

Conclusions
 Increase 53% no transpl still only 23/million
 Modest rise from 66% - 72% but in 3 of 8 centres still
 less than 70% - first yr graft survival
 No increase in pt survival but 91% @ 1 yr.
 Only 7% are diabetic
 more than 60% wait for more than 1 yr

Conta effect
 1 yr graft 54 - 81% but closer than before
 death infection 0 - 11%
 immediate function 12 - 80%
 good function at 1 yr.
 > 55 2% - 30%



Try to:
 better match
 obtain immediate function
 shorten cold ischaemia
 try to reduce infection

Notes: are high centres still low still low?
 A Some change from high to medium low?
 Tony from Nicholl CytA effect disappear?
 CytA helped post centres, not if already high
 Would it accept this

THE
 BRITISH TRANSPLANTATION SOCIETY

AUTUMN MEETING

Wednesday, 29th November 1989

Royal Society of Medicine
 London

PAPER 1

A PROSPECTIVE STUDY OF ANTI-THYMOCYTE GLOBULIN PLUS HYDROCORTISONE (ATG) VERSUS CYCLOSPORIN A PLUS PREDNISOLONE (CYA) IN SENSITISED AND REGRAFTED RENAL ALLOGRAFT RECIPIENTS

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Steroid resistant rejection is more common in highly sensitised and regrafted renal allograft recipients, and leads to graft loss in a significant proportion. 30 such recipients were randomly allocated at the time of transplantation to receive sequential immunotherapy, (ATG followed by Cyclosporin A + low dose prednisolone, n=15), or CYA + low dose prednisolone alone (n=15). There were no significant differences in recipient or donor characteristics or HLA matching. More patients on CYA had delayed function (n=7, 4 dialysis dependent) than on ATG (n=2, not dialysis dependent). There were no early immunological graft losses. 3 patients have lost grafts, all from the ATG group (myocardial infarction 4 months, chronic vascular rejection 11 months, transplant microabscesses 5 weeks). All other kidneys are functioning at 3-25 months with no differences in mean serum creatinine (ATG 154µmol/l, sem 9.5, NS t test). Early graft rejection was similar in each group (ATG 3/15, CYA 6/15, p=0.2 X²). Rejection after ATG was associated with infection, and reversed by steroids (2) or OKT3 (1). In the CYA group, 4 patients had steroid sensitive rejection, 2 required ATG. Complications, including infection were only associated with ATG (8 UTI, 3 viral infection, 1 candida, 3 septicaemia, 9 pyrexia, 2 rash, 2 arthralgia, 2 serum sickness). ATG reduced the incidence of early graft dysfunction in sensitised and regrafted patients; the price in terms of infection and complications was unacceptably high.

Rabbit ATG 2.5mg/kg controlled by leucocyte count
Same immunosuppression as other group.
3 pt triple therapy for less than 10 days.
Salaman: group in US who use ATG give antimicrobials + antifungals. Given here?
No: a single shot on table.

PAPER 2

DOES THROMBOXANE RECEPTOR BLOCKADE AMELIORATE CYCLOSPORIN A (CyA) NEPHROTOXICITY?

Sp dark hair, specs
dark suit -

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Chronic nephrotoxicity is the major limitation to the usefulness of CyA in transplantation and autoimmune disease. Thromboxane A₂ (TXA₂) is a potent vasoconstrictor which has been implicated in the mechanism of CyA nephrotoxicity. Renal production of its stable metabolite Thromboxane B₂ (TXB₂) is significantly increased in animals and humans treated with CyA. Animal studies have demonstrated partial reversal of nephrotoxicity by combining a Thromboxane Synthetase Inhibitor with CyA.

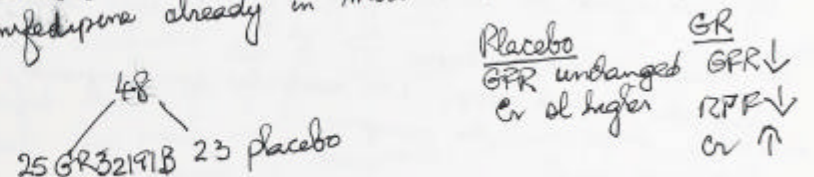
A group of renal transplant recipients between 6 weeks and 2 years post transplantation was treated for 3 months with a TXA₂ receptor antagonist, (Glaxo: GR32191B) in a double blind controlled trial.

Glomerular Filtration Rate (GFR) and Effective Renal Plasma Flow (ERPF) were measured using a single isotope injection technique. Creatinine clearance (Cl_{Cr}), serum creatinine (SeCr) and urinary prostanoid concentrations were also monitored.

Treatment with GR32191B produced no improvement in renal function: an overall decrease in GFR being demonstrated during the study period (p=0.025). Despite this unexpected decrease in GFR, deterioration in ERPF, Cl_{Cr} and SeCr did not reach statistical significance.

We conclude that TXA₂ receptor blockade does not appear to ameliorate clinical CyA nephrotoxicity. Combined Thromboxane Synthetase Inhibition and TXA₂ receptor antagonism may provide a more physiological approach to this problem.

* * * Excellent 1yr graft survival with BiA but progressive deterioration in the following years, preceded by gradual rise serum Cr
Traced at (to have stable patients) - stability at 6/52 challenged by 11m
6/52 - 2 yrs post transp; serum Cr < 300; > 14 yrs old.
On nifedipine already in most instances



Hyp Nifedipine has already utilized any reversible element of renal function available - but in fact GFR actually fell.

BT alone > 5 units 10-15%
 BT + new graft 50%
 BT + pregnancies 30%

THE EFFECT OF CYCLOSPORIN ON IMMUNOGLOBULIN-CLASS SWITCH IN PATIENTS RECEIVING BLOOD TRANSFUSIONS *Random*

Barbara B.K. Weber, M. C. Jones, G. Hillis, G.R.D. Catto, A.M. MacLeod Department of Medicine and Therapeutics, University of Aberdeen

Middle European. long hair tied back with long bow

IgG antibodies detected only by flow cytometry against donor lymphocytes in potential graft recipients correlate with an increased number of rejections and impaired graft function. Dialysis patients whose sera contains such antibodies risk developing cytotoxic antibodies following further transfusions. The effect of cyclosporin A (CyA) on the alloantibody response to blood transfusions (BT) was investigated by flow cytometry in 15 previously untransfused dialysis patients. 7 (group 1) received 5 third party BT at 2 weekly intervals with CyA (10mg/kg/day). 8 (group 2) received BT alone. The development of IgG and IgM antibodies was monitored by flow cytometry after each transfusion against lymphocytes from 6 normal donors (564 serum/cell combinations tested).

All antibodies detected in group 1 were IgM. 0/7 of patients in group 1 but 6/8 in group 2 developed IgG antibodies (p<0.02). IgG antibodies occurred in 0/270 serum/cell combinations in group 1 but in 29/294 in group 2 (p<0.001, Table). Cytotoxic antibodies occurred in 2 serum/cell combinations in group 2.

BT + Cy			Group 2 BT alone	IgM	IgG
Group 1	IgM	IgG			
BT CyA			1	2	9
1	2*	-	2	2	1
2	3	-	3	2	4
3	8	-	4	-	8
4	-	-	5	-	4
5	-	-	6	-	3
6	-	-	7	-	4
7	-	-	8	-	-

*number of serum/cell combinations

We conclude: A) CyA abrogates the switch from IgM to IgG antibody production in patients receiving random BT. B) Giving CyA with BT may thus prevent the occurrence of antibodies which are potentially harmful to a subsequent graft and are early evidence of developing sensitisation.

CyA abrogates the switch from IgM to IgG after transfusion

*Ben Bradley: need to know if; etc
 3 females in each group, 1 in each had been pregnant; no diff in DRB1/6 + DR1 in the group.
 But nos. small.*

Some people regard IgM as innocent if against

PAPER 4 Immediate non-function

NIFEDIPINE AND OTHER FACTORS INFLUENCING IMMEDIATE RENAL ALLOGRAFT FUNCTION

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We performed a retrospective analysis to examine factors affecting immediate function of renal allografts including drugs given to this end and the incidental administration of calcium channel blocking drugs. We defined failure of immediate function as the need for dialysis or a fall in creatinine > 15% within four days of transplantation. The drug histories of 172 patients transplanted over 32 months were examined. After exclusions 138 patients were analysed. 16% of those taking Nifedipine (n=31) for hypertension or angina at the time of transplantation failed to achieve immediate function compared with 40% for the rest (X²p<0.05). Sex, cause of death of the donor, warm ischaemic time < 30 minutes, administration to the donor of Mannitol, Steroids, Heparin and Phenoxybenzamine had no effect on the rate of immediate function. Administration of Dopamine to the recipient also had no effect. The immediate function rate was significantly worse when the donor age was > 50 years, the total ischaemic time was > 24 hours and when Dopamine had been given to the donor (X²p<0.05). Elevated trough whole blood Cyclosporin levels (>600nmol/l polyclonal radioimmunoassay) in the first week were associated with reduced immediate function (M.W.U.p<0.05).

In conclusion we suggest that Nifedipine reduces the incidence of primary non function whereas the donor's age, the length of ischaemia and the level of CyA contributes to primary non function.

Many pts given channel blockers pre transplants either for hypertension or angina.

*Immed non-function 30-70% in diff centres
 all pts cy 10mg/kg loading dose prior to operation for first graft
 other drugs eg steroid added if rejection*

Recipients received low dose dopamine for 3 dys

post op

*172 pts excluded 10 LD; used left with 138 pts
 84% Nifed had 1yr graft survival of 74% without it
 but low log rank not sign.*

*Low dose dopamine to recipient no benefit in terms of immediate function
 Tony Nichol: the state of hydration of recipients important in whether immediate graft function*

THE CLINICAL COURSE OF SOLID ORGAN TRANSPLANTATION IN HIV(+) PATIENTS

Mark

MH Cooper BSc MB ChB, AG Tzakis MD, TE Starzl MD (GR Giles MD FRCS) Department of Surgery, University of Pittsburgh, 3601 Fifth Avenue, Pittsburgh, PA United States of America.

Since organ transplantation in HIV(+) patients is contentious, we have undertaken a survival study of 25 patients transplanted between 1981 and 1988 who were or became HIV(+). Fifteen underwent liver transplantation and 5 each heart and renal transplantation. Eleven patients (44%) were HIV(+) prior to transplantation (prevalent group) although in only 3 (12%) was this known before operation. Of the remaining 14 patients (56%) seven converted following transplant. Ten patients were children (mean age 7.8 years) and 15 were adults (mean age 43 years). Nine patients (36%) developed AIDS after a mean time (AFT) of 2.4 years following transplant, of whom 6 are now dead. The children did well with a mean AFT of 3.12 years and mean survival of 3.8 years. By life table analysis there was a 58% 2 year survival in the HIV(+) patients compared to a 72% 2 year survival in 1491 HIV(-) liver transplant patients (NS). The HIV (+) transplant patients had a shorter AFT than 28 HIV (+) haemophiliacs (p=0.005) but similar to 42 transfusion acquired HIV(+) patients. Of the 13 current survivors, 12 have normally functioning grafts and 9 are employed.

Thus, although transplantation may shorten AFT in some HIV(+) patients, many benefit from the procedure. HIV is therefore not an absolute contra-indication to transplantation.

Immunosuppression not diff between pos + neg pts

Dummer et al Transpl 1989

3023 transpl'd in Pittsburgh Jan 81 - Sept 88, 25 HIV pos (0.8%) - 11 before transpl., 14 converted after transpl.

Deaths, TB, CMV, hepatitis

Control groups

28 haemophiliacs 14 transplanted pts of w/it the transpl., only after 3 yr the transpl had higher seroconversion rate

Rejection rate of 28

up to 15 mths no diff in liver survival in HIV+ & - but differs after that graft survival also good in HIV+ (not due to death) Mortality was similar in both positive and neg HIV groups

Bob Johnston donor who was transplanted was HIV neg but 5 organs transpl and still accepts courses know HIV pos, 1 death in 22 yr recipient

Esoter will ventilate for purpose of harvesting organs; when decided that death is inevitable, ask relatives and if agree will ventilate

PAPER 6

Nottingham "expected deaths" actual death ventilated about 3 extra yr.

DEATH, BRAIN DEATH, AND ORGAN DONATION IN A NEUROSURGICAL UNIT

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We present an analysis of potential and actual organ donors among 403 patients who died in the Glasgow neurosurgical unit in the three years 1986-88, and we assess the scope for increasing the number of donors from this source.

Brain death was diagnosed in 88 patients, and detailed information will be presented on these cases. Permission for organ donation was sought in 57 (84%) of the 68 potential donors, and granted in 41 (72% of these asked). Donation was not requested in 11 patients, but in five of them there were cogent reasons. Thirty-eight ventilated patients became asystolic without brain death testing, but only six of them might have been potential organ donors. Ventilation was discontinued before death in 65 patients, and 212 were never ventilated; considering any of these 277 patients as potential donors would have required major changes in their management and raised ethical and practical dilemmas.

Neurosurgeons are already familiar with the concept of brain death and the practicalities of organ donation, and 60% of suitable brain-dead patients in this unit donated organs in 1986-88. Any increase in the number of donors from this source would be limited without possible undesirable changes in practice. Campaigns to encourage organ donation in the intensive care units of general hospitals may have greater potential for increasing the number of organ donors.

But 40% did not 22 pot donors 11 died within 6 mths 6 not tested might have been w/ 12 kidneys 3 yrs

2.7 million pop. 100 beds (8 ICU beds)

assumed 500 head injury 150 150 spon. intra haemorrhage. 403 ventilated, 126 until death.

210 never ventilated 88 brain death cuts 38 " " not fulfilled.

showed 24 died within 24hrs after taking off ventilator

Unsuitable for donors 20 pot. donors 68 all except 6 were dead requested donors 57 (57%) success 30 out of 4 who asked? 14/20 cons; 22/30 SR 6/7 not successful failed to ask 11, refused 15, proc formal 1

EVIDENCE FOR PROCESSING OF WAG ANTIGENS BY THE RT1-D
CLASS II MHC MOLECULES OF PVG RECIPIENTS DURING
KIDNEY GRAFT REJECTION

Dark haired girl C.A. Priestley, S.C. Spencer, G.J. Sawyer, J.W. Fabre.
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The allograft response has been seen primarily as a direct reaction by recipient lymphocytes against the cells of the graft. A role for processing of graft antigens by recipient presenting cells has been postulated, but much less extensively studied. We have examined this question by blocking with monoclonal antibodies the class II MHC antigens of the recipient during rejection responses, using recipient-specific anti class II antibodies. For this purpose, we raised a new polymorphic mouse IgG₁ antibody (BMAC-4). Binding studies to purified RT1-B and RT1-D class II MHC antigens demonstrated that BMAC-4 recognises rat RT1-D class II antigens. BMAC-4 reacts with the DA (RT1^b) LEW (RT1^l) and PVG (RT1^e) strains, but is entirely negative on the WAG (RT1^u) strain as assessed by immunohistology, flow cytometry and radioimmunoassays. BMAC-4 is therefore recipient-specific in the WAG to PVG strain combination.

Treatment of PVG recipients of WAG kidney grafts with BMAC-4 (anti-recipient RT1-D class II) on the day of grafting and on the first and third post operative days increased the median survival time from 10 days to 34 days. Treatment with the MRC OX6 antibody (monomorphic IgG₁, anti RT1-B class II) was without effect. The dose of antibody used was sufficient to maintain free serum levels for 24 hours in the treated animals. These data suggest that processing and presentation of WAG antigens by PVG RT1-D class II antigens makes an important contribution to the rejection response.

BMAC₄ treated 0.1 ml sub mab 0.1 ml all prolonged survival (though all had rejection episodes)

LOW DOSE FK506 ABROGATES THE MHC CLASS I RESPONSE
TO BLOOD TRANSFUSION

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Blood transfusions (BT) may include cytotoxic antibodies to HLA class I antigens so precluding transplantation from certain donors. We therefore assessed the ability of the novel immunosuppressive drug FK506 to prevent sensitisation to rat MHC class I antigens (RT1.A) when given with blood transfusions.

Three groups of 6 AO rats (RT1.A^u), high responders to RT1.A^a, received two initial BT of 0.5ml DA (RT1.A^a) whole blood, on days 1 and 7. In addition group 1 received FK506 0.3mg/kg/day in olive oil, and group 2 FK506 0.3mg/kg/day in saline suspension, both i.m. for 14 days following the first BT. On days 42 and 49 all rats received a further DA BT. Sera from all rats were obtained at weekly intervals throughout the study.

All rats in groups 1 and 3 developed antibodies to DA class I antigens, detectable in the indirect haemagglutination (IHA) and the ⁵¹Cr release cytotoxicity assays at dilutions >2⁸, which persisted throughout the study. Activity in group 1 was, however, significantly less than that in group 3 (p<0.05). In contrast, in group 2, alloantibody responses, detected by either assay were completely suppressed during the initial BT period. When rechallenged with DA blood there was a transient rise in low titre (<2³) antibodies detected by IHA but cytotoxic antibodies did not develop.

We conclude (a) low dose FK506 prevents sensitisation, yet at the same time induces suppressor activity to class I antigens, and (b) the drug vehicle has a critical influence on efficacy at this dosage.

Jones et al Transpl 1988 rats CyA abolished IgG response + reduced IgM

High responder grouping of rats for transfusion.

*FK506 10% in olive oil } given IM (would now use orally)
given 10% in saline*

Trans dys 0, 7 and ab end

<i>4 groups</i>	<i>FK in oil + CyA</i>	<i>Reduction in antibody activity</i>
	<i>in saline + CyA</i>	<i>↓</i>
	<i>CyA alone</i>	<i>↓↓</i>
	<i>Vehicle alone</i>	<i>-</i>

Rechallenge in oil not abrogated, saline did, CyA did

Dose 0.3 mg/kg : when made up in oil goes lumpy

Multicentre trial : held back by the relationship between Japanese + Swiss

PAPER 9

WATER-SOLUBLE CLASSICAL CLASS I MHC MOLECULES DO NOT INFLUENCE THE GENERATION OR EFFECTOR FUNCTION OF CLASS I ALLOSPECIFIC OR SELF CLASS I RESTRICTED CYTOTOXIC T CELLS

2nd paper!

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Water soluble, classical (RT1-A) class I MHC molecules were bulk purified from aqueous extracts of DA (RT1^a) strain liver using a combination of monoclonal antibody affinity, lentil lectin affinity and gel permeation chromatography. These class I molecules were tested for their effect on both the generation and the effector function of allospecific PVG and LEW anti DA RT1-A class I cytotoxic T cells and TNP specific, self RT1-A^b restricted cytotoxic T cells.

MLC cultures were incubated with soluble class I MHC molecules in concentrations up to 5µg/ml (10⁻⁷M), equivalent to the class I antigenic activity of 10⁹ spleen cells per ml (-250 times the concentration of class I on the stimulating cells). However, no suppression of the generation of allospecific or self class I restricted cytotoxic T cells was seen.

Following the generation of cytotoxic T cells by normal MLC, the addition of soluble class I MHC molecules in concentrations up to 5µg/ml (-300,000 times the concentration of class I on the target cells) during the effector stage of the assay did not inhibit the allospecific or self class I restricted cytotoxic T cells effector function.

We conclude that water soluble classical class I MHC molecules have little potential for influencing the cytotoxic T cell response, either by functional inactivation of the responding T cells or by competitive inhibition of essential receptor-ligand interactions such as those between CD8 and class I or the T cell receptor and class I.

PAPER 10

THE INDUCTION OF TRANSPLANTATION TOLERANCE USING DONOR ANTIGEN AND CD4 MONOCLONAL ANTIBODY

Area unglycylated by post

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The aim of this study was to develop a protocol for the manipulation of the immune system that is effective in eliminating the rejection response and specific for the foreign histocompatibility antigens expressed on the allograft. We describe a technique whereby adult mice were made specifically unresponsive to vascularised cardiac allografts by pretreating the recipient with donor blood under the cover of a brief course of mAb against CD4.

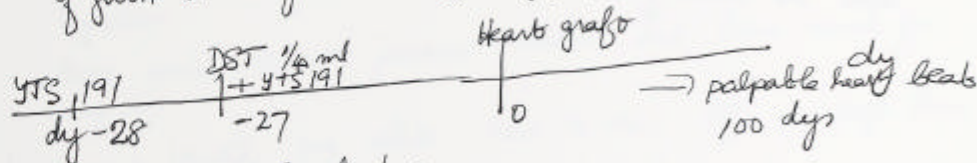
The anti-CD4 mAb, YTS 191.1, was given intravenously at 28 and 27 days preoperatively. The second dose of anti-CD4 mAb was combined with a donor specific blood transfusion (DST). Heterotopic cardiac transplantation was done on day 0. Grafts were followed by daily palpation.

Several full MHC mismatched strain combinations were tested over a range of anti-CD4 mAb doses (5µg-150µg/dose). In the C57BL/10(H-2^b) to C3H/He ((H-2^k) combination at the optimal dose, tolerance was induced as defined by indefinite graft survival. Higher and lower doses were less effective. Neither the YTS 191.1 or the DST alone when given 28 days prior to transplantation were as effective in prolonging graft function. Tolerance was further tested in recipients with functional heart grafts by skin grafting.

This new protocol can achieve transplantation tolerance in the adult murine cardiac allograft model.

DST 80% reg grafts by 50 days post-op, no only partially effective

Anti-CD4 antibody (YTS 191.1) effective up to 100 days if given in adequate dose (25µg), but this is non-specific



Dose 50 µg optimal dose
Skin grafts after 100 days non-spec donor skin - non-specific were rejected donor spec skin survived (when skin rejected hearts remained functional).
At least 5 animals in each group.

The protocol design is for the animals to see the DST under cover of YTS 191, but as time of transplant the YTS effect worn off and only DST effects present.

SERUM C-REACTIVE PROTEIN IN RENAL TRANSPLANT RECIPIENTS
AS AN AID TO DISCRIMINATION BETWEEN EARLY REJECTION AND
CYCLOSPORIN (CYA) TOXICITY.

Kate: very fat

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Immunology Dept, Southampton⁽⁺⁾.

The clinical course of patients receiving renal transplants is often complex and difficult to manage. One problem is in discriminating clearly between an early rejection episode and toxicity due to CYA, and cytokine measurements have been used to assist with this. Cytokines however also include release of C-reactive protein (CRP) and we have developed a highly sensitive, economical ELISA (sensitivity < 1 ng/ml) for this serum protein. 35p (sample + labour) 5-6 hrs

CRP was measured in sera from 104 normal controls, 95 haemodialysis and 55 CAPD patients, and in 57 patients during hospital admissions in the first 90 days post transplant.

In 24 episodes of rejection, peak levels of CRP were in the range 27 - >210 µg/ml., whereas in 11 episodes of CYA toxicity levels were 0.3 - 18 µg/ml i.e. within the normal ranges. CRP levels also fell with successful response to antirejection therapy.

Serum CRP was also useful in detecting and monitoring infection in patients with stable renal function. This highly sensitive assay provides a useful, economical, additional test for detection of rejection and infection and for differentiation between rejection and CYA toxicity in renal transplant recipients.

Rob: are you now acting on CRP results? No not yet
when does rise in CRP in relation onset of rejection?
A - difficult in getting early samples - sometimes pulled before she knew of them.

ENCAPSULATION OF PORCINE ISLETS IN
ALGINATE/POLY-L-LYSINE/ALGINATE MICROSPHERES

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The complications of long term immunosuppressive therapy preclude pancreas allo-transplantation as an acceptable treatment for uncomplicated Type 1 diabetes. However, encapsulation of rodent pancreatic islets within biocompatible membranes will prevent their rejection following xenotransplantation.

A simple method for the isolation and encapsulation of islets from the porcine pancreas has been developed. The pancreas was removed from anaesthetised weanling pigs and disrupted by sequential collagenase digestion. Islets were separated from the digested gland by sedimentation through a discontinuous gradient of Percoll at unit gravity. Individual islets were encapsulated within membranes comprised of calcium-alginate/poly-L-lysine/calcium alginate. not centrifuge

Glucose evoked insulin secretion of individual islets was assessed in-vitro. Eleven out of fourteen non-encapsulated islets and fourteen out of fourteen encapsulated islets responded to 16mM glucose: The median percentage increase in insulin output by non-encapsulated islets was 112% (range 0-1251%) and by encapsulated islets 154% (range 82-1333%).

Encapsulated islets were injected into three diabetic rats. Circulating levels (2.6-9.9 µU/ml) of porcine insulin were detected in serum 21 days post-transplant, effects on blood sugar variables stable to serum

This study demonstrates that isolated islets withstand encapsulation within alginate microspheres and retain their ability to secrete insulin in response to glucose stimulation.

Islets yields first successful Keith Reamster in diabetic rats
Rejection
Chose pork because porcine insulin has been used for many yrs
How to identify pig islets: hard to do: After 100 pigs have bount.
Salaman any reaction detected: foreign body reaction observed but difficult to identify the spheres.

CAN IMMUNOTOXINS DEplete GRAFT INTERSTITIAL DENDRITIC CELLS PRIOR TO TRANSPLANTATION AND THEREBY IMPROVE ALLOGRAFT SURVIVAL?

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- 2 Renal Transplant Unit, Royal Hallamshire Hospital.
- 3 Department of Pathology, University of Sheffield.
- 4 Department of Medical Microbiology, University of Sheffield.

The removal of interstitial dendritic cells (DC) from an allograft prior to transplantation into a recipient has been shown to improve graft survival in a number of experimental studies, but current regimes used to achieve this cannot be employed clinically. We therefore investigated in a rat experimental model whether this could be achieved by hypothermic perfusion of isolated grafts with anti DC immunotoxins (IT). ITs directed at rat class II MHC and leucocyte common antigen molecules were prepared by conjugating monoclonal antibodies and ricin A chain. In vitro experiments showed that only the anti class II ITs were capable of inhibiting alloantigen presentation and in the absence of responder cell treatment this was most effective when the stimulator and responder strains shared a haplotype (1). Subsequently isolated F1 hybrid (AGUS x WAG) kidneys were hypothermically perfused with an anti class II IT Prior to transplantation into AGUS recipients. This resulted in prolonged graft survival (9,11,13,30,>100,>100 days) when compared with perfusate alone perfused allografts (8,9,9,10,10,10 days) ($P < 0.05$, Wilcoxon's signed rank test) and no nephrotoxicity was observed even though the proximal tubular cells expressed the target antigen. The clinical implications of this work will be discussed.

1. J Clin. Exp. Immunol (1989) 76, 132-137.

IMMUNOHISTOCHEMICAL CHARACTERISATION OF THE MONONUCLEAR CELL (MNC) INFILTRATE DURING REJECTION OF ORTHOTOPIC LIVER TRANSPLANT (OLT)

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We have characterised the MNC infiltrate in 15 liver biopsies from 8 children (7 female, mean age 6.5 years, range 3 - 12) during acute or chronic rejection after OLT. Acute rejection (AR) was present on 7 occasions and chronic rejection (CR) on 8, on 3 of which it was accompanied by the appearance of vanishing bile duct syndrome. One child underwent three biopsies during the first two months post-OLT, for clinical suspicion of AR. Cryostat sections were stained in a two-step immunoperoxidase technique with a panel of monoclonal antibodies (OKT3, WT/31, STCS1, OKT4, OKT8, THB-5, HNK-1, B73.1, OKM1, L243, OKT9, anti-TAC) to MNC functional and activation markers. In AR and MNCs in portal tracts and perivenular zones are exclusively T-cells displaying the α/β Ti, most being cytotoxic/suppressor and expressing the activation markers HLA-DR and transferrin receptor, but not the receptor for interleukin 2 (IL-2). MNCs with these characteristics were found in all liver specimens from the child who underwent 3 biopsies, extent of infiltration being only mild in the first, severe in the second and dramatically decreased in the third after high dose steroids. In CR the infiltrate was similar, though less conspicuous than AR, but in contrast to AR T-lymphocytes expressing IL-2 receptor were detected. Activated cytotoxic/suppressor T-lymphocytes predominate in acute and chronic rejection of OLT and may be directly involved in liver damage.

MARKED DIFFERENCES BETWEEN ORTHOTOPIC AND HETEROTOPIC
AUXILIARY LIVER ALLOGRAFTS IN THE INDUCTION OF CLASS II MHC
ANTIGENS ON HEPATOCYTES.

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The reason for the apparent immunological privilege of liver grafts in comparison with other organ grafts is not known. In a previous study of donor induction in orthotopically transplanted liver allografts, we found that the hepatocyte (but not biliary epithelium) was markedly resistant to class II MHC induction. We postulated that a resistance to class II induction in hepatocytes might contribute to the immunological privilege of liver grafts.

In the DA to PVG strain combination, it is interesting to note that heterotopic auxiliary liver allografts are acutely rejected, in contrast to the spontaneous indefinite survival of orthotopic liver allografts in this strain combination. We therefore studied 2 heterotopic DA to DA liver isografts and 3 heterotopic DA to PVG Liver allografts at days, 1, 3, 5, 7 and 10 after grafting for donor class I and class II MHC induction using immunohistological techniques with donor-specific polymorphic mouse monoclonal antibodies. In contrast to our previous results with orthotopic liver allografts, we found that hepatocytes in the heterotopic liver allografts show strong class II MHC induction. This begins focally at day 5, and is widespread throughout the graft at days 7 and 10.

The major difference between orthotopic and heterotopic auxiliary liver grafts is that, in the former but not the latter, liver failure occurs pari passu with rejection. Liver failure is known to cause immunosuppression, and it is possible that it does so by inhibiting production essential to hepatocyte class II induction. These data need to be considered when heterotopic liver allografts are contemplated in the clinic.

There is Kupfer cell dysfunction to some extent

ANTI-EPITHELIAL CELL ANTIBODY ASSOCIATED
WITH GRAFT LOSS

Long hair *long manom*
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Sixteen children received 17 renal transplants over a period of 7 months were screened for the presence of non-HLA antibodies. Pre and post transplant serum samples were incubated with cultured epithelial cells and antibody binding to the cells was investigated by flow cytometry. Eight patients were found to have IgM or IgM and IgG antibodies directed against epithelial cells. A characteristic histological pattern was observed on biopsy sections in all these patients. Five of the children shown to have the antibody lost 6 grafts, including 1 HLA identical kidney. The antibody was present prior to transplantation in these patients. The remaining 3 patients with antibody experienced severe rejection which responded to treatment with plasmapheresis and cyclophosphamide. None of the 6 children who have functioning grafts and did not experience severe rejection were found to have anti-epithelial antibodies.

Antibody directed against epithelial cells correlates with severe rejection ($p < 0.001$) and is associated with graft loss in this group of patients. Further, identification of this antibody during a severe rejection episode, followed by plasma exchange/cyclophosphamide may prevent graft loss.

Jan-July 16 children received 17 Φ succeeded 5 Φ lost
grafts. Much higher graft loss than previously. loss within
2 days - 1 mths
IgM antibody present in 7, absent in 9
8 of the rejectors had antibody, none with functioning
grafts had antibody, one treated with plasmapheresis &
graft survived.
Distal tubules + arterioles stained
CMV pos or neg kidneys - doesn't know but none had a
rise in CMV titre

IDENTIFICATION OF ANTIGEN PRESENTING CELLS ON
NORMAL AND TRANSPLANTED HUMAN HEART - IMPORTANCE OF
ENDOTHELIAL CELLS

long nose, new committee member
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An essential requirement for antigen presentation is expression of class II and possibly ICAM-1 molecules on the cell surface. Here we have quantitated the nature of the class II positive cells in normal and transplanted heart using immunocytochemistry. In normal heart approximately 85% of DR expression (42.1 +/- 10.6 cells/unit area) can be accounted for by EN4 positive endothelial cells, all of which constitutively express ICAM-1. Relatively few cells bear the Leucocyte Common Antigen (7.7 +/- 3.1) and most of these are RFD7 positive macrophages or T cells. There is a paucity of RFD1 positive dendritic cells (0.4 +/- 0.6). In transplanted heart the increase in class II expression (99.0 +/- 31.8) is confined to infiltrating cells and interstitial structures. Only 42% of the DR is now accounted for by EN4 positive endothelial cells. The remaining DR is present on RFD7, and T cells. Dual immunofluorescence demonstrates that nearly all the RF1 cells are from the recipient. In conclusion, in normal heart presentation of allogeneic class II is by ICAM-1 positive endothelial cells. After transplantation, the influx of recipient cells of the macrophage/dendritic series are probably able to process allogeneic class II.

In long term survivors there is still donor class II around.

IS U.W. THE IDEAL SOLUTION FOR RENAL PRESERVATION?

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Univeristy of Wisconsin (UW) solution has been proposed as a universal solution for organ storage. Although the liver and pancreas preservation results appear to be impressive, the published results on UW stored kidneys do not yet justify its cost, and hyperosmolar citrate (HOC) remains the most commonly used solution for renal preservation in the U.K. UW appears to adequately preserve canine kidneys for 72 hours but our results with phosphate buffered sucrose (PBS140), a much cheaper solution, are comparable (1,2). The pig kidney, a better model than the dog, is more susceptible to preservation injury. In this prospective trial 15 consecutive pigs underwent renal autotransplantation and contralateral nephrectomy following 24 hour preservation in UW, PBS140 or HOC. Renal function was studied by measurement of perioperative blood flow; post-operative urine output; serum and urine electrolytes and osmolaity; inulin, P.A.H. and Lithium clearances for 2 weeks. The preliminary results of this detailed study indicate that PBS140 offers improved renal preservation when compared to both UW and HOC. (Results: means + SEM).

GROUP	UW	PBS140	HOC
Number transplanted to date	5	4	4
Death in renal failure	2	0	2
Peak Creatinine (umol/l)	1225±74	643±166	1023±117
Peak Potassium (mmol/l)	7.8±0.9	4.9±0.5	6.5±0.5
GFR at 2 weeks (ml/min)	40.9±0.0	106.4±22.4	44.0±1.6

- (1) Ploeg RJ et al. Successful 72-hour cold storage of dog kidneys with UW solution. Transplantation 1988; 46: 191-196.
- (2) Lam FT et al. Improved 72-hour renal preservation with phosphate-buffered sucrose. Transplantation 1989; 47: 767-71.

Na *HOC* *PBS140* *UW*
K *80* *120* *30*
UW vastly more expensive than others - about £120 per use
PBS140 did best by all the parameters of graft function measured.

*Were they all perfused at standard pressure and for same time
Yes from his 1m. Volume of perfusion / units time the same? Yes
In dog have got 72 hr preservation. Thinks porcine kidney
better model - more fragile, 27 more like human.*