11. N. Transplant Board

MEETING OF

BRITISH TRANSPLANTATION SOCIETY

3rd January, 1974

CHARING CROSS HOSPITAL (FULHAM), FULHAM PALACE ROAD, LONDON W6 8RF.

10.00 a.m. A.D. Mee and J.E. Castro (Urology and Transplantation Unit, Hammersmith Hospital, London)

'Inhibitory factors of the M.L.C. in patients with renal failure'

10.15 a.m. Valerie E. Jones and J. Abbosh (Division of Surgical Sciences, Clinical Research Centre, Harrow)

'Effects of equine anti-lymphocyte globulin treatment in multiple sclerosis patients: Tolerance to equine IgG'

10.30 a.m. Stella C. Knight and J. Abbosh (Division of Surgical Sciences, Clinical Research Centre, Harrow)

'Effects of equine anti-lymphocyte globulin treatment in multiple sclerosis patients: Changes of in vitro lymphocyte transformation'

10.45 a.m. J.S.F. Canavan, P.W. Horton, J.D. Briggs, P.R.F. Bell (Western Infirmary, Glasgow)

'Evaluation of the human renal allograft using technetium 99^{m} and the gamma camera'

11.00 a.m. COFFEE

11.30 a.m. D. Miller and J.R. Salaman (Transplantation Laboratory, K.R.U.F. Institute of Renal Disease, Cardiff Royal Infirmary)

'Is there an immunogen in renal perfusate?'

11.45 a.m. A. Ebringer, Immunology Unit, Queen Elizabeth College, University of London)

'The F1 response in high and low responder animals and tolerance'

12.00 noon Elizabeth Simpson (Clinical Research Centre, Watford Road, Harrow)

'Induction of cytotoxic T cell responses in vitro: effect of B cells primed to alloantigens'

12.15 a.m. B. Delor, T. Reme, R. Silva, B. Serrou (Department of Clinical and Experimental Immunology, Cliniques Saint-Eloi, Montpellier, France)

'Extraction and partial purification of an inhibitor product of the thymic epithelial cells'

BRITISH TRANSPLANTATION SOCIETY

A B S T R A C T S

(not for publication).

Enhanced survival of renal allografts in rats treated with low doses of ALG and bone marrow (BM) cells

Annette Byng, G.D. Pegrum, Grant B Williams & R.A. Risdon

The ability to induce unresponsiveness in human recipients in the period shortly before transplantation of a cadaveric renal allograft may be desirable since it is possible to maintain the donor kidney on a preservation machine during the period of pretreatment.

We have investigated the effects of pretreatment with ALG and BM cells either alone or in combination using as a model the transplantation of donor (Lewis x BN F_1 hybrid) kidneys into recipient (Lewis) rats.

It was found that pretreatment with low doses of ALG (10mg/Kg) and BM (10^5 cells) in combination either one or three days before grafting significantly prolonged survival whereas pretreatment with low doses of either ALG or BM alone were ineffective. High doses of BM alone (10^9 cells), as well as ALG (30 mg/Kg), also prolonged survival.

EFFECTS OF EQUINE ANTI-LYMPHOCYTE GLOBULIN TREATMENT IN MULTIPLE SCLEROSIS PATIENTS

I. Tolerance to equine IgG

Valerie E. Jones., J. Abbosh.

II. Changes of *in vitro* lymphocyte transformation

Stella C. Knight., J. Abbosh

I. Tolerance to equine IgG.

To induce tolerance to horse IgG, large doses, together with azathioprine and corticosteroids, were given to multiple sclerosis patients before treatment with antilymphocyte globulin (ALG).

Twelve of the fourteen patients had no clinical reactions to equine IgG but, of the other two, one developed serum sickness and the other systemic anaphylaxis. Antibody could not be detected in the twelve patients at any time by most methods.

At the time of ALG treatment, immune elimination studies and radioimmune gel filtration showed that the twelve patients were tolerant but this was 'partial' because concommitant immunity could be detected in some patients by radioimmune gel diffusion.

In the months after ALG therapy, this partial tolerance was lost; all patients who were skin tested gave immediate wheal and flare reactions. Nonetheless, all antibody was tissue bound because *in vitro* tests did not reveal circulating antibody. Immune elimination studies and fractional catabolic rate measurements confirmed this conclusion.

II. Changes of in vitro lymphocyte transformation.

Lymphocyte responses *in vitro* to phytohaemagglutinin Concanavalin A (Con.A), pokeweed mitogen and allogeneic lymphoid cell line cells were studied in the multiple sclerosis patients undergoing immunosuppressive therapy. The mitogen responses of the lymphocytes cultured in the presence of normal allogeneic serum or autochthonous serum were in the normal range before treatment.

Most patients showed a severe depression of responses to all mitogens during an initial week of treatment with Azathioprine and Prednisone. These remained low or were further depressed during the three weeks of

treatment with ALG. . Patients with no change in responsiveness during the Azathioprine and Prednisone treatment showed a very marked lowering of all responses when ALG was administered.

Responses mainly returned to the normal range at the end of the ALG treatment despite a 'maintenance' therapy of Azathioprine and Prednisone. The only indication of a more prolonged effect was observed in a few patients where low Con A responsiveness was still found several months after the end of ALG treatment.

Other studies showed that the levels of the five immunoglobulin classes changed very little during therapy and complement levels, which were reduced early in treatment, returned to pre-treatment levels some months later.

INHIBITORY FACTORS OF THE M.L.C. IN PATIENTS WITH RENAL FAILURE

A. D. Mee and J. E. Castro
Urology and Transplantation Unit,
Hammersmith Hospital,
London, W.12.

Circulating factors which inhibit immunological reactivity between the recipient and the graft may be responsible for prolonged allograft survival in some patients. The mixed lymphocyte culture (M.L.C.) has been used as an in vitro model of cell mediated immunity in which to study the effect of test sera from selected groups of patients.

Control experiments were performed with test sera from healthy untransfused males. Parallel M.L.Cs were set up, one using 30% AB serum, the other using 10% AB serum plus the test serum. Good reproducibility has been shown and the validity of the method established.

To date, ten live donor transplant patients have had M.L.Cs, three months or more after transplantation. In each case immunosuppression has been stopped for 48 hours prior to the M.L.C. In two cases there is non-specific blocking to donor, recipient and unrelated mismatch in M.L.C.

Three patients who have had skin grafts as a predictive test prior to live donor renal transplantation have had M.L.C. In none of these was blocking demonstrable.

Eleven of the twelve regular haemodialysis patients studied have shown non-specific blocking in M.L.C. The chemical nature of this blocking factor is being studied.

THE F1 RESPONSE IN HIGH AND LOW RESPONDER ANIMALS AND TOLERANCE

(A. Ebringer)
(Immunology Unit, Department of Biochemistry)
(Queen Elizabeth College - University of London)
(Kensington, W.8.)

Immune responses to various synthetic polypeptides and cellular antigens produces in some strains a high response and in other strains of the same species of animals, a low response.

About 30 such high-and-low immune response systems have been described and they have been linked to the transplantation antigens of the responder animals.

High immune responses have been ascribed to the presence and low immune responses to the absence of so-called "Ir genes" in T-cells[1].

An alternative explanation has been proposed, where the low responses are ascribed to cross-reactivity between the test antigen and the transplantation antigens of the low responder animals[2].

The F1 hybrid animals, resulting from a cross between a high responder and a low responder parents, have been found, in over 20 of such systems so far investigated, to give a quantitatively intermediate response.

Furthermore, F1 back-crosses to parental strains give either a 50% parental response or a 50% F1 hybrid intermediate response.

These results are difficult to reconcile with current theories of immunity, either the theory of acquired tolerance or the theory of clonal selection.

It is suggested self-tolerance in normal F1 animals is not produced by a process involving inactivation or elimination of self-reactive clones.

1) Benacerraf, B., and McDevitt, H.O. Science, 175, 273 (1972).

2) Ebringer, A., and Davies, D.A.L. Nature, 241, 144 (1973).

EXTRACTION AND PARTIAL PURIFICATION OF AN INHIBITOR PRODUCT OF THE THYMIC EPITHELIAL CELLS.

B. DELOR, T. REME, R. SILVA, B. SERROU

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In animals bearing spontaneous tumors (dogs, pigs) or transplanted tumors (New-Zealand rabbits, C57 B1/6 X DBA2 and BALB/c mice), we noticed two chronological steps in the evolution of their thymuses : firstly, massive cortical epithelial cystic formations, observed under light and electron microscopy, secondly complete atrophy of the thymuses. The cystic substance was a glycoprotein as shown by PAS coloration. We used thymuses of treated animals in order to isolate the cystic epithelial substance. Each thymus was homogenized and centrifuged at 20,000 x g for 1 hour. The supernatant was purified by fractionation with increasing concentrations of ethanol. Then the fraction were dialysed and lyophilized. For the controls, we used this same extraction procedure on kidney and spleen. We tested all the fractions with both spontaneous and PHA blastic stimulation tests of thymic lymphocytes from C57 B1/6 X DBA2 mice. The thymic fraction soluble in 75 % ethanol induced a more than 90 % decrease of thymidine up-take. No significant cytotoxicity was observed. FUrthermore, as compared with the controls, the substance provoked a decrease in the number of plaque forming cells and a delayed skin allograft rejection in normal mice (C57 B1/6 X DBA2 versus BALB/c mice). When injected in C57 B1/6 X DBA2 mice with Lewis tumors, it enhanced tumor growth as measured by the time of the appearance of the tumor, the tumor surface, the weight of the tumor, and the survival time of the mice. Further purifications are now being performed, by means of ion exchange chromatography and isoelectric focusing, and are verified by polyacrylamide gel electrophoresis. These results suggest that cortical epithelial cells of the thymus secrete a substance depressing the T-lymphocyte function.

EVALUATION OF THE HUMAN RENAL ALLOGRAFT USING TECHNETIUM 99^m AND THE GAMMA CAMERA

J.S.F. Canavan, P.W. Horton, J.D. Briggs, P.R.F. Bell.

Western Infirmary, Glasgow, G11 6NT.

The results of 53 gamma scans of renal allografts in 35 patients are reported, using technetium 99^m. The results were analysed as previously described by Aquino, et al. and the functions P/P (ratio of peak to plateau counts over the graft), $T\frac{1}{2}$ (time in seconds for counts to reach half their peak value) and $\triangle T$ (delay between peak of iliac artery and peak of activity over the kidney) were derived.

Rejection was found to be associated with diminished values for P/P (< 1.15) with increased values for $T\frac{1}{2}$ (> 80 seconds); $\triangle T$ also showed high values (> 5 seconds).

In four oliguric patients whose initial gamma scans showed good vascularity, subsequent scans showed absence of radioactivity over the renal area. Subsequent investigation demonstrated arterial thrombosis in one, advanced chronic rejection in another, and total necrosis of the graft despite patent renal vessels in the remaining two.

In conclusion, we have found the gamma scan using technetium 99^m to be of considerable value in determining the viability of the transplanted kidney and in the differential diagnosis of acute rejection in the oliguric phase.

Induction of cytotoxic T cell responses *in vitro*: effect of B cells primed to alloantigens.

Elizabeth Simpson, C.R.C., Watford Road, Harrow, Middlesex.

Mice grafted with allogeneic skin across an H-2 barrier develop cytotoxic T cells in the spleen. Using a short term (4 hour) chromium release assay, peak cytotoxic activity can be shown to occur between 10 and 14 days post grafting, but after 3 weeks no activity is detectable. If spleen cells from previously grafted mice are placed in culture for 4 days with additional antigen, they can be induced to make a cytotoxic response which is considerably greater than that of similarly cultured spleen cells from unprimed mice. This amplified activity of primed spleen cells has the hall marks of a secondary response, and has been termed 'memory'. It is dependent on the presence of primed T cells. Memory activity first appears 14 days after skin grafting, and persists at least until 3 months post grafting. Thereafter it can only be detected if B cells are removed from the spleen cell suspension prior to sensitization *in vitro*. This suggests that splenic B cells from primed mice exert an inhibitory effect on the induction phase of the cytotoxic T cell response. It also raises the possibility that blocking factors, which are probably antibody/antigen complexes, exert their suppressive effect on the induction phase of the immune response, rather than on the effector phase.