A's. H K. R S. W Z

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WOODS, PRINTER, SOUTHWELL

British Transplantation Society

Summer Meeting 1979

Leicester Royal Infirmary

Tuesday, 3rd July, 1979

TIMETABLE

10.00 a.m.	Coffee
10.30	PAPERS 1 — 10 Chairman: R. W. Blamey
1.00 p.m.	Lunch
2.00	Business Meeting
2.45	SYMPOSIUM ON IMMUNOLOGICAL MONITERING Chairman: Professor P. Bell
3.45	Tea
5.30	Close
6.30	Society Dinner at Leicester University Local Organiser: Richard Wood

10.30 a.m.

1. A COMPARISON OF TECHNIQUES OF DUCT MANAGEMENT IN CANINE PANCREATIC ALLOTRANSPLANTATION

A. Procyshyn, P. McMaster, R. Y. Calne, R. Valdes, K. Rolles, B. Herbertson and D. Smith.

Cambridge.

Continued exocrine secretion by the pancreatic allograft has proven to be a major obstacle to successful whole organ pancreatic transplantation for the amelioration of diabetes mellitus. We have evaluated four techniques of management of the ductal system in pancreatic allografts performed in 35 pancreatectomized mongrel dogs. With two techniques, the allograft was placed orthotopically. In 9 dogs (Group 1), the donor graft included the duodenal loop which was interposed between divided recipient jejunum; in 8 dogs (Group 2) a modified Aquino duodenal patch was anastomosed to recipient jejunum. With the other two techniques, the allograft (without duodenum) was placed heterotopically. In 12 dogs (Group 3) the pancreatic ducts were ligated, and in 6 dogs (Group 4) obliterated by injection with Neoprene. All dogs were immunosuppressed with Cyclosporin A at 10 mg/kg/day, 18 mg/kg/day or 25 mg/kg/day. In Group 1, 3/9 animals died with graft vascular thrombosis, 2/9 of pancreatitis and 1/9 of duodenal bleeding. Median survival was 3 days. In Group 2, 3/8 dogs died of vascular thrombosis, 2/8 of pancreatitis and 1/8 with duodenal disruption. Median survival was 16.5 days. In Group 3, 3/12 died of vascular thrombosis and 3/12 of pancreatitis. Median survival was 17 days. In Group 4, 2/6 dogs died of vascular thrombosis and 1/6 of pancreatitis. Median survival was 30.5 days. The results show that attempts at pancreatic exocrine drainage using duodenum introduces the hazard of complications of intestinal anastomoses and does not reduce the risk of pancreatitis. Ductal obliteration with Neoprene shows promising potential for ductal management in pancreatic transplantation.

10.45 a.m.

INBRED RATS FOLLOWING BLOOD TRANSFUSION 2. CHANGES IN CELLULAR IMMUNE COMPETENCE IN

P. S. Veitch, B. K. Shenton, C. Proud, R. M. R. Taylor.

Newcastle.

Blood transfusions prior to renal transplantation are beneficial to subsequent kidney graft survival (Murray et al 1974). We have previously reported (Proud et al In press Brit. J. Surg.) prolonged cardiac allograft survival in inbred rats following third party blood transfusion. Using the same rat strain combination (DA—blood donor; PVG/C heart donor; WAG recipient) we have studied the effects of (A) blood transfusion on recipient lymphocyte immune reactivity and (B) passive transfer of lymphocytes from a DA blood transfused WAG to syngeneic WAG rats which subsequently received PVG/C cardiac allografts.

A) WAG rats received iv either 2 ml (1) DA blood or (2) syngeneic WAG blood (controls). Changes in recipient lymphocyte response to mitogens and DA and PVG/C lymphocytes were measured using a cytopherometric technique (Shenton et al 1977). No significant changes in lymphocyte responses were observed in the control group. A significant suppression of DA transfused WAG lymphocyte responses to mitogens and PVG/C lymphocytes was observed at day 7, was maximal at day 14 and by 56 days had returned to control values.

B) In two further groups 10^8 washed splenic lymphocytes were (1) transfered from WAG rats 14 days after DA transfusion to non transfused syngeneic WAG rats. (2) Control WAG rats received syngeneic non transfused cells. Both groups were transplanted with PVG/C hearts and received minimal post operative immuno-suppression. (Azathioprine and Prednisone).

Group	n	Median Graft Survival (Days)	Range (Days)
1	8	14	8 — 37
2 (control	8	8	8 — 26

Wilcoxon Rank sum test $p<0.05$

Conclusions (A) Following transfusion there is a suppression of recipient lymphocyte reactivity to mitogens and lymphocytes unrelated to the blood donor. (B) Passive transfer of lymphocytes from DA transfused WAG rats to non transfused WAG rats prolonged subsequent graft survival in these rats.

MURRAY et al. (1974) Tissue Antigens, 4, 548 — 557.
SHENTON et al. (1977) J. Immunol. Meth. 14, 123 — 139.

11.00 a.m.

3. THE IMMUNOSUPPRESSIVE PROPERTIES OF TWO COMMONLY USED BENZIMIDAZOLES, MICONAZOLE AND MEBENDAZOLE

J. J. Miller E. Couhig, S. C. Reeves, J. R. Salamam.

Cardiff.

The benzimidazoles miconazole and mebendazole are used clinically to treat fungal and hydatid infections. This study was carried out to see if they, like other imidazoles, also posses immunosuppressive properties. Both drugs were found to be capable of suppressing phytohaemagglutinin (PHA) and poke weed mitogen (PWM) stimulated responses of human lymphocytes but in case of miconazole this was due to a cytotoxic action of the drug. Both drugs suppressed mixed lymphocyte reactions (MLR) without cytotoxicity and prolonged the survival of skin allografts in rats. Mebendazole also extended the survival of rat heterotopic allografts. The greatest prolongation of skin and heart allografts was achieved with a combination of mebendazole, imuran and prednisolone.

The drugs were given orally at 200 mg/Kg and 15 mg/Kg respectively. Imuran and prednisolone were given at 4 mg/Kg I.P.

HEARTS Wistar (AgB — 2) → AS (AgB — 1)

NO. OF ANIMALS		TREATMENT	SURVIVAL TIME (days)	RANGE	p (v. Control)
1.	9	Saline	7.6 ± 0.87	(6 — 9)	
2.	6	Miconazole	8.3 ± 1.3	(7 — 10)	NS
3.	10	Mebendazole	12.4 ± 4.6	(8 — 22)	<0.005
4.	8	Meb/Imuran/Pred.	15.8 ± 9.1	(8 — 35)	<0.001
5.	7	Imuran/Pred.	8.6 ± .97	(7 — 10)	NS
SKIN					
1.	11	Saline	8.3 ± 0.5	(8 — 9)	
2.	7	Miconazole	12.8 ± 4.0	(10 — 12)	<0.005
3.	9	Mebendazole	17.7 ± 2.5	(12 — 21)	<0.001
4.	7	Meb/Imuran/Pred.	24.7 ± 11.7	(10 — 36)	<0.001
5.	6	Imuran/Pred.	10.8 ± 2.5	(9 — 14)	<0.005

11.15 a.m.

4. DONOR SPECIFIC SUPPRESSOR CELLS IN HUMAN RENAL ALLOGRAFTS

J. L. B. Dossetor, E. M. Liburd. T. Kovithavongs, M. R. Higgins, V, Pazderka.

University of Alberta, Edmonton, Alberta, Canada.

The low requirement for immunosuppressive medication after the first few months in successfully transplanted renal allograft recipients suggests that donor (D) specific immunologic "tolerance" may have been induced. We have sought eveidence of this by 2 *in vitro* parameters in sequential post-transplant sampling of recipient (R) patients' peripheal blood lymphocytes (PBL): a) changes in the capacity of mitomycin treated D cells (Dm) to generate cytotoxic lymphocytes (CTL) in R cells, in a 6 day mixed leukocyte culture when then tested in cell mediated lympholysis (MLC → CML) assay against D target cells; and b) suppression of CTL generation by Dm in third party normal cells are (X, Y, or Z) when small numbers of Rm cells are added to 6 day MCL when tested in CML against D target cells. Cell proportions in [X/Dm + Rm] culture are 1:1.25:0.25. These two tests were designed to pick up donor-specific suppressor cells (SL) in R.PBL.

Clear evidenc of SL was obtained in all patients (5 CD and 3 LD) studied so far. SL appeared within 3 weeks and remained thereafter. No major rejections occurred in these 8 so we cannot say if SL, and donor specification R unresponsiveness, disappear before rejection though these aspects are under study. This suppression by R.PBL is mediated by the T cell subpopulation. SL develop in HLA-identical sibling recipients after transplant so HLA differences are not responsible for generation of SL, in these.

11.30 a.m.

5. THE TIMING OF ANTI-REJECTION THERAPY

J. R. Salaman & E. Couhig

Cardiff.

It is often assumed that early treatment of a rejection episode is important and that if treatment is delayed rejection may not be reversed. Much time and ingenuity has been expended on devising tests that might warn of impended rejection so that treatment might be given earlier. As we know of no evidence to show that early treatment is important, the following experiments were carried out.

The pattern of rejection of rat heart allografts was studied in 9 SA rats (AgB — 2) transplanted with Wistar (AgB — 1) hearts. Biopsies and E.C.G. recordings were obtained on days 2, 4, 6 and 8 after transplantation and these were compared with similar studies obtained from a group of rats with cardiac isografts. All rats received Imuran 4 mg/Kg and Prednisone 4 mg/Kg daily. The allografts showed a significant degree of destruction histologically and a reduced potential on E.C.G. by day 6, which was even more evident on day 8. Four other groups of immunosuppressed rats (groups 3 — 6 below) were then treated with a single dose of Methylprednisolone (16 mg/Kg) on either day 0, 2, 4 or 6.

SURVIVAL OF HETEROTOPIC HEART ALLOGRAFTS

	Survival (days)	Mean (days)	p (v. control)
1. Nil	6, 8, 8, 8, 8, 8, 9	7.9	
2. Imuran (I) + Prednisone (P)	7, 8, 8, 9, 9, 9, 10	8.6	
3. I + P + Methylprednisolone on day 0 (MO)	9, 9, 10, 19, 34, 50+	21.8	(<0.05)
4. I + P + M2	8, 9, 9, 9, 10, 10	9.2	
5. I + P + M4	8, 9, 9, 9, 18, 25	13.0	
6. I + P + M6	8, 9, 12, 14, 15, 25, 34	16.7	(<0.05)

Methylprednisoloe significantly extended graft survival when given on the day of transplantation and on day 6 when the first study showed rejection to be well advanced. "Early" treatment of rejection on days 2 or 4 was largely ineffective.

11.45 a.m.

6. SHOULD REJECTION EPISODES RECEIVE EARLY TREATMENT?

M. J. Thompson, R. W. Blamey

Nottingham.

In a Lewis → D.A. heart graft system, immunosuppression on a pulsed basis has been found as effective as continous immunosuppression:

Immunosuppression	Rejection (Day)	Mean
Nil	11, 11, 11, 11, 12, 14, 14	12
Continous steroid plus cyclophosphamide (Day 1 — 19)	10, 28, 31, 49, 100, 100, 100	59.7
Pulsed steroid plus cyclophosphamide (Days 5, 12, 19)	13, 33, 46, 100, 100, 100, 100, 100 100, 100	79.2

Pulsed cyclophosphamide alone (Days, 5, 12, 19) was then used, giving survival of 50 days.

Further experiments have revolved around this to examine whether pulsed suppression should given early or later in a rejection episode:

Cyclophosphamide (50 mgms/kg)	Rejection (Day)	Histology of rejection	Mean
Pulse at day 3 only	14, 28, 67 +, 63 +, 56 +	Mild	48.5 +
5 only	15, 18, 19, 21, 25, 26, 27	Advanced	21.5
7 only	11, 12, 12, 13, 14, 35	Advanced	16.1
9 only	9, 10, 10, 11, 20	Advanced	12.0
	(3 day and 5 day $t = 3.1$, $p < 0.01$)		

It appears that rejection is well controlled by a pulse of immunosuppressive therapy given early in a rejection episode (i.e.) when the histogical picture of rejection is mild.

12.00 a.m.

7. RENAL TRANSPLANT BLOOD FLOW MEASURED BY DOPPLER ULTRASOUND

R. F. M. Wood, D. H. Evens, and D. G. Nasmyth

Leicester.

Vascular accidents are a potentially avoidable cause of transplant failure. By the time they are suspected and diagnosed it is often too late to save the graft. In this study Doppler Ultrasound has been used to investigate progressive vascular occlusion in canin renal auto-transplants.

A directional Doppler flow probe connected to a spectrum analyser was used to measure renal artery blood velocity. The renal vessels were connected to the iliac vessels using silastic tubing. Stenoses of unknown dimensions were inserted on either the venous or arterial sides of the circuit. The venous outflow was designed to enable the pressure drop across the stenosis to be measured. Renal blood flow was monitored by an in-line electro-magnetic flow meter.

In transplants auto-grafts observed blood velocity had a characteristic wave-form with a Pulsatility Index (PI) of 1.01 (s.d. ± 0.18). Renal vein obstruction and twisting of the kidney produced more pulsatile waveforms with higher PIs. A 94% venous stenosis caused a significant increase in PI to 2.06 (s.d. ± 0.96), with complete venous occlusion consistently raising PI above 15. Renal artery occlusion produced a progressive reduction in PI to a level of 0.46 (s.d. ± 0.09) at greater than 90% stenosis.

These studies demonstrate that Doppler Ultrasound is of potential value in the early detection of vascular problems in renal transplantation.

12.15 p.m.

8. PLASMA EXCHANGE IN RENAL ALLOGRAFT REJECTION

J. D. Briggs, R. L. Cumming and R. B. Hogg

Glasgow.

Plasma exchange was carried out in 21 acute rejection episodes in 18 patients which had failed to respond 2 or 3g of IV prednisolone. Four litre exchanges with plasma protein solution were carried out between two and five times in each case and were associated with improvement in graft function in 16 of the 21 episodes. The graft survival at six months was compared with that in a group of 14 patients with acute rejection treated during the previous two years with similar doses of IV prednisolone.

In the patients treated by plasma exchange and IV prednisolone the graft survival was 65% compared with 29% in those receiving prednisolone only. The incidence of infection and its related mortality did not differ significantly between the two groups of patients. This study suggests that plasma exchange is beneficial in the treatment of acute rejection and indicates the need for a controlled trial.

12.30 p.m.

9. SCREENING OF SERA FROM RENAL TRANSPLANT RECIPIENTS FOR BIOLOGICAL ACTIVITY

Carol Grey, Veronica van Heyningen, Margaret Ennis, C. M. Steel and J. T. Anderton

Edinburgh.

Sera from more than sixty renal transplant recipients have been screened for complement-dependent cytotoxic activity against a panel of HLA-typed human blood lymphocytes and against cultured human cells including B and T lymphoid lines, a myeloma line on two or more occasions and an attempt has been made to relate the findings to their clinical course. Cytotoxic activity could be detected in only about 10% of sera and few reactions could be attributed to anti-HLA A, B, C or DR specificity. In two patients, one of whom had been transplanted many years earlier, transient appearance of non-HLA-related strong cytotoxic antibody activity correlated in time with episodes of acute rejection. Both of these sera, as well as samples from several other patients, suppressed lymphocyte activation *in vitro* by mitogens and/or by allogeneic cells.

12.45 p.m.

10. SERIAL CYTOLOGY, CERVICAL AND VIRAL STUDIES ON FEMALE PATIENTS UNDERGOING TRANSPLANTATION

C. J. H. Ingoldby, N. McWhinney, J. E. Castro, J. Wachtel

Hammersmith.

Cancer is 100 times more frequent in renal transplant recipients than age matched controls (Penn).The short latent period of 38 months between transplantation and tumour appearance may be related to the high incidence of virus infections and result from activation of oncogenic viruses which is known to occur in immunodeficient animals (Nehlesen, S.L., Clin. Exp. Biol., 9, 63, 1971). Urinary cytology can reveal occult virus infection.

During a 3 year period, we studied cervical and urinary cytology in all females who survived more than 6 months after transplantation.

Fifty patients, average age 42.5 years, on immunosuppression for 4.82 years average (range 0.5 — 10 years) were followed serially.

Cervical cytology showed one patient with trichomonas, eight with monilia and one with herpes. There was one case of dysplasia and one carcinoma-in-situ present on initial screening.

Examination of the urine showed one patient with monilia, two with CMV, one with polyona virus and one with papavovirus. None of these infections were clinically apparent.

The low incidence of abnormality contrasts with other studies and the reasons for this will be discussed.

Penn, I. (1977) Transpl. Proc. 9. 1121.