MEETING OF BRITISH TRANSPLANTATION SOCIETY

16th October, 1974

THE WELLCOME BUILDING, EUSTON ROAD, LONDON

9.45 a.m. **C. G. Brooks and R. New** (*Department of Immunology, St. Mary's Hospital Medical School, London*):
'Studies on the mechanism mediating specific unresponsiveness to skin allografts induced in adult mice.'

10.05 a.m. **C. G. Brooks** (*Department of Immunology, St. Mary's Hospital Medical School, London*):
'*In vitro* studies on tolerance to transplantation antigens induced in neonatal mice.'

10.25 a.m. **M. Jeannet, P. Vassalli and F. Botella** (*Hospital Cantonal, Geneva*):
'Lymphocyte dependent cytotoxic antibody in kidney transplantation.'

10.45 a.m. **R. A. Sells, J. Bradley and P. J. Reilly** (*Department of Surgery, University of Liverpool, Liverpool*):
'The effect of enhancing antibody on spleen cell re-activity in graft versus host reaction.'

11.05 a.m. Coffee.

11.20 a.m. **J. R. Salaman and D. Miller** (*K.R.U.F. Institute of Renal Disease, Cardiff Royal Infirmary*):
'An *in vivo* test of immune responsiveness in man.'

11.40 a.m. **J. J. van Rood and A. van Leeuwen** (*Department of Immunohaematology, University Hospital, Leiden, The Netherlands*):
'Serotyping for MLC.'

12 noon **P. J. Morris** (*Nuffield Department of Surgery, University of Oxford, Radcliffe Infirmary, Oxford*):
'Comments on HL-A in cadaveric renal transplantation.'

12.20 p.m. **A. Blussé van Oud Alblas, J. G. van den Tweel, J. J. Keuning, A. Termijtelen and J. J. van Rood** (*Department of Immunohaematology, University Hospital, Leiden, The Netherlands*):
'A new method of typing for LD determinants.'

12.40 p.m. **J. S. F. Canavan, J. D. Briggs, J. Graham and D. N. H. Hamilton** (*Departments of Surgery and Orthopaedics and Renal Unit, Western Infirmary, Glasgow*):
'Bone necrosis following renal transplantation.'

1.00 p.m. LUNCH.

2.00 p.m. BUSINESS MEETING OF THE SOCIETY.

2.45 p.m. **J. W. Fabre and J. R. Batchelor** (*McIndoe Memorial Research Unit, Queen Victoria Hospital, East Grinstead, Sussex*):

'The role of the spleen in the rejection and enhancement of renal allografts in the rat.'

3.05 p.m. **D. K. C. Cooper** (*Department of Surgery, Cardiothoracic Institute, University of London*):

'The haematoxylin-basic fuchsin-picric acid staining reaction as a test of myocardial viability in resuscitated and preserved hearts.'

3.25 p.m. **J. Collin, R. M. R. Taylor and I. D. A. Johnston** (*Department of Surgery, University of Newcastle-upon-Tyne*):

'Warm ischaemic tolerance of canine pancreas.'

3.45 p.m. TEA.

4.00 p.m. Discussion of report by Working Party on "Donor Supply."

FUTURE MEETINGS

16th APRIL, 1975—Spring Meeting, to be held jointly with the French Transplantation Society.
JUNE/JULY, 1975 (date yet to be determined)—meeting in Cardiff.
15th OCTOBER, 1975—Autumn Meeting in London.

ABSTRACTS (not for publication)

C. G. Brooks and R. New

Cells from mice made specifically unresponsive to H-2 incompatible skin allografts by antigen pretreatment and a short course of ALS and bearing perfect grafts for more than two months were found to be reactive in both splenomegaly and popliteal lymph node GVH assays. Lymph node and spleen cells from unresponsive mice were reactive in MLC, too, and the generation of cytotoxic cells during MLC could be clearly demonstrated. No cytotoxic cells (assayed by ^{51}Cr-release test) were detectable in these mice, and although injection of relatively large numbers of F_1 cells i.p. did generate some cytotoxic cells the numbers produced were distinctly less than in control mice. Unresponsive mice, as distinct from controls, did not respond to injection of small numbers of F_1 cells into the footpad as judged by popliteal lymph node enlargement.

Concentrations of "unresponsive" serum up to 6% failed to cause any inhibition of MLC reactivity of either normal or "unresponsive" cells, or to inhibit the generation of cytotoxic cells in MLC. Neither did serum concentrations of up to 11% inhibit the action of cytotoxic cells, but a preliminary experiment suggests that at a concentration of 20% unresponsive serum may be inhibitory in this system.

These and other results strongly suggest an active suppressor mechanism. We believe that this acts primarily at the level of initiation. At present it is not possible to say whether serum factors or suppressor cells, or both, are responsible for the unresponsive state.

C. G. Brooks

It has been suggested by Hellström *et al.*[1] that CBA and A mice made tolerant by neonatal injection of 50 million (CBA × A) F_1 spleen cells have both antigen-reactive cells and serum blocking factors in the micro-cytotoxic assay. Attempts to repeat these experiments in mice demonstrably fully tolerant showed no evidence of either reactive cells or blocking factors.[2]

Preliminary results on the induction of tolerance in various mouse combinations suggest that the LD rather than the SD antigens control tolerance induction, a result which may argue against the participation of Ag-Ab complexes in the maintenance of tolerance. An extensive investigation of fully tolerant mice showed no sign of MLC-reactive cells in either lymph nodes or spleens, and cells from tolerant mice did not suppress the mixed lymphocyte reaction of normal cells. Cells from tolerant mice had no cytotoxic activity in a ^{51}Cr-release test, nor could cytotoxic cells be induced during MLC culture. By contrast, mice given ¼ of the fully tolerogenic dose of cells at birth behaved as partially tolerant, many mice showing a chronic form of graft breakdown. Cells from such mice often had partial MLC reactivity. Serum from fully tolerant mice did not inhibit MLC or cytotoxic reactions of normal cells. Only serum from old tolerant mice, where there was some evidence of graft breakdown, or occasionally from partially tolerant mice, inhibited MLC responses.

These results support the concept that in mice made fully tolerant at birth there is a clonal deletion or inactivation. Results presented here and elsewhere[2] suggest that in mice given insufficient cells at birth to cause complete tolerance an active form of suppression may be responsible for the partial tolerance observed.

1. I. Hellström *et al.* Nature *236*, 69, 1971.
2. P. C. L. Beverley *et al.* Transpl. Proc. *5*, 679, 1973.

M. Jeannet, P. Vassalli and F. Botella

The lymphocyte-dependent antibody (LDA or LALI) assay has been used in parallel with the complement-mediated micro-lymphocytotoxicity (MLCT) test in 48 cadaver kidney transplant recipients. In most patients sera have been tested both before and after transplantation using as target cells the kidney donor lymphocytes. In twenty cases acid eluates were prepared from the kidney graft after rejection or at autopsy and tested in parallel with pre- and post-transplantation sera. The results indicate a marked increase in sensitivity of the LDA assay compared with the standard micro-lymphocytotoxicity test. In seven cases the pretransplant serum sample was found retrospectively positive, using as target the kidney donor lymphocytes or allogeneic lymphocytes bearing the same incompatible antigen(s) for the recipient. These seven patients rejected their graft during the first month after transplantation. In three additional cases an antibody detectable by the LDA assay but not by the MLCT test was found only after the acute rejection of the graft. In two, the kidney donor shared four HL-A antigens with the recipient, which suggests that new HL-A (third locus?) or non HL-A antigens may be revealed by the LDA assay. Antibodies were detected in the graft evaluates in 9 or 20 cases and again the LDA assay appeared to be more sensitive than the MLCT assay. So far all recipients with a functioning graft 6 months after transplantation have a negative LDA test. The data suggest the possible importance of the LDA cytotoxic mechanism in allograft rejection in man and the potential usefulness of the LDA assay for the prevention of acute rejection of kidney grafts.

R. A. Sells, J. Bradley and P. J. Reilly

The local graft versus host reaction induced by the injection of parental strain spleen cells into the footpads of F_1 offspring, has been used in the rat[1] as an indicator of cellular immune status, and more recently has been applied to the mouse. Recent evidence has been put forward that enhancing antibody may exert its suppressive action through receptor-site (ideotype) affinity. This paper describes the effect of enhancing antibody (of proven value in the mouse heart preparation) on the local GVHR in the mouse.

(1) The injection of normal CBA spleen cells causes dose-dependent enlargement of the popliteal node; 5×10^6 cells produced a reactivity index of 5.1 ± 0.65 (control 1.1 ± 0.12); $p<0.001$.

(2) Pretreatment of CBA donors with 0.2 ml. CBA anti-Balb/c skin graft serum (known to be enhancing in the auxiliary heart graft system) significantly reduced the reactivity index; pretreated cell index 3.24 ± 0.8; normal cell index 5.48 ± 0.65; $p<0.01$.

(3) Pretreatment of CBA donors with 0.2 ml. of CBA anti-Balb/c with serum which was not effective in enhancing heart grafts had no significant effect on GVH reactivity of treated donor spleen cells.

Findings: The pre-treatment of parental strain mice with enhancing allo-antiserum causes significant impairment of spleen cell reactivity as measured by GVHR. The immunosuppressive properties in the GVHR assay appear to correlate with immunosuppressive ability in the heart graft system.

1. Ford, W. L., Burr, W., Simonsen M. (1970) Transplantation 10, 258.

J. R. Salaman and D. Miller

A test has been devised with the object of assaying immune responsiveness in normal and immunodepressed persons. It has been based of the Graft versus Host model of Ford[1] and employs rat antigens as the immunological stimuli. Peripheral blood lymphocytes were injected into the hind feet of young Wistar rats, and seven days later the popliteal lymph nodes were removed and weighed. It was found that the unwanted Host-versus-Graft activity could be suppressed in the rats by total body irradiation, 300 rads being determined as the optimum dose. Inactivated lymphocytes were injected into the right hind foot as a control. Inactivation by irradiation (2,400 r) was not completely successful and formalisation was found to be a more satisfactory alternative.

In each test formalised and unformalised cells (in the same dosage) were injected into the right and left hind feet respectively and the result expressed as a ratio: weight of L. node/weight of R. node. Lymphocytes from 45 healthy individuals were examined in this way. Experiments were performed in triplicate and the mean ratio plotted against the dose of cells injected. A close linear correlation was found ($r=0.86$) which could be expressed as: log response $= 0.234 + 0.741$ log dose ± 0.538.

All normal individuals reacted similarly and a "poor responder" group could not be identified. Patients with immune depression, however, could be shown to be statistically distinct.

1. Ford, W. L., Burr, W., Simonsen M., Transplantation 10, 258, 1970.

J. J. van Rood and A. van Leeuwen

We have previously reported (Transpl. Proc. V, 4: 1539, 1973) on a method to identify sera which might contain antibodies against MLC (LD) determinants. In brief this method was a modification of the MLC inhibition test introduced by Ceppellini (Transpl. Proc. III: 63, 1971) but now using SD identical stimulator cells (MISIS). MISIS positive sera could be shown to contain antibodies which would react maximally with 30% of the lymphocytes in peripheral blood in a double sandwich fluorescent antibody assay.

Further studies have revealed that these antibodies react with determinants present on B-cell enriched but not T-cell enriched cell populations. Family and population data indicate that one of these sera recognise a determinant which is coded for by the HL-A supergene region, but not by the Four, LA or AJ loci. The locus coding for this determinant is either the MLC locus or a locus more closely linked to the MLC than the Four locus.

In short this antibody might be the human equivalent of the anti-Ia antibodies in the mouse.

[redacted] Morris

Several comments on HL-A typing in cadaveric renal transplantation are presented. These comments are based on an analysis of 314 consecutive transplants over a six year period in Melbourne as this represents a relatively uniform experience from one centre.

1. The sharing of 3 or 4 antigens between donor and recipient results in a better survival than pairs where no antigens are shared. It has not been possible to distinguish degrees of incompatibility between these two extremes based on survival.
2. Second locus incompatibilities do not appear to be more important than first locus incompatibilities as measured by survival.
3. The sharing of one antigen at the first locus and one antigen at the second locus (shared "haplotype") between donor and recipient results in improved survival.
4. The major blood groups of donors and recipients do seem to affect graft survival but the results are different to those reported by Joysey *et al.* (1973).
5. Sensitisation of a recipient as indicated by the presence of lymphocytotoxins before transplantation does indicate a group with a poorer prognosis than non-sensitised recipients as measured by survival.
6. An analysis of matching for HL-A in sensitised patients is not more impressive than in the total group.
7. Second cadaveric renal transplants do as well as first transplants and it is postulated that this is due to avoidance of incompatibilities in the second graft that were present in the first donor-recipient situation.
8. A policy of a low blood transfusion rate for patients on dialysis does not appear to diminish the frequency of cytotoxin formation.

A. Blussé van Oud Alblas, J. G. van den Tweel, J. J. Keuning, A. Termijtelen and J. J. van Rood

The LD determinants on a lymphocyte can be recognized with the mixed lymphocyte culture (MLC test) using a panel of cells homozygous for LD (typing cells) as stimulator cells (Transpl. Proc. V, 4: 1535, 1973). At this moment the sum of gene frequencies of the recognized LD determinants does not exceed 0.62.

In order to recognize LD determinants independent of typing cells we developed an analysis of MLC results which provides discrimination between reactions of cells differing for 0, 1 or 2 LD determinants. This approach was then tested in order to discover LD determinants for which no typing cells were available.

We selected cells carrying LD determinants of relatively high frequency. 28 of these cells were tested in one experiment in all combinations. Results were separated in 3 groups as sharing 0, 1 and 2 determinants. Because the results were internally consistent, LD determinants could be assigned to the individual cells in the test to a total of 9 different LD determinants. Of these, two determinants were in linkage disequilibrium with the HL-A haplotypes 1-8 and 3-7 and could be recognized by our typing cell panel. They have a relatively high gene frequency. Four determinants, in linkage disequilibrium with the haplotypes 2-7, 2-12, 2-W10 and 2-W15, could not be recognized by the typing cells, though apparently they have a high frequency, while for 3 determinants too few data are available to estimate their gene frequency. This approach makes it thus possible to identify and even type for LD determinants for which typing cells are not available.

J. S. F. Canavan, J. D. Briggs, J. Graham and D. N. H. Hamilton

Between May, 1969 and December, 1972, 52 patients received renal allografts in Glasgow. After a minimum follow-up period of 18 months, 11 of these patients have developed radiological evidence of bone necrosis at intervals varying from 4 to 47 months from the time of transplantation. The total number of sites so far detected is 39, the commonest being the femoral head. Pain has been associated with 62 per cent of the lesions. Bone necrosis has occurred more commonly in females, obese patients and those with hyperlipidaemia, but no association has been found with hyperparathyroidism. While the lesions are almost certainly steroid induced, prednisolone dosages have not been significantly greater in patients with bone necrosis when compared with other transplant patients. Rectilinear and gamma camera scans have not been found to be of any additional value over conventional X-rays in the diagnosis of the lesions. Histological examination has been carried out in 5 patients with bone necrosis. In contrast with bone necrosis from other causes, fibrous and bony proliferation is much less prominent. Surgery has been carried out in 3 patients with encouraging results.

J. W. Fabre and J. R. Batchelor

The role of the spleen in the rejection of renal allografts is an unresolved question which clinical trials have not clarified and on which little experimental work has been done (1). As regards the importance of the spleen in the enhancement of organ grafts, recent experimental work has given conflicting results (2, 3). We have examined these questions with respect to renal allografts in the August to AS (Ag-B^5 to Ag-B1) combination, using both F_1 hybrid and homozygous donors.

With the F_1 hybrid donor, splenectomy at the time of grafting, 7 days pregraft, or 6-7 weeks pregraft had a markedly suppressive effect on graft rejection. 14 out of 19 animals survived for >12 weeks, whereas unmodified recipients survived less than 12 days. Furthermore, the lymphocytotoxic antibody response to the graft was completely abolished in most splenectomised recipients. Attempts to reconstitute splenectomised recipients with large doses of normal syngeneic spleen cells were only partially effective. Splenectomy at the time of grafting or 7 days pregraft did not interfere at all with passive enhancement.

With the homozygous donor, splenectomy at the time of grafting had only a marginal effect on graft rejection, prolonging animal survival by only 1-2 days. Splenectomy at the time of grafting neither increased nor decreased the effectiveness of passive enhancement, which by itself delays but does not prevent rejection of the homozygous renal allograft.

These studies, which are currently being extended, have a number of theoretical and clinical implications which it is proposed to discuss.

1. Opelz, G. and Terasaki, P. I. Transplantation *15*, 605 (1973).
2. Enomoto, K., Lucas, Z. J. Transplantation *15*, 8 (1973).
3. Tilney, N. L., Bell, P. R. F. Transplantation *18*, 31 (1974).

D. K. C. Cooper

In 1971 Lie and his colleagues described a histochemical technique, the HBFP stain, applicable to formalin-fixed paraffin-embedded material, which provides a clear and striking demonstration of early myocardial ischaemia, the ischaemic fibres staining a vivid crimson colour in contrast to the light brown colour of non-ischaemic tissue. This technique is simple and rapid to perform as a routine histological procedure and is reliable, reproducible, and not affected by reasonable post-mortem intervals (up to 36 hours). It provides selective staining of early ischaemic myocardium, but not normal myocardium or infarcted myocardium that can be identified by the conventional haemotoxylin and eosin stain.

In the present study, sections of myocardium taken immediately after anoxic cardiac arrest were "negative" when stained with HBFP, as were sections taken after 15 minutes anoxic arrest, though occasional very small areas of positive staining were seen. By 30 minutes, however, there were scattered areas of crimson fibres, which increased in number at 45 and 60 minutes after death. These "positive" sections also showed the vesicular nuclei seen in H and E-stained sections: the nuclei were not hypertrophied.

In hearts which had been in a state of anoxic arrest for 30 minutes and then resuscitated and preserved as an auto-perfusing heart-lung preparation, the "positive" response after 30 minutes anoxic arrest reverted to a "negative" response during 2 hours myocardial perfusion. In hearts which had been preserved as an autoperfusing heart-lung preparation with no interim period of anoxic arrest, the HBFP stain response remained "negative" throughout, confirming satisfactory myocardial perfusion.

Though more experience of this simple, rapid test is required, results obtained in the present study suggest that the HBFP test may prove to be of considerable value in the assessment of myocardial viability in the field of cadaver heart resuscitation and preservation.

J. Collin, R. M. R. Taylor and D. A. Johnston

The duration of the warm ischaemic insult is of undoubted importance to the results of pancreatic transplantation and yet the ability of the pancreas to tolerate warm ischaemia is still unknown.

Experiments were designed to study the effects of periods of warm ischaemia of the pancreas on carbohydrate tolerance and plasma insulin levels. Three groups of dogs were studied. The head and uncinate process of each dog studied was excised leaving the duct ligated tail. The blood supply to the pancreatic tail was occluded for varying periods of time up to 120 minutes. Ischaemia during occlusion of the blood supply was confirmed by injection of disulphan blue.

Group I dogs (no warm ischaemia) showed markedly raised serum amylase levels maximal on the second or third postoperative day. Glucose tolerance was impaired but remained within the normal range. Fasting glucose and insulin levels remained unchanged.

In Group II (60 minutes warm ischaemia) four of the five dogs maintained no diabetic glucose tolerance and normal fasting glucose and insulin levels. Serum amylase levels were raised for the first seven post-operative days.

In Group III (120 minutes warm ischaemia) all dogs displayed markedly impaired glucose tolerance, fasting glucose levels were in excess of 300 mg%, fasting insulin levels were undetectable and there was no insulin output in response to intravenous glucose.

AGENDA FOR THE ANNUAL BUSINESS MEETING

TO BE HELD ON WEDNESDAY, 16th OCTOBER, 1974

1. Minutes of Business Meeting held on April 17th, 1974 (see below).
2. Matters arising from the Minutes.
3. Election of Officers and Committee members.

Present Committee	*Elected*	*Due to retire*
General Secretary: L. Brent	1972	1974
Meetings Secretary: A. D. Barnes	1972	1974
Treasurer: J. Hopewell	1972	1975
R. Y. Calne	1972	1974
Heather M. Dick	1973	1975
H. Festenstein	1972	1975
E. M. Lance	1972	1975
P. B. Medawar (Chairman)	1972	1974
R. A. Sells	1973	1975
A. R. Sanderson (B.S.I. representative)	—	—

As E. M. Lance has resigned from the Committee because of his departure from the U.K. there are *three Committee vacancies;* in addition, the posts of *General Secretary* and *Meetings Secretary* need to be filled.

The following nominations have been received:

POSITION	NOMINEE	PROPOSER	SECONDER
General Secretary:	L. BRENT, Department of Immunology, St. Mary's Hosp. Medical School, London	J. R. SALAMAN	P. B. MEDAWAR
Meetings Secretary:	A. D. BARNES, Queen Elizabeth Hospital, Birmingham	R. A. SELLS	J. BRADLEY
Committee members:	J. R. BATCHELOR, McIndoe Memorial Research Unit, Queen Victoria Hospital, East Grinstead	A. R. SANDERSON	L. BRENT
	P. R. F. BELL, Department of Surgery, General Hospital, Gwendolin Road, Leicester	R. A. SELLS	J. R. SALAMAN
	D. B. EVANS, Renal Unit, Addenbrooke's Hospital, Cambridge	VALERIE C. JOYSEY	R. Y. CALNE
	D. N. H. HAMILTON, Department of Surgery, Western Infirmary, Glasgow	HEATHER M. DICK	J. D. BRIGGS
	ELIZABETH SIMPSON, Clinical Research Centre, Northwick Park Hospital, Middlesex	P. B. MEDAWAR	R. A. SELLS