BLOOD COMPONENTS

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The whole blood transfusion effect is probably beneficial in spite of the presence of potentially harmful components. Therefore, in a strongly incompatible rat model [DA (RT1<sup>n</sup>) → Lewis (RT1<sup>1</sup>)], we have studied the effect of whole blood and purified blood cell types, including dendritic cells, on renal allograft function and survival. Recipient pretreatment with donor cell types was carried out 2 weeks prior to transplantation. The results were as follows: (+ mean survival in days). Dendritic cell preimmunised animals were studied as three groups ie + 10<sup>3</sup>, 10<sup>4</sup> and 10<sup>5</sup> cells.

- |                                 |   |                  |
|---------------------------------|---|------------------|
| 1) Controls (6.9)               | 2) Whole blood (14.5)                       | 3) Plasma (7.4)  |
| 4) Platelets (8.5)              | 5) B cells (16.0)                           | 6) T cells (7.2) |
| 7) Pure RBCs (27.6)             | 8) (10 <sup>4</sup> ) Dendritic cells (7.8) |                  |
| 9) Peritoneal macrophages (8.0) | 10) Dendritic cells + RBCs (26.3)           |                  |

These results indicate that RBCs and B cells (and, to a lesser degree, platelets) were enhancing. T cells and dendritic cells led to a more severe degree of rejection as judged by day 7 creatinine values. The effect of dendritic cells was abrogated by the addition of RBCs.

The blood transfusion effect may be potentiated by the removal of T cells and dendritic cells. The effect of T cells may be of some relevance to human transplantation.

FC RECEPTOR-BLOCKING ANTIBODIES : THEIR ASSOCIATION WITH MULTIPARITY AND ALLOGRAFT ENHANCEMENT IN THE RAT RENAL TRANSPLANT MODEL

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We recently reported a significantly improved one year graft survival rate in kidney transplant recipients who, in pre-transplant sera, had Fc receptor-blocking antibodies directed to donor B lymphocytes<sup>1</sup>. Similar antibodies were detected against paternal B lymphocytes in sera from women with normal pregnancies but were absent from women subjected to spontaneous abortions<sup>2</sup>.

These reports prompted us to study the relationship between these antibodies, pregnancy and allograft enhancement in the animal model. Inbred female Lewis (RT1<sup>1</sup>) and DA (RT1<sup>a</sup>) rats were used as recipients of kidneys from their (LE x DA)F<sub>1</sub> hybrids. Virgin and multiparous rats (2-3 litters) at approximately 2 weeks post-partum were given a left orthotopic kidney transplant with immediate right nephrectomy. The EA rosette inhibition assay was used to detect Fc receptor-blocking antibodies against B lymphocytes. Survival times were significantly longer in the multiparous recipients than the virgins in both strains with many long survivals (> 100 days) :

	Mean survival times (days)		
	Multiparous	Virgins	P
(LE x DA)F <sub>1</sub> → LE	53.4 ± 45	15.4 ± 22.9	<.02
(LE x DA)F <sub>1</sub> → DA	89 ± 26.5	19.5 ± 26.3	<.002

Antibodies against paternal B lymphocytes were detected in sera from all post-partum but not virgin rats.

These data indicate that Fc receptor-blocking antibodies which develop during pregnancy are associated with enhanced survival of renal allografts in this rat renal transplant model.

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MONITORING OF REJECTION OF RAT LIVER ALLOGRAFTS USING FINE NEEDLE BIOPSIES AND ASPIRATION CYTOLOGY

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DA (allografts) or BN (isografts) liver were transplanted orthotopically into BN recipients. Half of the allografts were treated with Cyclosporin A (3mg/kg). In overlapping intervals transplanted and non transplanted livers (controls) were punctured with a thin spinal needle, cells were aspirated, cytocentrifuged, fixed on slides and stained. The counts of the peripheral white blood cells and of the aspirated cells were compared and the increment calculated. The data of aspiration biopsies were compared with those of normal histological specimens of the grafts taken in similar overlapping intervals. The frequent fine needle biopsies as well as the normal biopsies were well tolerated by the animals. Isogenic and CyA treated grafts survived over 200 days, however allogenic grafts were rejected within 15 days. In the control group the increment was zero. The punctures and the normal biopsies did not cause inflammation. In the isograft group, there was a slight inflammatory infiltration consisting of granulocytes and lymphocytes. The allograft group showed a continuous increase of monocytes and macrophages and various forms of lymphoid cells. In the CyA group there was a slight increase of granulocytes but later the increment returned toward zero. If CyA was withdrawn for a short period the increment reversibly increased. In summary, cytologic and histologic data correlated well and we feel that this method deserves further investigation.

VASCULAR ENDOTHELIAL CELLS PRESENT ALLOANTIGEN EFFICIENTLY

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Presentation of antigen by accessory cells is an important step in the activation of T-lymphocytes. In the presentation of alloantigens, e.g. during kidney allograft rejection, many cells have been demonstrated to have an accessory cell function, such as monocytes, dendritic cells, and Langerhans cells of the skin. Vascular endothelial cells have been shown to have an antigen presenting capacity for soluble antigens. We have tested the antigen presenting capacity for alloantigens of both cultured arterial and venous endothelial cells in dogs. Unidirectional mixed leucocyte cultures were performed with responder and stimulator cell suspension depleted of accessory cells by carbonyl iron treatment and adherence to nylon wool. Depletion was complete as demonstrated by absence of stimulation of lymphocytes in these cultures. Addition of arterial or venous endothelial cells of the responder to these cultures, restored the proliferative response of responder lymphocytes to alloantigens to normal. These data on the alloantigen presenting capacity of vascular endothelial cells in vitro suggest at least one common function of known alloantigen presenting cells and endothelial cells. As endothelial cells are widely present in vascularised organ allografts, compared to scarcely present dendritic cells, this might be of utmost importance in clinical transplantation.

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Recent studies have shown the presence of strongly Ia positive cells in several non-lymphoid tissues, including kidneys. A proportion of these cells is almost certainly comprised of dendritic cells, which in vitro, are potent stimulators of allogenic lymphocytes. We have attempted to visualise the time-dependant, in situ relationships between invading host lymphocytes and Ia positive cells resident in donor kidneys in a rat transplant model (DA/Lewis), using double layer immunofluorescence and strain specific anti Ia monoclonal antibodies on transplant biopsies. Data on survival, renal function, and quantitation of graft infiltrates is also presented.

The results of our studies may be summarised as follows:-

- 1) Ia positive cells in normal rat kidneys are present in the interstitium, mesangium and in perivascular tissue.
- 2) Clustering of invading host lymphocytes is seen particularly where donor Ia positive cells are present.
- 3) Donor kidneys depleted of Ia positive cells by sublethal irradiation show diminished graft infiltration. (Not explained by the effect of irradiation only). The survival of Ia cell depleted kidneys was  $12.2 \pm 0.8$  (5) days of  $6.88 \pm 0.6$  (9) in controls. The number of lymphocytes isolated from dissaggregated grafts 4 days after transplantation was  $25 \pm 5.5 \times 10^6$  in controls and  $8.3 \pm 2.08 \times 10^6$  in Ia depleted kidneys. On the basis of these studies, we propose a model of the sequence of events in rejecting kidneys, with emphasis on the role of Ia positive cells resident in the donor graft.

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Monoclonal antibody OKT 3 appears to be useful for the treatment of kidney allograft rejection. One of its biological effects is the induction of mitosis in T lymphocytes. We have recently produced two monoclonal antibodies which appear to be reactive with the T 3 antigen, since the binding of these antibodies to T cells is completely blocked by OKT 3. When we tested these antibodies for their mitogenic effect, we found that one of them (WT 32, subclass IgG2A) was mitogenic for T cells from all individuals tested while the mitogenic effect of the other antibody (WT 31, subclass IgG1) showed great variability between individuals. Monoclonal antibody WT 31 is a potent mitogen for T lymphocytes from most normal subjects, but in 30% (15 out of 52 individuals) there is virtually no mitogenic response to WT 31. This "non-responsiveness" is reproducible with different blood samples and with frozen cells from the same donor. Immunofluorescence studies indicate that T lymphocytes from "non-responders" do bind antibody WT 31 to a similar extent as "responders". Preincubation of non-responder cells with WT 31 decreases the mitogenic response to WT 32. Population studies revealed no clear-cut relation between one of the HLA-A, B, or DR antigens and the non-responsiveness to WT 31. Preliminary data from family studies indicates that unresponsiveness to WT 31 does not segregate with HLA. In those individuals who are non-responsive to WT 31, another IgG1 antibody against the T 3 antigen (UCHT 1 from Dr. Beverley, London) is not mitogenic either. Therefore, the Fc moiety of the IgG1 antibodies might be involved. Addition of purified monocytes from a responder to purified lymphocytes from a non-responder restores the responsiveness to WT 31 and UCHT 1, suggesting that the polymorphism in the mitogenic effect of these antibodies is related to polymorphism in monocyte function (possibly the Fc receptor).

HAPTEN SPECIFIC SUPPRESSOR CELLS CAN PREVENT KIDNEY ALLOGRAFT REJECTION IN  
RECIPIENTS PRETREATED WITH HAPTENATED DONOR ALLOANTIGEN

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Associative recognition via antigen bridging among T-lymphocyte sub-populations has been proposed as a fundamental event in both augmentation and suppression of immune responses<sup>1</sup>. Extensive work in hapten-carrier systems has shown that suppressor T-cells specific for one epitope on a multideterminant antigen can diminish antibody responses against other epitopes on the same antigen<sup>2</sup>. We have extended these findings by demonstrating that similar lymphocyte interactions can be manipulated to suppress cellular immune responses and to enhance kidney allografts in rats. In our experiments the suppressor determinant was trinitrophenyl (TNP), while the other epitopes were histo-compatibility antigens. Suppressor cells (Cs) generated in DA (Rt1<sup>a</sup>) rats by injection of nascent TNP, were adoptively transferred into 400r irradiated DA recipients along with TNP-haptenated LEW(Rt1<sup>l</sup>) or PVG (Rt1<sup>C</sup>) spleen cell membrane. Recipients were then grafted with either a LEW or PVG kidney one day later. Rats given Cs and TNP-donor antigen tolerated their allografts (MST>100d) in contrast to those which were given normal cells and TNP-donor antigen (MST 17d), Cs and third party antigen (MST 17d), or normal cells or Cs alone (MST 11). The suppressor population was active for at least 3 months after induction and its phenotype is under investigation. Regulation of allograft responses via suppressor epitopes may be an important step in suppressor pathways. Such lymphocyte interactions may explain phenomena such as the blood transfusion effect, and an understanding of suppressor cell mechanisms will enable clinical manipulation of the immune response.

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THERMOSTABLE ERYTHROCYTE-ROSETTES IN RENAL FAILURE AND ALLOGRAFT REJECTION

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Allograft rejection is usually preceded by activation of T lymphocytes in which state such cells may be identified by their ability to form thermostable rosettes with sheep erythrocytes (TE-R). The objective of the present work, therefore, was to determine whether or not enumeration of TE-R in peripheral blood was of any value in the diagnosis of rejection.

TE-R levels were abnormally high in uraemic patients maintained by chronic haemodialysis but fell within the normal range shortly after renal transplantation. TE-R levels remained normal thereafter unless a rejection episode occurred, in which case there was a significant rise 1 - 2 weeks prior to that diagnosis by clinical and chemical criteria (p<0.001). Furthermore, TE-R levels remained high if the rejection episode turned out to be irreversible but fell if anti-rejection therapy was effective. Normal TE-R levels were noted in recipients without rejections, those with graft loss unrelated to rejection, those with acute tubular necrosis requiring acute haemodialysis, and patients with acute infections but no evidence of renal failure.

We conclude that TE-R elevation is a specific finding that may be used in the early diagnosis of allograft rejection and also in identification of its reversal.

CAN IMPENDING ISCHAEMIC NECROSIS OF BONE IN RENAL TRANSPLANTATION BE DIAGNOSED BEFORE IT OCCURS?

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Ischaemic bone necrosis (IBN) of various joints occurs in 4 - 41% of renal transplant patients receiving conventional immunosuppressive therapy (CIST). High dosage of steroids has been implicated. In this study 104 patients were studied radiologically and by scanning with a computer-linked 2-phase gamma camera after an injection of Technecium 99 Diphosphate (MDP), an isotope taken up by osteoblasts. Blood flow through the bone can be determined as well as normal accumulation of MDP. 90 patients had a renal transplant, 65 were receiving CIST, 12 Cyclosporin A (CyA) and steroids, 3 CyA alone. 10 haemodialysis (HD) patients who had previously received more than 2 kidney transplants with CIST were examined. 14 patients tested were on HD and had never received steroids or a transplant. An unsuspected incidence of 57% abnormal accumulation of  $^{99}\text{Tc}^{\text{m}}$ -MDP was seen in our transplant population. Even more surprisingly 3 patients receiving CyA only had scan abnormalities. 11 patients within this group had radiologically proven IBN and in 2 patients the bone scan abnormality preceded the X-ray finding by 2- 4 months. The discovery of such lesions before IBN may lead to its prevention as has been shown in an analogous situation<sup>1</sup>. It may also elucidate the pathogenesis of this grave complication of renal transplantation.

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WARM B-CELL ANTIBODIES ARE NOT A CONTRAINDICATION TO RENAL TRANSPLANTATION

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There is currently considerable variation between Tissue Typing Laboratories in the technique for pre-transplant B-cell crossmatching and the significance of a positive crossmatch is still unclear. Iwaki et al<sup>1</sup> have divided B-cell antibodies on the basis of the temperature of the initial incubation step. "Warm" antibodies react at 5<sup>0</sup> and 37<sup>0</sup> and are associated with poor graft survival, while "cold" antibodies react at 5<sup>0</sup> and may be non-damaging autoantibodies. In our unit, where all incubation steps are performed at 22<sup>0</sup>, 70 patients have received allografts in the presence of positive B or T and B cell crossmatches. In order to determine if the temperature of the initial incubation helped to identify harmful antibodies we have repeated the crossmatches on 27 of these patients at 5<sup>0</sup> and 37<sup>0</sup>.

Only two patients had antibodies reactive at 5<sup>0</sup> but not 37<sup>0</sup>. The other 25 patients had positive crossmatches at both 5<sup>0</sup> and 37<sup>0</sup>. The three month graft survival in this group was 84% (21/25). Thirteen patients had autoantibodies and all reactions were seen at 5<sup>0</sup> and 37<sup>0</sup>. Graft survival at three months was 85% (11/13) in this group. Fourteen patients either had no autoantibodies or were not tested for them. Twelve had positive reactions at 5<sup>0</sup> and 37<sup>0</sup> and graft survival was 83%. We conclude that incubation temperature does not help in identifying damaging antibodies. Other criteria are necessary for determining the significance of a positive B-cell crossmatch.

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THE FUNCTIONAL PARAMETERS POST TRANSPLANTATION OF PAIRED KIDNEYS FROM 60  
CADAVERIC DONORS. A COMPARISON OF POST TRANSPLANT FUNCTION ON DIFFERENT  
IMMUNOSUPPRESSIVE THERAPY

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120 cadaveric kidneys from 60 local donors were transplanted at St. Mary's Hospital, Portsmouth. Function was analysed at 1 and 3 months post transplantation as were the number of rejections, mean blood pressure at 7 days and HLA matching.

Results: At 1 month post transplant 66% of kidneys from the same donor were behaving similarly in terms of function whereas at 3 months the proportion was 45%. Further, in 18 cadaveric donors where 1 of the recipients was treated with conventional immunosuppressive therapy and 1 with Cyclosporin A. at 1 month 94% of the kidneys in the Cyclosporin A group were functional compared to 78% of the kidneys in the group receiving conventional immunosuppressive therapy (N.S.). However, at 3 months 94.4% of the Cyclosporin A group were still functional compared to 39% of the conventional immunosuppressive therapy group ( $p < 0.0005$ ). There was no statistical difference between the number of rejections, mean blood pressure or HLA matching.

We conclude that functional examination of kidneys at 1 month gives an indication of the predominantly preservation characteristics, as evidenced by the fact that a larger proportion of paired kidneys behave in similar fashion when monitored at that time. However, at 3 months immunological differences exerted by the recipients as well as the effect of immunosuppressive therapy is clearly indicated in the functional integrity of the transplanted graft when examined at that time.

DR TYPING OF CHRONIC RENAL FAILURE PATIENTS AND THE USE OF LYMPHOBLASTOID  
CELL LINES (LCLs)

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From recent reports of improved graft survival by the transplantation of DR matched kidneys, it follows that all CRF patients should be fully typed for A, B, C and DR antigens. However, DR typing of peripheral blood samples from CRF patients is notoriously difficult. To circumvent some of the technical problems associated with DR typing, we have used a relatively simple method to generate LCLs from small (10 - 15 ml) peripheral blood samples. Over the past year 58 individual cell lines have been formed, representing 47% of our total number of prospective transplant recipients. 90% of these cell lines have been easily and successfully DR typed. In most cases the DR type was confirmed if already known and the discrepant results were associated either with "difficult" DR antigens or LCLs from non-Caucasian patients. DR typing in 29% of these LCLs were from patients that had no DR type even after numerous (1-6) attempts on peripheral blood samples. In a number of patients, the LCL type enabled the second DR antigen to be specified. The generation of LCLs offers a relatively simple alternative to the use of peripheral blood samples for DR typing and may offer additional benefits in terms of ease and clarity of the DR type obtained.