The British Transplantation Society

April 11th and 12th, 1995

UNIVERSITY OF MANCHESTER
HULME HALL
MANCHESTER
# BRITISH TRANSPLANTATION SOCIETY

## STATEMENT OF ACCOUNTS FOR THE YEAR ENDED 30 SEPTEMBER 1994

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**MEETINGS**

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<td>Miscellaneous</td>
<td>700</td>
<td>400</td>
<td>Miscellaneous</td>
<td>615</td>
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**Excess receipts over payments**

| 17457 | 57891 |

Balance as at 30 September 1993: 52485

| Add excess of receipts over payments | 10524 |

53009

**Financed by:**

- Ulster Bank Ltd Business Reserve: 49027
- Current account: 12982

62009

The statement of Accounts and Balance are in accordance with information and records provided.

Honorary Treasurer: Honorary Auditor

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**ABSTRACTS SELECTED FOR PRESENTATION**
Efficacy of Mycophenolate Mofetil in Prevention of Acute Rejection in Renal Allografts: A Placebo Controlled Study.

S A Sadek
On Behalf of the European Mycophenolate Mofetil Co-operative Study.

Academic Unit of Surgery, St James University Hospital, Leeds

Mycophenolate Mofetil (MMF) is a selective inhibitor of both T and B lymphocyte proliferation.

A multicentre European randomized double-blind placebo-controlled study recruited 491 patients undergoing first or second cadaveric renal transplants. It compared Cyclosporin (Cya) and steroids with Cya, steroids, and MMF in two doses. There were three arms to the study: 1) Cya, Steroids + placebo n=160; 2) Cya, steroids, MMF 2gram/day n=160; 3) Cya, Steroids, MMF 3 gram/day n=160. All patients were followed up for a minimum of one year. The study end point was rigorously set as treatment failure (defined as premature withdrawal from the study for any reason), or biopsy proven rejection.

Analysis was conducted on intent-to-treat basis. All three groups were demographically similar. The following results are for the early phase encompassing the first 6 months of the study.

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>MMF 2g</th>
<th>MMF 3g</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=166</td>
<td>n=165</td>
<td>n=160</td>
<td></td>
</tr>
<tr>
<td>Treatment failure or Biopsy</td>
<td>93 (56.9%)</td>
<td>56 (30.3%)*</td>
<td>62 (38.8%)*</td>
</tr>
<tr>
<td>Proven Rejection</td>
<td>77 (46.9%)</td>
<td>28 (17.9%)</td>
<td>22 (13.8%)</td>
</tr>
<tr>
<td>Biopsy positive rejection</td>
<td>35 (21.1%)</td>
<td>9 (5.5%)</td>
<td>5 (3.1%)</td>
</tr>
<tr>
<td>Patients requiring OKT3, ATG or ALG</td>
<td>1.61</td>
<td>1.43</td>
<td>1.48</td>
</tr>
<tr>
<td>(mg/kg) n=114</td>
<td>n=126</td>
<td>n=108</td>
<td></td>
</tr>
</tbody>
</table>

* p<0.001 each MMF dose Vs Placebo

The addition of MMF to Cya and Steroids significantly reduces the incidence of acute rejection and substantially curtails the use of biological agents in the treatment of rejection.

Transfusion Induced Tolerance Is Effective Against an MHC Class I but Not a Class I Disparity in High Responder Rats

EB Bell, IA Cossens, C-P Yang, SL Hayes and PJ Wood

Immunology Research Group, Biological Sciences, Medical School, University of Manchester, Manchester M13 9PT

Previous investigations demonstrated that DA (RT11) cardiac allograft survival in the low responder PVG-RT1u strain was significantly prolonged (MST, 38-44 days) by injecting recipients with 1.5 ml DA blood 1, 2 or 4 weeks before transplantation. By giving PVG recipients a prior blood transfusion from PVG congenic strains of rats before DA heart grafting we show that both MHC and non-MHC antigens contribute in an additive fashion to the extended survival, but unequally. In order of importance MHC class I (Aβ) > MHC class II (B/β) > MHC class H-like (Cβ) > non-MHC antigen.

Attempts were made to prolong DA cardiac allografts in the high responder PVG-RT1u strain by donor specific blood transfusion (DST) but without success. DA hearts were rejected acutely with accelerated kinetics. We asked, therefore, whether this failure to induce tolerance was shared equally between MHC class I and II. PVG-RT1u recipients given a transfusion of blood from DA (a full mismatch) or PVG-R8 rats (RT1Aβ β/β/β) rejected MHC class I disparate PVG-R8 heart grafts acutely. However, PVG-RT1u rats given a DA or PVG-R23 (RT1Aβ B/β/β) blood transfusion and allografted with PVG-R23 hearts (a class II plus class H-like disparity) retained their heart allografts indefinitely (>150 days). Recent work (Morton et al. J Immunol 1993; 150: 2078) has demonstrated that rejection of PVG-R8 hearts in the PVG-RT1u strain can be induced by anti-Aβ alloantibody. However, we found that alloantibody against rat MHC class II (anti-B/β) was unable to induce rejection of PVG-R23 allografts by passive transfer in PVG-RT1u athymic nude recipients.

This study may help to define the circumstances (e.g. when transplants are matched at MHC class I) where DST is most likely to have a beneficial clinical effect.
FK506 AND GLUCOSE METABOLISM: A PROSPECTIVE STUDY IN LIVER TRANSPLANT RECIPIENTS


Liver Unit, Queen Elizabeth Hospital and Diabetic Clinic, General Hospital, Birmingham, UK.

We evaluated changes in glucose metabolism in FK506-treated liver graft recipients during the first year after transplantation (OGTTs). Serial 75g oral glucose tolerance tests (OGTTs) were performed in 16 consecutive patients at 2, 6 and 12 months after LTx. Ten matched healthy volunteers acted as the control group. Steroids were tapered off and discontinued by the end of the 12th postoperative month. The mean daily FK506 dose (mg/kg) was progressively reduced during the study period (6.4 vs 0.6 at 2.5.6, 6 and 12 months, P=0.01). At 2 months OGTTs were diagnostic of diabetes mellitus (DM) in 5 patients and of impaired glucose tolerance (IGT) in 5 (WHO diagnostic criteria). Overall glucose concentrations during OGTTs were significantly higher in LTx group than in the controls (P<0.001). After steroid withdrawal OGTTs at 6 and 12 months demonstrated sustained improvement in patients overall response to glucose challenge (2 vs 6 vs 12 months, P=0.01). At 12 months only one patient had DM and 5 others had IGT. However overall glucose concentrations during OGTTs at 12 months were still significantly higher in the LTx group than in the controls (P=0.001). Plasma insulin concentrations during OGTTs in transplant recipients didn’t change during the study period (2 vs 6 vs 12 months, P=0.1). Significant fasting and overall hyperinsulinaemia were observed at each time point (P<0.001 vs control at 2, 6 and 12 months) implicating resistance to insulin action.

We conclude that steroid withdrawal is associated with improved glucose tolerance in FK506-treated liver transplant recipients. However, persistent derangement of glucose tolerance and insulin response suggests that the long-term adverse metabolic effect of FK506 is steroid-independent.

Evidence for deletion of antigen reactive T cells after intrathymic injection of allografts

N. D. Jones, N. C. Fluck, A. L. Meller, P. J. Morris and K. J. Wood

Nuffield Department of Surgery, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DU

We have established an intrathymic model of tolerance induction across a full major and minor histocompatibility antigens difference in mice. Recipient CBA (H-2b) mice were treated IV with a depleting anti CD4 monoclonal antibody (YTA3.1) on two consecutive days (day -28, -27) relative to heart transplantation on day 0. Coincident with the second dose of mab, 5x10^6 C57BL/10 (H-2b) spleen leukocytes were injected into the thymus (2.5x10^7 per lobe). Operational tolerance to C57BL/10 cardiac allografts was induced in 85% of recipients treated using this protocol (MST > 100 days (n=8)) compared to 32 days in mice treated with YTA 3.1 alone (n=9). Tolerance induction was antigen specific; third party BALB/c allotransplants were rejected at a rate comparable with the anti-CD4 control group.

To elucidate the mechanisms of tolerance induction following intrathymic injection of C57 allografts we used T cell receptor (TCR) transgenic mice (BM3.6) expressing TCR α and β genes specific for the donor class I antigens, H-2Kb. The clonotypic mab T98 was used to detect T cells expressing the transgenic TCR. As CD8^+T98^+T cells were present in the periphery of BM3.6 TCR transgenic mice a depleting anti-CD8 mab (YTS169) was included in the tolerance induction protocol to ensure depletion of peripheral T cells. The effects of intrathymic injection of C57 spleen leukocytes on T98^+ thymocyes was examined.

An 80% reduction in the CD8 single positive thymocytes population (CD4^-CD8^+) was observed 10 days after intrathymic delivery of C57 allografts (n=5). Double positive thymocytes (CD4^+CD8^+) were unaffected (n=3). This reduction in the CD4^-CD8^+ thymocyte population was antigen specific as injection of syngeneic (H-2b) or third party (H-2k) cells did not result in a comparable decrease (n=3). Mab therapy alone had no effect on the thymocyte population. We were able to confirm that the reduction in the CD4^-CD8^+ cells was due to deletion of this population and not downregulation of either CD8 or TCR. T98 and CD8 expression on the remaining thymocytes was identical to that found on thymocytes in naive or control BM3.6 mice and tissue sections of injected thymus showed a definite deletion of thymocytes compared to control thymus.

In conclusion, deletion of donor reactive single positive thymocytes may be responsible for the induction of tolerance following intrathymic delivery of donor allograft.
CYTOKINE INDUCIBLE ADHESION MOLECULES: AN INDICATOR OF ISCHEMIA-REPERFUSION INJURY IN LIVER ALLOGRAFTS

Bhargava A., Bradley N.J., Burroughs A.K., Dhillon A.P.,
Rolles K., Davidson B.R.†

University Department of Surgery, Liver Transplant Unit, and Department of Histopathology, Royal Free Hospital School of Medicine Pond Street London NW3

Interleukin Adhesion Molecule-1 (ICAM-1) and E-Selectin are cytokine inducible polyepitides involved in leukocyte/endothelial cell interaction. Activated donor Kupffer cells, and endothelial cells may participate in cytokine production.

The aim of this study was to investigate the expression of ICAM-1 and E-selectin on liver biopsies following graft storage and warm ischemia/reperfusion. Change in expression was correlated with degree of graft injury by measuring levels of AST were upon arrival at ITU.

Following cold storage (723 ± 31 mins) and reperfusion, at 90 mins, liver biopsies from 22 grafts were snap-frozen. 5 μm frozen sections were stained immunohistochemically for ICAM-1 and E-selectin: Expression, following reperfusion, compared to the stored allograft was analysed by light microscopy, and by computer-aided image analysis, measuring integrated optical density (IOD).

<table>
<thead>
<tr>
<th>ICAM-1 Expression Following Reperefusion</th>
<th>*AST &gt;1000</th>
<th>*AST &lt;1000</th>
</tr>
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<tbody>
<tr>
<td>Increase</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>No Change</td>
<td>2</td>
<td>11</td>
</tr>
</tbody>
</table>

(4 Fisher's Exact Test p = 0.05)

Expression ICAM-1 and E-selectin was parallel. Activation of ICAM-1 on sinusoids and hepatocytes occurred following graft storage. A further increase in ICAM-1 expression, following reperfusion, was associated with severe ischemia/reperfusion injury. E-selectin expression on endothelium increased significantly, mean IOD 1.07 after storage, compared to 2.69 (p=0.05 test) following reperfusion.

Induction of both molecules is probably as a result of ischemic injury rather than an immune mechanism. These results support the concept that Kupffer cell activation and endothelial cell injury occur following reperfusion. There is evidence, however, to suggest that cytokine activity continues during storage and may be enhanced by a period of warm ischemia, resulting in graft injury.

CELL INFILTRATION AND INDUCTION OF MHC CLASS II AND ADHESION MOLECULES ON RAT CORNEAL ENDOTHELIAL CELLS DURING REJECTION

Figuiriredo, FC; Nicholls, SM; Zhang, L; Easty, DL.

Dept. of Ophthalmology, University of Bristol, Bristol, U.K.

Purpose. The endothelial cell layer at the back of the cornea is essential for corneal transparency and in humans is easily damaged beyond repair by rejection. The processes involved in its destruction are not well understood. We have therefore examined flat preparations of rat corneal endothelium during rejection for leukocyte infiltration and MHC class II and ICAM-1 expression. Methods. Penetrating keratoplasty was performed on 81 rats. The animals were divided into two groups: immunosuppressed (PVG→PVG, n=25) and allografts (LEW→PVG, n=56). Eight normal corneas were also examined. Animals from each group were killed on days 10 and 15 after transplantation (i.e., just before and during rejection). The corneas were removed, the endothelia were carefully peeled off and mounted on glass slides. They were stained by the avidin biotinylated peroxidase (ABC) method with monoclonal antibodies against T cells (OX34), macrophages (ED3), MHC class II (OX6) or ICAM1. Samples were coded, the presence of leukocyte infiltrate was determined and the intensity of staining were measured using a Quincke 500 image analysis system. Results. There was no cell infiltration, class II or ICAM-1 expression on the normal endothelium. Patchy de novo expression of class II and ICAM-1 was evident on endothelial cells of allografts and in grafts by day 10, on both donor and host. Two-way analysis of variance revealed a significantly greater expression of both MHC class II (p=0.004) and ICAM-1 (p=0.01) in allografts compared with isografts in the donor tissue, which increased at day 15. Macrophages and T cells, accompanied by donor endothelial damage, were present during rejection in all allografts.

Conclusions. Flat preparation is a useful method for assessing changes in the endothelium after transplantation, as the whole tissue can be seen. MHC class II and ICAM-1 are not normally expressed on normal corneal endothelial cells. De novo expressions of these molecules is induced in allografts before and during rejection and this may be important in corneal endothelial cell immunogenicity, because of the low expression of MHC class I antigens by these cells. Cell infiltration may suggest a DTH reaction, although a direct CD4+ cytotoxic T cell response may also occur, especially in cases of MHC class II matched grafts.
THE IDENTIFICATION OF NON-HLA SPECIFIC ANTIBODIES
BY FLOW CYTOMETRY

AMANDA ROBSON AND SUSAN MARTIN

Tissue Typing Laboratory, St. Mary’s Hospital, Fallowfield Road, Manchester

The flow cytometry crossmatch (FCXM) is a highly sensitive technique which detects the binding of both complement fixing and non-complement fixing antibodies to donor cells. A positive FCXM is associated with increased numbers of rejection episodes and early graft failure. However, some centres have reported "false positive" FCXM suggesting that flow cytometry is too sensitive or is detecting antibodies irrelevant to transplant outcome.

Cytotoxic studies which have inhibited alloantibody binding to HLA class I by blocking target cells with a class I specific monoclonal antibody, showed that a positive crossmatch due to non-HLA specific antibodies was not associated with graft failure. However, cytotoxic blocking assays are limited by the availability of suitable non-complement fixing monoclonal antibodies. Flow cytometry does not involve complement fixation and can therefore use any monoclonal antibody of appropriate specificity as a blocking agent.

We have used the monoclonal antibodies W6/32 and MCA477 to block antibodies to HLA class I and class II respectively. By incubating donor cells with these antibodies prior to three-colour flow cytometry crossmatching we have been able to demonstrate the presence or absence of HLA specific antibodies in 48 sera from 12 cytotoxic negative, FCXM positive donor/recipient pairs. Of the 12 pairs tested, 7 patients were shown to have HLA specific antibodies to their respective donors. By performing a flow cytometry autocrossmatch on the 5 patients with no evidence of donor specific HLA antibodies we have identified 3 patients with low levels of IgG alloantibodies. It is possible that the presence of non-HLA antibodies in positive FCXM may account for a significant proportion of the "false positive" results that have been reported. Using this method to define antibodies giving a FCXM positive cytotoxic negative result, patients with non-HLA antibodies can be successfully transplanted whilst avoiding graft failure due to low levels of HLA specific antibodies.

CELLULAR LOCALISATION OF COMPLEMENT C3 SYNTHESISED IN HUMAN RENAL ALLOGRAFTS


Renal Unit, Guy’s Hospital, London SEI 9RT and Department of Medicine, Addenbrooke’s Hospital, Cambridge, CB2 2QQ

The role of complement in renal allograft injury still remains to be defined. Earlier we found that C3 mRNA was expressed in human renal allografts and that expression of C3 mRNA was increased during renal allograft injury both in ischaemic kidney and during the cellular allograft response. It was clear from the determination of the allografts of C3 mRNA expressed in renal allograft biopsies that the C3 synthesised was in part of donor type, but it was not clear which cells in the graft expressed C3 or whether the expression of C3 mRNA resulted in protein synthesis. To investigate this further we took advantage of the two main C3 allootypes: C3A and C3B. These can be distinguished on western blots using the C3F selective monoclonal antibody HAV 4.1. Using this antibody as an immunohistological reagent we analysed the expression of C3F in the kidneys of 12 C3F donors transplanted into C3F recipients. This revealed almost exclusively renal tubular cytoplasmic staining of C3, indicating that the tubules were the prominent source of tissue protein synthesis during allograft dysfunction. There was no staining of mononuclear cells, indicating that donor C3 synthesis was not attributable to passenger leukocytes. In contrast, staining with polyclonal anti C3 antibody, detecting both donor and recipient C3 allootypes, identified a predominantly glomerular capillary and peritubular pattern of staining. With donor C3F kidneys transplanted into C3F recipients, HAV 4.1 produced a similar pattern of staining to polyclonal anti-C3, because in this case both donor and recipient C3 types were detected with both reagents.

The conclusion from this study is that renal tubules are the main source of C3 synthesised within transplanted kidney, whereas extrinsic C3 produced by the recipient seems to be deposited in a capillary and peritubular distribution. The synthesis of complement within renal grafts displaying ATN and cellular rejection may represent an important adaptation of the mechanism of allograft injury and may play a different role from plasma C3 fixed in renal transplant tissue.
AN IN VITRO MODEL OF HYPERACUTE REJECTION OF XENOGENIC ISLETS

Gray DMR, Song Z, Clover L, Wolech KI, Morris DJ.

Nuffield Department of Surgery, University of Oxford, John Radcliffe and Churchill Hospitals, Oxford

We have recently shown that freshly isolated rabbit islets transplanted into untreated cynomolgus monkey recipients undergo extensive destruction within 6 hours with histological features typical of hyperacute rejection of vascularised organs, namely blinding of natural antibody, infiltration by neutrophils and cell lysis. Interestingly, incubation of the same islets with fresh human serum containing both natural antibody and complement did not result in decreased viability (as assessed by FDA/EB quenching) after culture for up to 2 days, suggesting a further factor was necessary to initiate islet destruction. Since early neutrophil infiltration was a constant feature of our in vivo model, we added human neutrophils to freshly isolated rabbit islets in the presence of fresh human serum and saw a significant decrease in islet viability after 2 hours incubation. This finding was repeatable using both human and cynomolgus monkey serum and neutrophils. We then demonstrated that the damage process was dependent on the presence of active complement, and was specific for the xenogenic serum but not for the source of neutrophils: thus rabbit, human or cynomolgus neutrophils will destroy rabbit islets only if fresh primate serum is present. However, human neutrophils have no effect on rabbit islets in the presence of rabbit serum. Dilution experiments showed that the number of neutrophils required was remarkably small (<5 neutrophils/islet).

We believe that this simple assay amounts to an in vitro model of hyperacute xenograft rejection (arguably the first ever described), and we have now used the model to develop a tissue-culture based strategy for preventing hyperacute rejection of xenografted islets, preliminary transplantation studies in primates indicate that this strategy does indeed significantly reduce the destruction of xenografted islets in vivo.

T CELL RESPONDING TO ALLOGENEIC HEART AND KIDNEY CELLS USE DIFFERENT T CELL RECEPTOR REPERTOIRES

Galil Tawfik1, Ian V. Hutchinson1, Richard Kay1

1University of Manchester, Immunology Research Group, 32-39 Stopford Building, Manchester M13 9PT. 2Dept. of Pathology, Ninewells Hospital, Dundee DD1 5HT.

Background & Aims: Alloantigens can be recognised by the T cell heterodimeric antigen receptor either directly on the surface of allogeneic cells or 'indirectly' as antigen-peptide fragments bound to MHC molecules. We have shown recently the predominant usage of certain TCR V genes by alloreactive T cells. Established lymph node T cell lines and clones predominantly used the Vβ 6.0 gene in 80.0 - 85.7% and 60.3 - 64.3% of cases respectively. Only one joining region, Jβ 2.1, was used by these T cells and a common motif could be discerned in the TCR N region. No Vδ gene segments could be identified.

The aim of this study was to compare the TCR β chain repertoire of T cells responding to alloantigens on different organs namely kidney and heart.

Methods: DA rat T cell lines were generated in culture by stimulation with isolated Lewis rat kidney and heart cells. Total RNA was extracted and the reverse transcription-polymerase chain reaction (RT-PCR) performed using a universal Vβ and a Jβ primers. The PCR product was cloned into a PBR322 vector and the TCR β chain was characterised by direct sequencing of the cloned plasmid DNA using dideoxy chain termination method.

Results: Several different Vβ gene segments (6.0, 8.3, 4.0, 5.2) were expressed by the kidney alloreactive T cell lines. Vβ 6.0 was most commonly used, present in 40 - 45% of lines, but Vβ 8.3 also accounted for a significant proportion Vβ usage (26% of lines). Jβ 2.1 accounted for 55 to 60% and Jβ 2.5 for approximately 29% of the total Jβ segments used by these T cells.

In contrast, a non-homogeneous pattern of Vβ gene usage was seen in T cell lines reactive against alloreactive heart. Vβ 4.0, 6.0, 5.2, 5.1 and 16.0 were all detected. Of these, the most frequently used Vβ was Vβ 4.0 (34%) followed by Vβ 6.0 (25%). The Jβ gene segments 2.1 and 2.5 accounted for approximately 55% of Jβ segments used and Jβ 3.1 was detected in the remainder of the T cell lines.

Conclusion: The pattern of Vβ gene usage in kidney alloreactive T cells was similar to that observed for lymph node-stimulated cells. This suggests that the T cell response to both allogenic lymph node and kidney cells is directed toward a single immunodominant epitope and that this epitope reacts with a restricted range of T cell receptors. Vβ 6.0 T cells also accounted for approximately 30% of heart alloreactive lines. However, almost 33% of heart alloreactive T cells used Vβ 4.0 and over 25% of kidney-reactive T cells use Vβ 8.3, suggesting that organ specific peptides also play a role in determining the alloreactive T cell repertoire. The limited Vβ and Jβ usage along with a common N region motifs still points to a very restricted response. Selective immune intervention directed at specific TCR structures would appear to be a viable strategy in preventing allograft rejection.
HEPARIN MODULATES EXPRESSION OF HLA-DR AND ICAM-1 BY CYTOKINE STIMULATED HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS (HUVEC).

D Rix, LVP Karipara, D Talbot, RMR Taylor, JLR Forsythe & JA Kirby

Department of Surgery, The Medical School, University of Newcastle upon Tyne, NE2 4HH, UK.

Endothelial expression of Class II MHC antigens and of the adhesion molecule ICAM-1 may play an important role during initiation of the rejection process following allergenic renal transplantation. The proinflammatory cytokines IFN-γ and TNF-α upregulate expression of these molecules. Many cytokines are sequestered prior to engaging their specific receptor by interaction with specific domains on cell-surface or matrix glycosaminoglycans such as heparan sulphate. Soluble heparin may modulate this initial presentation.

HUVECs were cultured in medium supplemented with varying concentrations of heparin and were stimulated with IFN-γ and TNF-α. After culture for 72 hours the expression of Class II MHC antigens and ICAM-1 were assessed by quantitative flow cytometry. A sensitive lymphocyte adhesion assay was then used to measure the ability of these cells to bind activated lymphocytes.

It was shown that the upregulation by IFN-γ of both Class II MHC antigens and ICAM-1 was reduced in the presence of increasing concentrations of heparin (Table). A similar effect was not seen following the activation of HUVECs by TNF-α.

<table>
<thead>
<tr>
<th>Cell Stimulation</th>
<th>Flow Cytometric Antigen Quantification (MFI ± SD; n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MHC Class II Antigens</td>
</tr>
<tr>
<td>No heparin</td>
<td>10,341 ± 182</td>
</tr>
<tr>
<td>500 ng/ml IFN-γ</td>
<td>500,510 ± 37,073</td>
</tr>
<tr>
<td>500 ng/ml IFN-γ</td>
<td>189,722 ± 19,251</td>
</tr>
<tr>
<td>100μg/ml heparin</td>
<td>18,529 ± 204</td>
</tr>
<tr>
<td>100μg/ml IFN-γ, 100μg/ml heparin</td>
<td>10,341 ± 182</td>
</tr>
</tbody>
</table>

The adhesion between IFN-γ stimulated HUVECs and mitogen activated lymphocytes was reduced significantly when the HUVECs were treated with IFN-γ in the presence of heparin. Heparin also reduced the synergistic activation of HUVECs produced by mixture of IFN-γ and TNF-α.

These results suggest an explanation for the immunomodulatory properties that have been reported for heparin. It is known that IFN-γ has a higher affinity for purified heparin than for physiological glycosaminoglycans. Therefore, heparin may competitively displace this important pro-inflammatory cytokine from the endothelium and reduce its biological activity by allowing gross dilution into the blood.

ALLOANTIGEN PRESENTATION BY B LYMPHOCYTES: REQUIREMENT FOR ACTIVATION.

J.L. Wilson, A.C. Cunningham, P.M.R. Taylor and J.A. Kirby

Department of Surgery, University of Newcastle, NE2 4HH, UK.

The role of B cells in antigen presentation is controversial. In this study the ability of B cells to present alloantigen was studied in MLR. Lymphoproliferation and IL-2 production were measured. EBV-transformed B cell lines (EBV-LCL) and purified resting B cells were capable of initiating a primary MLR. However only EBV-LCL retained this ability after fixation. Preactivation of resting B cells with a Fab′, anti-human IgM enabled these cells to induce lymphoproliferation and stimulate cytokine production after fixation. Flow cytometry revealed that resting B cells constitutively express Class II MHC antigens but not the ligands B7-1 (CD80) and B7-2 (CD86). These molecules were induced by activation and peaked after 72 hours but were expressed constitutively at high levels by EBV-LCL. Serial fixation where B cells were trapped at various times during activation demonstrated a correlation between constitutive ligand expression and APC function.

<table>
<thead>
<tr>
<th>Period of B cell activation with Fab′ anti-IgM</th>
<th>Ligand expression of flow cytometry (MFI)</th>
<th>5 Day MLR with stimulation by fixed B cells (n=5)</th>
<th>E.2 (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>B7-1 (CD80)</td>
<td>810 (1.212)</td>
<td>886±107</td>
</tr>
<tr>
<td>3 days</td>
<td>B7-2 (CD86)</td>
<td>4,150 (11,881)</td>
<td>23,775±4,617</td>
</tr>
</tbody>
</table>

The importance of these ligands was further evidenced by inhibition of MLR with mAb to B7-1, B7-2 and Class II MHC and CTLA4-Ig. Furthermore, the hyperactivity observed in MLR with resting B cells can be overcome by stimulation of CD28 on lymphocytes using an anti-CD28 mAb. This study suggests that only activated B cells have the ability to stimulate naive CD4+ T cells as they can provide both antigen presentation and the necessary costimulatory signals.
A MULTI CENTRE STUDY OF FLOW CYTOMETRIC CROSSMATCHING
IN THE UK

A.W. Harmer*, B.K. Shaner†, A.E. Bail†, S. Garner*, S. Martin* on behalf of the British
Society for Histocompatibility and Immunogenetics (BSH) Flow Cytometry Group

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Flow cytometric crossmatching (FCXM) is a highly sensitive method for the
detection of HLA specific antibodies in patients’ sera and positive recipient-donor
crossmatches are considered a contraindication to renal transplantation by some
transplant centres. However, the clinical significance of positive crossmatches is not
clear as the association with transplant outcome differs between centres. The BSH flow
cytometry group initiated a study to determine whether differences in techniques
and interpretation of results affects the definition of a positive crossmatch and thus
accounts for apparent variation in clinical significance.

Five cell/serum exchanges have been carried out with 12 centres participating in the
crossmatching exercises. A total 17 sera and 6 cells, giving 25 cell/serum combinations,
were tested for IgG antibodies. With each laboratory using their own criteria for
positivity there was complete agreement for 10 crossmatches and over 85% agreement
for a further 8. Seven crossmatches did not produce clear agreement between centres.
Based on the fluorescence data obtained for positive and negative results where there
was agreement, and the use of calibration beads, an independent definition of positivity
was determined. When this was applied to the uncalibrated results the concordance
increased from 51% to 77%. In addition tests on doubling dilutions of 4 sera also
showed differences in sensitivity between centres.

Cytotoxic testing of 19 combinations was carried out at the same time as the
FCXM by 4 centres, confirming the greater sensitivity of flow cytometric assay. Three
sera collected from patients with previous transplant failure were negative by
cytotoxicity but were reported at FCXM positive by the majority of laboratories. This
indicates the clinical significance of the greater sensitivity of the flow cytometric assay.

In conclusion this study demonstrated that there is agreement on the FCXM result
for a majority of undiluted sera. There were however apparent differences in sensitivity
between centres shown by 27% of sera tested at neat and by dilutions of sera. The
BSH group has shown that some of these differences can be attributed to the definition
of positivity used by individual centres. The group is continuing to work towards
optimising the FCXM.

POLYMORPHISM WITHIN THE HUMAN IgG ANTI-PIG REPERTOIRE

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It is accepted in pig-to-human xenotransplantation research that a dominant proporion of human IgG and IgM anti-pig is against epitope(s) carried on the
pentasaccharide Galα1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc [the ‘Galili’ antigen(s)].
The development of two possible therapeutic strategies (antigen-specific
immunoadsorption and soluble antigen inhibition) will depend on the extent to which
individuals share the same anti-pig specificities. We have tested sera from individuals
from all 4 ABO groups to assess whether IgG and IgM anti-Galili specificities within
the population are monomorphic. 20 sera (5 from each ABO group) were tested by
ELISA for IgG and IgM binding to 3 separate HSA-conjugated antigens derived from
the parent Galili molecule: the terminal disaccharide Galα1-3Galβ1-4HSA (‘di-HSA’),
the terminal triasaccharide Galα1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc (‘tri-HSA’), and the
whole pentasaccharide Galα1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-4Glc (‘penta-
HSA’). All sera had equivalent binding of IgM to the three antigens. However, for
IgG there were 3 distinct patterns of binding:

<table>
<thead>
<tr>
<th>Antigen binding pattern</th>
<th>Number of sera (20)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>di-HSA alone</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>di- and penta-HSA only</td>
<td>7</td>
<td>35</td>
</tr>
<tr>
<td>di-, tri- and penta-HSA</td>
<td>11</td>
<td>55</td>
</tr>
</tbody>
</table>

45% of sera therefore, did not bind to tri-HSA. In keeping with this, it was found in 8
sera tested that whilst in 4 soluble tri-HSA readily inhibited IgG binding to penta-
HSA by ELISA (50% inhibition point at c.0.05 mM tri-HSA), the other 4 sera were
resistant to inhibition by tri-HSA. There are therefore distinct subgroups of IgG anti-
Galili antibodies. Nevertheless, absorption using a disaccharide-Sepharose column
showed that IgG binding to penta-HSA by ELISA can be at least partly removed by
disaccharide in the 6 sera tested. This study shows for the first time that human IgG
anti-Galili specificities are polymorphic, but that disaccharide immunoadsorbents may
still be capable of removing anti-Galili activity.
APPLICATION TO TRANSPLANTATION OF HLA CLASS I DNA TECHNIQUES

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We have previously, in joint studies with the Collaborative Transplant Study (CTS), shown the benefit of the detection of HLA-B8 alleles by DNA techniques (1) in assessing the differences in DNA and serological determinations and (ii) in the improved graft survival (2) found using DNA techniques to determine the HLA-B8 allele. We have now developed techniques to determine HLA-A and -B alleles by amplification of DNA by PCR and detection by sequence specific oligonucleotide probes.

We have applied the system to test 77 renal donors and 19 recipients used at this unit. Four differences in SS0 typing from serological typing for donors were found as follows: HLA-A74 instead of HLA-A31; HLA-B56 on two occasions instead of HLA-B57 and HLA-B70 was confirmed when HLA-B70 had been queried by serology. One recipient had been designated as positive for HLA-B47 but was negative by SS0.

In addition this new procedure has been applied to serologically homozygous donors on our Bone Marrow Registry of 5,000 donors. The homozygous antigen was detected in all occasions. However for HLA-A13 13% of 695 serologically homozygous donors were found to have an extra allele. For HLA-A we only tested donors homozygous for the less common antigen and excluded HLA-A1, -A2, -A3. We found 3% of the 114 homozygous donors had an allele not detected by serology.

The results are not surprising. The sera used to type potential bone marrow donors differs in quality from the sera we use for solid organ transplantation. Most of the alleles missed have a low frequency in this population. It is important they are detected in order to have available bone marrow donors with these alleles.

At present we are testing zero mismatch (HLA-A, -B, -DR) renal transplant pairs from the CTS study in order to verify the class I serological type and to determine if detection of class I alleles by DNA techniques would improve graft survival.

ANTITRANCYCYTE GAMMAGLOBULIN INDUCTION THERAPY IN THE MANAGEMENT OF HIGHLY SENSITISED RENAL TRANSPLANT RECIPIENTS.

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Department of Urology & Transplantation, Beaumont Hospital, 99 Rep. of Ireland.

Between 1st November 1980 and 1st October 1983, we performed 35 cadaveric renal transplants in 35 highly sensitised patients with a measured peak panel reactive antibody levels (PRA) of greater than 80%. Eight were primary transplants, 19 were second, 6 were third and 2 were fourth transplants. The mean number of HLA antigen mismatches was 2.8.

Post-operative immunosuppression consisted of polyclonal antibody therapy using intravenous antithymocyte globulin (ATG) Fresenius R 2.5mg/kg daily and standard triple therapy, cyclosporine 1mg/kg orally, azathioprine 1-2 mgs/kg orally and prednisone 60mgs/day orally, tapered over 3 months to 2.5mg/daily. The ATG dosage was adjusted to maintain the absolute lymphocyte count between 0.1-0.2 x 10^9/L. The mean duration of ATG treatment was 38.4 days (range 2-14 days; median 10 days).

Three allografts were lost due to technical reasons in the immediate post-operative period and two patients suffered accelerated acute vascular rejection, necessitating allograft nephrectomy within the first week. Two patients have suffered graft loss due to acute rejection on days 222 and 601 post-transplant and a further graft was lost on day 1155 due to chronic rejection. If those allografts that have suffered technical loss or failed within one week of transplantation are excluded from analysis, the one year actual patient and allograft survival was 100% and 96.6% respectively.

Mean post-transplant serum creatinine at 1 month, 3 months and currently is 165µmol/L, 162µmol/L and 159µmol/L respectively.

Ten patients (29.4%) developed serological evidence cytomegalovirus infection, with 6 patients manifesting evidence of active clinical disease. All cases responded to intravenous gancyclovir treatment.

In conclusion, we felt that excellent results can be safely achieved in highly sensitised renal transplant recipients using prophylactic induction therapy with ATG in combination with cyclosporine based triple therapy instituted immediately post-transplantation.
KIDNEY TRANSPLANTATION IN PATIENTS OLDER THAN 60 YEARS OF AGE - IS IT WORTH IT?

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Hahnemann University, Philadelphia, PA, USA, 19102-1192

With recent increase in the age of the population, there is a rising number of dialysis patients who are older than 60 years, and many of these are being considered for transplantation, particularly when their survival results on dialysis are inferior to those of younger individuals. During the past 3 years we have accepted for transplantation 32 patients age 60-76. Before being accepted for transplantation, all patients underwent thorough medical assessment, particularly with regard to pulmonary and cardiac functions, which included thallium stress tests and sometimes coronary angiography. Many patients were found to have additional risk factors besides renal failure, which included hypertension (54%), cardiac disease (38%), diabetes (21%), previous surgery (20%), and a history of malignancy (11%).

Three patients had previous failed transplantation. Two patients received kidneys from living related donors, and 30 from cadaveric donors aged 10-74 years with a cold ischemia time of 19-41 hours. Whenever possible, kidneys from older donors were given to these recipients. After transplantation there was no primary nonfunction, but 15% of the grafts showed delayed function. In living donor recipients, patient and graft survival was 100% at 1-3 years. In recipients of cadaveric donors, patient survival was 93.7%, at 1, 2, and 3 years, and graft survival was 75%, 66%, and 66% at the same time intervals, respectively. One patient was lost to cerebral lymphoma with a functioning kidney, and another to overwhelming CMV infection. Five other grafts were lost to rejection. A number of non-fatal post-transplant complications were seen in these patients which included cardiac arrhythmia, pulmonary and urinary tract infections, de novo malignancy, late onset diabetes mellitus, and wound dehiscence. All patients with functioning grafts are active and enjoying a good quality life. From this study and from the known lower survival rates and poor quality of life in elderly patients on dialysis therapy, we believe that kidney transplantation in the elderly recipient is a very valuable, cost effective and worthwhile effort.

ONE THOUSAND LIVER TRANSPLANTS: SURGICAL COMPLICATIONS AND OUTCOME.

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The marked improvement in results of liver transplantation have been attributed to better recipient selection, refinements in organ preservation and procurement, improved operative and anaesthetic techniques aided by technological advances, superior immunosuppressive regimens and better post-operative management.

The results of 1000 consecutive orthotopic liver transplants (OLT) performed in this centre (721 adults; 160 children) were evaluated, comparing early (1982-1987; 131 OLT) with more recent (1988-1994; 867 OLT) experience. Changing trends in indications (primary tumours, fulminant hepatic failure, alcoholic liver disease and retransplantation) and in vascular, biliary and infective morbidity and mortality were analysed. Thirty day mortality improved from 28% to 10%. Operative time (7 hours (range 3-12) vs 6 hours (range 2-11)), blood replacement (17 units (0.2-141) vs 5 units (0.54)), hospitalisation (26 days (0-153) vs 18 days (0-142)) were all significantly reduced (p<0.05). One and five year actuarial survival improved from 51% and 41% to 79% and 74% in adults and from 47% and 33% to 78% and 73% in children.

Despite liberalisation of recipient selection criteria, use of more marginal donors, and major surgical complications, patient survival continues to improve.
The lung contains a large pool of lymphocytes which have a phenotype of chronically activated cells. During rejection, lymphocytes are observed to infiltrate a transplanted lung. The aim of this study was to investigate the function of epithelial adhesion molecules in lymphocyte binding.

Monolayers of primary human alveolar epithelial cells (AEC; type I pneumocytes) were cultured in vitro and incubated with $^{51}$Cr labelled lymphocytes. Non-adherent lymphocytes were removed; the adherent cells lysed and the released $^{51}$Cr measured by $\gamma$-spectrometry. The effects of antibody blockade of LFA-1 or CD2 on lymphocytes, or of their ligands on the AEC were determined.

The effect of in vitro activation of lymphocytes resulted in a significant increase in binding ($p=0.0025$). Lymphocytes activated with PHA for 2-3 days bound to AEC with a mean of 19.6% ($n=10$), whereas 43.7% of lymphocytes cultured more than 6 days with PHA bound to similar monolayers ($n=9$). The binding capacity of morphological variants of cultured alveolar epithelial cells was different; monolayers consisting of flat, thin cells (type I pneumocyte-like) reproducibly bound a greater proportion of lymphocytes than those with a dense classical type II pneumocyte-like morphology. The results of 3 separate experiments demonstrated a significant increase ($p=0.0005$) in binding of the same populations of lymphocytes to flat, thin alveolar epithelial cells (mean binding of 33%) compared to those with a dense type II-like morphology (mean binding of 4%).

Antibodies to CD18 reproducibly inhibited lymphocyte adherence ($p<0.05$), whereas the CD2 antibody failed to modulate binding. Antibodies specific to the epithelial adhesion molecules ICAM-1 and LFA-3 also inhibited the binding of lymphocytes ($p<0.05$).

In conclusion, AEC express functional ICAM-1 and LFA-3, and the adherence of allogeneic lymphocytes is affected by their activation state, and the differentiation state of the epithelial cell.
QUANTIFICATION OF ADHESION BETWEEN CULTURED HUMAN INTRAHEPATIC BILIARY EPITHELIAL CELLS (HIBECE) AND ALLOGENIC LYMPHOCYTES

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HIBECE are major immunological targets during acute and chronic liver allograft rejection. It is known that cytokine-stimulated HIBECE express potentially immunogenic Class I and Class II MHC antigens and the adhesion molecules ICAM-1 and LFA-3. Our previous studies have shown that comparable levels of these molecules are expressed by HIBECE and immunocompetent ‘professional’ antigen-presenting cells.

Cytokine-mediated immune responses are dependent on initial establishment of static cell-cell contact between lymphocytes and their targets. For this reason it was decided that the adhesion interaction between cultured HIBECE and mitogen-activated T lymphocytes should be studied. Static adhesion assays were used to measure the adhesion of activated T cells to monolayer-cultured HIBECE under basal conditions and after stimulation of HIBECE with pro-inflammatory cytokines. Studies were also performed to examine modulation of this adhesion using monoclonal antibodies to block the interaction between specific adhesion molecules.

A novel flow cytometric assay was designed to compensate for variations in HIBECE confluent after cytokine stimulation. This assay allowed evaluation of the mean number of lymphocytes resected at each epithelial cell. Each staining HIBECE bound 2.7 ± 0.3 lymphocytes, IFN-γ-stimulated HIBECE bound 2.6 ± 0.3 lymphocytes, TNF-α-stimulated HIBECE bound 4.2 ± 0.5 lymphocytes and HIBECE treated with both cytokines bound 5.9 ± 1.1 lymphocytes. These values represent an increase in cell-cell binding of 33% following IFN-γ treatment, 63% following TNF-α treatment, and 110% following stimulation with the cytokine combination.

Antibody blocking experiments showed that pre-treatment of the activated T cells with antibodies to CD16, CD11a and CD2 and with ATG (Meloy) significantly blocked their binding to cytokine-stimulated HIBECE (91% with CD16 and CD11a, 21% with CD2, and 44% with ATG, all p<0.02). These results demonstrate the importance of the LFA-1–ICAM and the CD2–LFA-3 adhesion systems in stabilising binding between lymphocytes and HIBECE.

The increased adhesion between activated T cells and cytokine-stimulated HIBECE may be related to the susceptibility of these cells to immunological damage during hepatic allograft rejection.

MONOCLONAL ANTIBODY THERAPY IN SMALL BOWEL TRANSPLANTATION

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An attractive immunosuppressive strategy in highly immunogenic transplant models is to combine monoclonal antibodies (MAb) in order to block more than one inter-cellular interaction. In this study we used a combination of an anti-CD4 MAb (OX38) and an anti-LFA-1–ICAM (WT.1) in an effort to prolong survival following small bowel transplantation (SBT) in the rat.

Fully allogenic vascularised heterotopic SBT was performed between PVG (RT1a) donors and DA (RT1b) recipients. The recipients were given the following daily doses of MAb by intravenous injection on days –1, 0 (day of SBT) and +T.

<table>
<thead>
<tr>
<th></th>
<th>OX38</th>
<th>WT.1</th>
<th>Survival (days)</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>7, 7, 7, 8, 9</td>
<td>7</td>
</tr>
<tr>
<td>Group A</td>
<td>1mg/kg</td>
<td>-</td>
<td>6, 6, 6, 8, 10</td>
<td>7</td>
</tr>
<tr>
<td>Group B</td>
<td>5mg/kg</td>
<td>-</td>
<td>5, 11, 12, 13, 14, 17, 20</td>
<td>13 *</td>
</tr>
<tr>
<td>Group C</td>
<td>-</td>
<td>1mg/kg</td>
<td>6, 10, 14, 16</td>
<td>14</td>
</tr>
<tr>
<td>Group AC</td>
<td>1mg/kg</td>
<td>1mg/kg</td>
<td>7, 7, 7, 13, 16</td>
<td>7</td>
</tr>
<tr>
<td>Group BC</td>
<td>5mg/kg</td>
<td>1mg/kg</td>
<td>6, 9, 9, 12, 13</td>
<td>0</td>
</tr>
</tbody>
</table>

* p = 0.05 vs. Controls and vs. Group A (Wilcoxon rank sum test)

The effects of these protocols on circulating cell populations was investigated in parallel groups of untransplanted rats (T) receiving the same MAb regimens. Regular blood samples were taken for total and differential leucocyte counts and for analysis by flow cytometry. The absolute CD4+ count of group B(6) fell by more than 75%, but by less than 50% in group A(t). Maximal OX38 binding site blockade was approximately 40% and 25% respectively. In contrast, WT.1 blocked more than 80% of its binding sites and appeared to reverse the effects of OX38 because the CD4+ counts of groups A(t) and B(t) both increased by more than 50%.

In this study, therefore, combination MAb therapy was not beneficial, possibly because of the increase in circulating CD4+ cells induced by WT.1.
T AND B CELL RESPONSIVENESS TO DONOR CLASS I MHC MOLECULES AND PEPTIDES IN LONG SURVIVORS OF KIDNEY ALLOGRAFTS

A M Berham, G J Sawyer and J W Fabre

Division of Cell & Molecular Biology, Institute of Child Health, London

Nineteen LEW rats with long surviving (>100 days) (DA x LEW/F, kidney allografts were generated by treating the recipients with cyclosporin A for 10-14 days after grafting. Cyclosporin A completely suppressed the early antibody response to intact DA class I molecules in all 19 LEW rats, but 17 of the 19 rats developed antibodies between four and six weeks after grafting, i.e., between two and four weeks after the cessation of cyclosporin A immunosuppression. Thus, in these 17 recipients, T cell recognition for indirect recognition of donor class I molecules (for provision of help to B cells) were obviously functional.

Two of the 19 long survivors did not produce antibodies to donor class I molecules at any stage. These 2 rats, and one of the 17 rats which did produce antibodies, were immunized with synthetic peptides corresponding to regions of the DA class I molecule known to be recognized by LEW CD4+ T cells. None of these long-survivors suffered rejection episodes as a consequence of the peptide immunization. However, 3 developed self-APC-dependent CD8+ T cell proliferation to the immunizing donor peptides, and strong antibody responses to these peptides. In one of these long-surviving rats, the peptide priming resulted in the prompt development of strong antibodies to intact donor class I molecules. This long-survivor therefore had potentially reactive, but quiescent, T helper and B lymphocytes to the donor class I molecule. In the other long-surviving rat without antibodies to intact donor class I, peptide priming did not stimulate an antibody response to the intact donor class I molecule. This suggests B cell anergy or tolerance in this long survivor. Thus, in this model of kidney allograft tolerance, B cell and indirect CD4+ T cell reactivity to donor antigens is the norm, in spite of long-term exposure of the recipient to donor antigens without evidence of rejection. None of the animals develop tolerance for the indirect T cell recognition of donor class I antigen. In occasional animals, potentially reactive B cells are present but quiescent because of the absence of T cell help, perhaps as a consequence of suppressor phenomena. And in other occasional animals, B cell non-reactivity (anergy or tolerance) develops.

Rapsynecin Prevents the Infuobation of Chronic Rejection in Renal Transplantation in the F344-LEW Model

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Transplantation of kidneys from F344 to Lewis rats (which are MHC antigen identical, non-MHC antigen disparate strain) results in the development of clinical (proteinuria, hypertension, slowly rising creatinine) and histological features (glomerulosclerosis, tubulo-interstitial fibrosis and arterial animal thickening) of chronic rejection. We have previously used this model to show the importance of immunoglobulin, macrophages and growth factors in the development of these features, confirming that chronic rejection is an immunologically mediated process.

To test this hypothesis, we used drugs with differing effects on the immune system to block various steps in these pathways. Cyclosporin, which is immunosuppressive by virtue of its ability to block IL-2 receptors on T lymphocytes, failed to prevent the development of chronic rejection at any dosage. Rapsynecin, which as well as inhibiting T-cell function, diminished endothelial IgG1 deposition and growth factor receptor expression, prevented the development of chronic rejection. Prevention was dependent on pretreatment with rapsynecin and administration after the development of the disease did not produce remission. Blockade of growth factor receptors alone, using high doses of angiotensin (a non-immunosuppressive somanostatin analogue) also prevented the development of chronic rejection.

Identification of the pathogenesis of chronic rejection explains why current immunosuppressive regimens have been ineffective in reducing the continuous rate of long term graft loss. The use of immunosuppressive agents which inhibit immunoglobulin production, or which modulate growth factors offers hope of a treatment for this condition for the first time.
THE RISK OF NEOPLASIA IN THE EUROPEAN RENAL TRANSPLANT POPULATION HAS BEEN UNDERESTIMATED


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Renal allograft recipients are at an increased risk of neoplasia, although the extent of the problem has not been established in the typical European transplant population. To assess this risk, a comprehensive retrospective study was performed of 938 recipients transplanted at one centre over 24 years. Eight local, regional, national and international record sources were accessed for each patient in order to attempt maximum yield.

The search revealed 70 patients (7.5%) who had developed a neoplastic lesion with 18 having more than one type. More than half (42) were cutaneous lesions: 71% squamous cell carcinoma, 29% basal cell carcinoma.

The risk of developing neoplasia in the first 10 years following transplantation was 14%. By 20 years this had risen to 40%, compared to a 6% cumulative risk of neoplasia in an age-matched control population (p<0.005, Kaplan-Meier analysis, Kolmogorov-Smirnov test).

The full extent of this problem in the European transplant population has been underestimated and, as recipients and grafts survive longer, there is a clear need for both life-long surveillance and closer investigation of these patients.

ANTICENCE INHIBITION OF THE MAJOR PIG EPITOME FOR HUMAN NATURAL ANTIBODIES

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Human natural antibodies (NAb) which can bind to carbohydrate antigens of pig endothelial cells invariably initiate the destruction of pig tissues. This currently constitutes the main barrier to the use of pig organs in transplantation. The pig Galα1,3Gal epitope has been suggested to be the major target for these NAb. We have used affinity purified human Galα1,3Gal antibody and anti-human IgM and IgG, secondary reagents in FACS analysis to determine the target density of this and other epitopes on pig endothelial cells.

Our results show that over 90% of the human IgM antibody targets consist of the Galα1,3Gal epitope whereas less than 5% consist of other epitopes. Human IgG antibody also binds to less than 5% of epitopes other than Galα1,3Gal.

The pig Galα1,3Gal epitope is made by a specific α1,3galactosyltransferase. We have previously isolated cDNA and genomic clones coding for pig α1,3galactosyltransferase. Expression of the cDNA clone in green monkey COS cells reconstitutes the expression of the Galα1,3Gal epitope in these cells as indicated by binding of E84 lectin as well as affinity purified human anti-Galα1,3Gal NAb. Antiserum oligonucleotides designed to bind to the region for initiation of translation of the pig α1,3galactosyltransferase mRNA markedly inhibit the expression of the Galα1,3Gal epitope on pig endothelial cells. Since this epitope appears to constitute almost all of the target structures for human NAb on pig endothelial cells efficient inhibition of this epitope using anti-epitope technology in vivo or ex vivo should obviate the hypersensitive response to pig tissues.
THE POSITIVE INFLUENCE OF MULTI ORGAN PROCUREMENT ON RENAL GRAFT SURVIVAL

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Purpose: In view of the increasing tendency towards multi organ procurement (1988: 38%; 1993: 61%), this observational study was undertaken to evaluate whether the renal graft survival was affected by the procurement policy. Methods: All first renal cadaveric transplants performed between 1988 and 1993 within Eurotransplant (Austria, Belgium, Germany, Luxembourg) were analyzed (N = 9076). Results: MCD (Multi Organ Donor) kidneys came from younger, mainly male, donors who more often died of a known disease than the SOD (Single Organ Donor) kidneys. The MCD kidneys were transplanted into younger recipients with a lower pre-transplant immunization, with a shorter cold ischemic time period, over a less good HLA-DQ mismatch gradient and were more often preserved with the UW solution compared to SOD kidneys.

<table>
<thead>
<tr>
<th>Type</th>
<th>N</th>
<th>Donors</th>
<th>Recipients</th>
<th>Transplants</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCD</td>
<td>1791</td>
<td>18 yr</td>
<td>60 yr</td>
<td>75 yr</td>
</tr>
<tr>
<td>SOD</td>
<td>5084</td>
<td>66 yr</td>
<td>60 yr</td>
<td>61 yr</td>
</tr>
</tbody>
</table>

The univariate analysis showed that kidneys from MCD's had a significantly better graft survival than kidneys from SOD's. The 1 year and 3 years graft survival for MCD kidneys was 89% and 81% respectively; for SOD kidneys, 83% and 73% respectively. Results of the multivariate analysis demonstrated that the relative risk of graft failure for a SOD kidney versus a MCD kidney was 1.13 (p = 0.04).

Conclusion: A statistically significant better outcome of a MCD versus a SOD kidney transplant is currently present, irrespective of the observed differences in donor-patient transplant characteristics. It is anticipated that a better and more attentive global management of the potential MCD donor at the ICU unit and the broader expertise of multi-organ explantation teams may contribute to the benefit.

OUTCOME OF KIDNEYS FROM OLDER DONORS IN OLDER AND IN YOUNGER RECIPIENTS

CA ROGERS, MA BELGER, RJ BAWDEN, PV FOCOCK, JD BRIGGS

For the UKTSOA Users' Kidney Advisory Group, UK Transplant Support Service Authority, Bristol, UK

While the demand for renal transplant grows, the potential donor pool is diminishing as deaths from head injury and intracranial haemorrhage decrease. This has led to the need to use kidneys from older donors and in the UK 12% were over 60 years in 1993, compared with 3% in 1984.

This analysis looks at 10233 cadaveric transplants using adult donor (19+ years) kidneys which were performed in 33 centres in the UK and Republic of Ireland between 1994 and 1995. Exploratory analysis identified three study age groups, 19-45 years, 46-60 years and 61+ years.

Multifactorial analysis of 3 year transplant survival indicated generally that the risk of failure increased with donor age and with recipient age. However, percentage transplant survival estimates at 3 years suggest a change in trend with kidneys from donors over 60 years faring better in recipients over 50 years than in recipients aged 46-60 years.

Among 5160 recipients with grafts functioning at 1 year for whom serum creatinine values were available, these values correlated directly with donor age and inversely with recipient age. Mean creatinine values (μmol/l) at one year for the 3 age groups (in ascending order) were 156, 120, 274 for donors and 198, 187, 172 for recipients.

This analysis provides support for age matching in recipients over 45 years so that kidneys from older donors are used preferentially for older recipients.
A SUCCESSFUL STRATEGY TO IDENTIFY ACCEPTABLE HLA MISMATCHES IN HIGHLY SENSITISED PATIENTS WAITING FOR A RELEANT TRANSPLANT.

S Martin, H Liggett, DG Tate, FA Dyer, CD Short, RWG Johnson

1Tissue Typing Laboratory, St. Mary's Hospital,
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Highly sensitised patients (HSP), by definition, have antibodies to a wide range of HLA specificities. Within the UK a scheme has been established whereby HSP are prioritised and kidneys are exchanged if a donor has only one HLA-A or B mismatch (MM) with the patient and the MM has been previously defined as acceptable. An acceptable mismatch (AMM) is an HLA-A or -B specificity to which the patient does not have antibodies. One accepted strategy for the identification of AMM is to screen HSP against a selected panel of lymphocytes having only one HLA-A or -B MM with the patient. A negative result indicates that MM as being acceptable. Such a strategy involves cytotoxic screening and requires a larger cell panel than is usually available at one centre. In addition when sensitised patients are crossmatched using flow cytometry (FC) that must also be incorporated into the screening programme.

Our protocol involved initial cytotoxic screening of one or two sera with peak antibody reactivity from each of the HSP (Screen 1). Patients known to have only IgM autoantibodies were excluded. An 80 cell panel was selected to include all common and some rare HLA specificities and to avoid common haplotypes. If a patient serum was negative against a panel cell then that serum and up to 10 others from that patient were tested against that cell using FC (Screen 2). When a patient was negative by FC then all available sera were cytotoxic crossmatched against that panel cell (Screen 3). Only after a negative result in Screen 3 were the HLA-A and -B MM between the panel cell and the patient defined as AMM.

Of 55 HSP entering Screen 1, 26 went on to Screen 2 but only 6 of those were then negative and went onto Screen 3. The 6 patients negative by FC had been tested against 8 to 16 cells but were negative only with between 1 and 5 cells. At the end of Screen 3, only 3 of the 6 patients had negative results with 2, 4 and 5 cells respectively and have now been registered in the HSP scheme with 1, 5 and 7 AMM. This was a rigorous but successful strategy for the definition of AMM. However, the workload and hence cost implications were considerable which is an issue that must be addressed when HSP are being considered for transplantation.

INTENSIVE INITIAL QUADRUPEUPLE IMMUNOSUPPRESSION IN THE MANAGEMENT OF SIMULTANEOUS PANCREAS KIDNEY RECIPIENTS

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Between December 1992 and December 1994, we performed 12 consecutive simultaneous pancreas kidney (SPK) transplants in 7 male and 5 female recipients. All patients had suffered long-term islet cell diabetes mellitus IDDM (mean duration 21 years) and were in established renal failure (mean duration of dialysis 12 months) prior to transplantation. Post-transplant, intensive immunosuppression consisting of antithymoglobulin (ATG Freserius R) cyclosporine (CSA), azathioprine (AZA) and prednisone was instituted in all cases. ATG was continued for a total of 16 - 18 days and a CSA whole blood level (inceptor R of > 400ng/ml) was maintained for the first 30 days post-transplantation. The CSA dosage was then adjusted to maintain whole blood levels in the range of 250-350 ng/ml.

No patient required dialysis following transplantation and all allografts functioned immediately. All patients received anticytomegalovirus (CMV) prophylaxis in the form of intravenous gancyclovir in the immediate post-operative period followed by high dose oral acyclovir. Only 1 patient developed clinical CMV infection which responded to intravenous gancyclovir therapy.

No patient has suffered any episode of acute rejection. With a mean follow up period of 10 months, all patients have excellent renal function (mean serum creatinine 153 mmol/L despite the high dose CSA regime employed. Eleven of the 12 patients are insulin independent. One patient developed Type II diabetes, 8 months post-transplantation.

In summary, we believe that initial intensive CSA based Immunosuppression is a safe and highly effective regime in the management of SPK recipients without compromising renal function.
THE DETECTION AND CLINICAL IMPORTANCE OF ANTI-ATG ANTIBODIES IN RENAL TRANSPLANTATION.

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Monoclonal and polyclonal anti-lymphocyte preparations are used in solid organ transplantation, both for the induction of immunosuppression and the treatment of steroid-resistant rejection. In our centre rabbit anti-lymphocyte globulin (ATG, Marsilix) has been used in 30% of patients over the past year and exposure to the xenogenic protein component of this preparation may lead to the development of antibodies directed against the anti-lymphocyte globulins. Since the ATG is administered with concurrent immunosuppression the likelihood of an individual patient mounting a significant antibody response has been hard to predict. The aim of the present study was to determine the clinical relevance of the presence of antibodies to ATG using both an ELISA and a flow cytometric assay. 27 sera from patients who had received ATG therapy were analysed with both assays and a hundred fold increased sensitivity of the flow cytometric method was found. The cytometric assay was also rapid and specific. Sequential testing of serum from a renal transplant patient given a second course of ATG for rejection, showed an initial elevated level of anti-ATG antibodies (titre 1/18000) before treatment which rose to a titre of >1/300000 after treatment. This was accompanied by T (CD3+) cell escape from the immunosuppressive regime. The use of a second ATG preparation raised in the horse permitted successful treatment of the rejection episode to be achieved. This study has shown that the newly developed flow cytometric assay can detect the presence of extremely low, but clinically relevant, levels of antibody to ATG to be detected thus allowing accurate monitoring of patients who require second courses of ATG therapy. Indeed, in one patient, ATG sensitisation occurred after a single dose and after a time interval of 20 months a rapid antibody response developed which abrogated the therapeutic effect of further ATG therapy.

LANGERHANS CELL MIGRATION FROM SKIN ALLOGRAFTS
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Immediate and permanent skin replacement with allografts would be desirable in the treatment of conditions involving massive skin loss, for example major burns. At present this is not feasible because skin provides one of the most powerful antigenic challenges of any transplant. The reasons for this immunogenicity are not clear but may include the following: tissue-specific alloantigens, delayed revascularisation, upregulation of major histocompatibility antigens on keratinocytes, and especially the presence of Langerhans cells (LC). Langerhans cells, members of the dendritic cell (DC) family, are highly specialised macrophages with important functions in the induction of skin allograft rejection. LC migrate from skin grafts, and are uniquely able to stimulate naive T cells, and initiate immune responses within the recipient. Although skin graft rejection has been shown to be dependent on intact lymphatic connections, the evidence that LC migrate to lymph nodes is largely indirect. Therefore, the aim of this study was to track LC migration directly into the lymphoid tissue of the mouse.

Skin was grafted between different strains of mice. Between 1 and 7 days after grafting, rare cells expressing donor major histocompatibility complex (MHC) class-II molecules were detected by immunocytochemistry in frozen sections of draining lymph nodes (frequency 1 cell/20 sections, p < 0.01 vs syngeneic grafts) and spleen (frequency 1 cell/2 sections, p < 0.001). This high frequency of detection may have been due to low numbers of LC leaving the graft (-1 x 10^6/graft), the frequency being high (1 cell/2 sections, p < 0.001). Therefore, various doses of fluorescently-labelled LC-enriched epidermal cells were instilled into an abdominal subcutaneous pocket to examine cell entry to a skin graft bed. Label was detected in draining but not control nodes 24 hours later providing that a minimum of 1 x 10^5 LC was instilled. Using the polymerase chain reaction (PCR) technique, we further demonstrated that cells containing donor MHC class-II mRNA were present in draining, but not control nodes, 24 hours after skin grafting or the instillation of donor LC. We conclude that epidermal LC can enter draining lymph nodes after skin grafting, the probable route by which a recipient may become sensitised to a skin allograft. The significance of donor MHC class-II positive cells in recipient spleen is being evaluated in current experiments.
SURVIVAL OF RENAL RETRANSPPLANTS CAN MATCH THAT OF PRIMARY TRANSPLANT:

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Recent reports show that survival rates for renal retransplantation have improved throughout the 1980s, with one year graft survival approaching that of primary transplants. The reasons for this are not clear. We have compared the results of 844 primary and 146 retransplant adult cadaveric transplants performed in our single centre over two periods from 1984-1988 and 1989-1993 to identify any improvement in survival and determine possible causes. Variables subjected to uni- and multivariate analysis were: time from death to transplantation (CTT), cold ischaemic time (CIT), delayed graft function (DGF), acute rejection, donor age, previous graft survival time and time on waiting list. Immunosuppressive protocols were the same throughout the study period.

Actuarial 1yr & 5yr patient survival for primary and retransplant groups were unchanged between epochs despite increased mean recipient age in the second epoch (39.2+24yr vs 42.3+27yr, p=0.0005). One year & 5yr actuarial graft survival in the first epoch for primary and retransplanted groups was 83.9% & 63% and 73.1% & 58.2% with a significant improvement in the second epoch to 87.5% & 75.8% and 88.6% & 76.4% (p=0.0001). There was no significant difference in the rate of DGF between primary and retransplant groups within or between epochs despite CIT being significantly increased in the second epoch (20.16+25.12h vs 25.12h, p<0.0001) and an increase in older donors (>49yr) during 1989-93 (p<0.0001).

During 1984-88, 0 DRmm transplants comprised 45% of primary and 48% of retransplants increasing to 58% and 61% respectively during 1989-93 (p=0.0001). Second epoch 0 DRmm grafts had significantly fewer rejection episodes that 0 DR mm grafts from 1984-88 (mean 0.93 vs 0.72, p=0.02). Apparent homogeneity of HLA-DR specificities was reduced from 21.4% in the period 1984-88 to 11.3% between 1989-93 (p=0.0001). From 1984-1993 significant improvements were made in lymphocyte count and antibody screening, retransplanting and HLA-DR definition.

We have shown that survival for retransplants can match primary transplants and propose that this can be achieved by both intensive pre and post-transplant antibody screening as well as mimicking HLA-DR mismatch.

PRE-EMPTIVE CADAVERIC RENAL TRANSPLANTATION: OPTIMAL MANAGEMENT OR UNWISE USE OF SCARCE RESOURCES

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Background and aims: Pre-emptive cadaveric renal transplantation (PCRT) maximises the chance of maintaining high quality of life and may avoid the morbidity of dialysis together with the associated financial costs. These benefits are offset by disadvantages which include the possibility of transplantation many months before the need for dialysis, resulting in wasted organ function, an immediate risk of graft failure with conversion to a dialysis-dependent state and uncertainty of the safety of PCRT: there is some evidence that the immunosuppressive effects of uraemia may improve graft survival.

We have performed a case-control analysis of the outcomes of PCRT in one centre and, with the aid of a computer model, have sought to predict the optimal time to place patients with chronic renal failure on a cadaveric transplant waiting list with respect to financial costs and the implications of PCRT on the utilisation of cadaveric organs.

Methods and results: Patients who underwent PCRT between 1976 and 1993 were compared to a matched cohort of contemporaneous first cadaveric transplant recipients who were dialysis-dependent when transplanted. 100 patients met the criteria for PCRT and they were well matched to the control group with respect to age, sex, blood group, HLA match, sensitisation, donor age, donor source (local/external) and year of transplantation. There were no significant differences in patient survival (5 year: 88.6% vs 88.7%), graft survival (5 year: 69.2% vs 71.9%) or graft function as assessed by 1, 2 and 3 year plasma creatinine (139.2, 139.7 & 150.2 vs 150.8, 146.7 & 144.0) for PCRT and controls respectively.

To examine the implications of placement on a transplant waiting list before the need for dialysis, a Markov-type computer-based model was established to predict the cumulative costs and organ usage associated with PCRT. Throughout a wide range of conditions, shorter time periods were associated with PCRT. Throughout a wide range of conditions, shorter time periods were associated with PCRT. Throughout a wide range of conditions, shorter time periods were associated with PCRT. Throughout a wide range of conditions, shorter time periods were associated with PCRT.

Conclusion: PCRT appears to be safe and can result in cost savings but may increase the total number of organs required. Early inclusion on a transplant waiting list with a view to PCRT can be justified if it allows better overall HLA matching and graft survival, to compensate for the greater organ usage that would otherwise occur.