Preliminary Communications

Serotyping for Homotransplantation. XXII, Specificity of Cytotoxic Antibodies Developing after Renal Transplantation

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Summary: Cytotoxic antibodies against allogeneic lymphocytes were detected in 13 patients after removal of a renal allograft. Antibodies were not detected in seven patients who had grafts in situ for less than two months, but were detected in 13 out of 17 patients who had grafts in situ for longer than two months. The HL-A specificity of these positive sera was compatible with the HL-A antigen mismatch of the donor-recipient pairs in most instances, providing further evidence that such antibodies result from direct sensitization by the allograft and may play some part in renal allograft rejection.

INTRODUCTION

Cytotoxic antibodies reacting with allogeneic lymphocytes can be detected after removal of a renal allograft in humans in many instances (Morris *et al.*, 1968). It was suggested that such antibodies arose as a result of sensitization by the allogeneic graft, as there was an increase in reactivity and titre of these antibodies after transplant nephrectomy. Furthermore, in three out of four instances the antisera were shown to react strongly with donor cells after removal of the rejected kidney, demonstrating the specificity of these antibodies against donor lymphocytes. If such cytotoxic antibodies developed as a result of sensitization by a renal allograft and played a part in graft rejection, then it should be possible to show that the specificity of the antisera for leucocyte groups of the HL-A system is the same or at least includes the group for which donor-recipient were mismatched. We have now been able to study sera from a number of patients who have had renal transplants removed where both donor and recipient were typed for leucocyte groups of the HL-A system.

METHODS AND MATERIALS

Twenty-four patients who had their first renal allografts removed at periods varying from 1 week to 51 months after transplantation provided the sera used in this study. Sera were kindly referred to us for testing by Drs. Hume, Williams, and Rolley, of Richmond; Drs. Goldman, Rubini, Martin, and Gonick, of U.C.L.A.; Drs. Merrill, Carpenter, and Murray, of Boston; and Dr. Nakamoto, of Cleveland. These patients had no demonstrable lymphocyte antibodies before transplantation.

Twenty-two out of 24 donor and recipient pairs were tissue-typed for HL-A antigens as described previously (Terasaki *et al.*, 1967). Leucocyte antigens present in the donor and absent in the recipient were considered to be mismatched. The microdroplet lymphocyte cytotoxicity test was used also for determining cytotoxicity in the post-transplant sera. All sera were tested against a panel of at least 80 unrelated donors. Strong antisera were tested in dilutions of 1:8 and 1:16 in order to help define specificities. The specificity of the antisera was defined by a statistical procedure of Mickey (in preparation).

Sera which did not show cytotoxic activity by the usual method were retested against a panel of 24 random donors,

with various modifications in the procedure as described below. The nomenclature of antigens is given according to the new W.H.O. agreement, and those antigens which have not yet been given an international designation are denoted by the prefix Te, referring to groups previously described by Terasaki, Mickey, and co-workers.

OCCURRENCE OF CYTOTOXINS

Of the 24 patients who had rejected their primary renal allograft, 13 had definite cytotoxins after transplantation (Table I). Unfortunately serum samples were not available at regular times after transplant removal, as indicated in Table I. It can be noted that 5 out of the 11 non-reactive sera had been obtained more than five months after nephrectomy, so that any activity could have been lost by the time the recipient was tested. The duration of the graft survival may have some bearing on the development of antibodies, for none of the seven patients whose grafts were removed at or before two months (some for technical complications) had cytotoxins, whereas 13 out of 17 patients whose grafts were removed after two months had cytotoxins.

Patient	Percentage Reactivity	Donor-Recipient Mismatch	Specificity of Antisera	Duration of Transplants	Time after Nephrectomy Serum Tested
J.B.	33	HL-A1	HL-A1	25 months	2 weeks
C.S.	40	HL-A1	HL-A1	50 months	12 weeks
E.					
Rad.	17	HL-A1	HL-A1	22 months	5 weeks
H.R.	50	HL-A2	HL-A2	46 months	8 weeks
H.H.	63	HL-A2	HL-A2, Te3, 9	36 months	24 weeks
S.S.	82	HL-A7, (?) HL-A1	HL-A1, HL-A7	8 months	8 weeks
N.M.	83	Te3	HL-A1, HL-A7, HL-A8	15 months	36 weeks
E.R.	66	HL-A1, HL-A7, Te4	Te4	15 months	8 weeks
M.D.	89	HL-A2, HL-A5	Multispecific	11 months	8 weeks
H.B.	98	Not tested	Multispecific	11 months	8 weeks
W.A.	80	HL-A1	HL-A2, Te4, 9	2 months	26 weeks
D.V.	10	HL-A1, HL-A5	0	4 months	14 weeks
B.C.	7	HL-A7	0	13 months	2 weeks
J.D.	0	Not tested	0	15 months	1 week
M.C.	0	Te3, HL-A5		31 months	8 weeks
P.H.	0	HL-A5		36 months	44 weeks
H.S.	0	0		12 months	12 weeks
W.J.	0	0		2 months	12 weeks
L.C.	0	0		6 weeks	32 weeks
G.R.	0	Te3, HL-A7		1 month	28 weeks
H.H.	0	Te3, HL-A5		3 weeks	10 weeks
H.K.	0	0		3 weeks	1 week
R.D.	0	HL-A1, HL-A7		2 weeks	28 weeks
R.B.	0	HL-A1, HL-A2		1 week	20 weeks

Some of the non-reactivity of sera from patients who had rejected grafts could be attributable to long-term storage, for many sera had been stored at -60 to -20° C. for periods of up to three years before testing. Attempts were made to determine whether the sera had lost potency by testing the sera at 4 and 20 times the usual test volume of 0.0005 ml. Such increase in serum volumes failed to show activity when tested with cells from 24 random individuals. The possibility that the negative results were being produced by anticomplementary factors was investigated by the following methods: (a) heating the sera at 56° C. for 30 minutes, (b) heating at 50° C. for 30 minutes, (c) shaking out the serum before addition of C^1 , (d) dilution of sera to 1:5 or 1:30, and (e) incubation with C^1 for three hours instead of the usual one hour. None of these treatments was effective in revealing cytotoxic activity in nine of the negative sera when tested with lymphocytes of 24 persons. Four positive results with sera heated at 56° C. for 30 minutes were rechecked with tests on 30 other cells with negative results. It thus appears that the initial four positive results may have been false-positives.

SPECIFICITY

In four instances the specificity of the antibodies were in exact agreement with the mismatched specificities. One patient (H. R.) who received a transplant from a brother had been identified as being mismatched for HL-A2 (Mac) (Vredevoe *et al.*, 1965). Four years after transplantation he rejected his transplant, and two months later was found to have strong monospecific HL-A2 antibodies. Three other patients with HL-A1 incompatibilities with their donors developed antibodies of additional specificities. For example, one patient (S. S.), who was mismatched for HL-A7 (Terasaki *et al.*, 1967) and doubtfully for HL-A1, developed HL-A7 and HL-A1 reactivities. In two instances the sera were too active (>89% positive) to be classified and in two other sera the activity was too low (<10%).

Although the number is small, the patients mismatched for antigens of the first sublocus ("LA") of the HL-A system (Dausset *et al.*, 1969; Terasaki *et al.*, 1969) seem to have produced antibodies whereas those incompatible for antigens of the second sublocus did not have cytotoxins. Five of the six patients mismatched only at a first sublocus antigen had antibodies, whereas one of the six patients incompatible only at the second sublocus had produced cytotoxins.

DISCUSSION

It is apparent from these results that the cytotoxins which appear after rejection of a kidney allograft (Morris *et al.*, 1968) often contain antibodies of the mismatched HL-A specificity of the donor. Such evidence supports the view that HL-A antigens are of importance in transplantation and that the antibodies against them have some role in the rejection of grafts. In seven instances the specificity of the antibodies which were found clearly included one in which the kidney donor was incompatible. Occurrence of other specificities can be accounted for by either transfusion with donors of other types or non-specific broadening reactivity.

Deliberate immunization with donors of limited HL-A differences by means of skin grafts or transfusions have not led uniformly to production of monospecific antisera (Ceppellini *et al.*, 1964; Amos, 1965; Walford *et al.*, 1965). In some instances a specific antiserum was produced that agreed with the antigenic discrepancy between donor-recipient, but very often an antiserum with additional specificities was obtained. Even in instances where a specific antibody results after skin grafting, further boosting of the recipient very often results not in a stronger monospecific antiserum but in a polyspecific antiserum (Hammond *et al.*, 1967). Thus during the terminal stages of renal graft rejection and after rejection, when the patient received supportive haemodialysis, the boosting effects of these transfusions may well result in polyspecific antiserum (Rolley *et al.*, 1969).

The existence of antecedent glomerulonephritis in the recipient could be regarded as an explanation for certain antisera having specificities broader than or different from those of the histocompatibility antigens of the donor. Documentation of this proved not feasible, because only test sera and not full case details were made available to us. However, we consider this possibility to be highly unlikely, because antibodies against histocompatibility antigens have never to our knowledge been detected in glomerulonephritis or in any other of the presumed autoimmune disorders.

Of the patients who failed to show antibodies in their sera after transplant nephrectomy some did not have the graft in place long enough to be sensitized to a level where cytotoxic antibodies could be detected. However, four patients who had a graft in situ for times ranging from 4 to 36 months did not show cytotoxic antibodies in their sera after transplant nephrectomy. The possibility that the cytotoxic activity in these sera was masked either by anticomplementary factors or by other

unknown inhibitory factors is probably excluded by the series of tests done in the present study. Heating sera to 56° C. for 30 minutes (Rolley *et al.*, 1969) was not effective with the sera tested here. The possibility that storage of serum over a long period of time may result in loss of activity was seen in this study, where at least in one instance (E. Rad.) all activity was lost after one year's storage. Thus some of the negative results may be attributable to prolonged storage of antisera.

If it is accepted that these sera were truly negative for cytotoxic activity, then either the renal grafts may have failed because of recurrent disease or cytotoxic antibodies may not invariably develop as a result of renal allograft rejection. There is some evidence to support the latter suggestion, for after a single allogeneic skin graft in humans further skin grafts or leucocyte booster injections are often necessary for the development of antibodies.

Nevertheless, the demonstration of cytotoxic antibodies in the sera of some of the patients who had a transplant nephrectomy some time after transplantation, together with a specificity of these antibodies which was in agreement with the original donor-recipient mismatch, further supports our previous suggestion that these antibodies do develop as a result of direct sensitization by the renal allograft and probably play a definite part in chronic graft rejection in the immunosuppressed patient. In an earlier report (Morris *et al.*, 1967) we indicated that sera from these sources might be useful for leucocyte typing in organ transplantation, as serum specificities would be directed against histocompatibility antigens. This would appear to be so in this study, though the additional specificities found in many of the positive sera would diminish their value for this purpose.

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