

Histocompatibility testing in human renal allografts. I. Evidence for a strong and a weak HL-A sublocus in recipients of allografts from living related donors

WILLIAM E. BRAUN, M.D.
JUDITH J. MURPHY, A.B.*

Division of Research

THE significant correlation of histocompatibility typing with the clinical status of patients who received renal allografts from living related donors has been demonstrated for sibling-sibling and for parent-child pairs.¹⁻³ Those who did not show such a correlation constituted two major groups of patients: those mismatched recipients whose allografts retained good function, and those matched recipients whose allografts failed. These discrepancies between histocompatibility testing and renal allograft survival may have been attributable to several major factors: (1) differences in strength of histocompatibility antigens, (2) effects of antigenic combinations, (3) differences in response of various hosts to the same histocompatibility antigens, (4) inadequacies of leukocyte typing sera for detecting HL-A histocompatibility antigens, (5) presence of other histocompatibility loci in addition to the HL-A locus.

By using well-defined leukocyte antisera capable of detecting 13 HL-A antigens and thus minimizing the fourth factor, we attempted to gain information in regard to the effect of the other factors, particularly the relative strength of histocompatibility antigens. Because this study was retrospective, there was a bias against finding a strong histocompatibility antigen, since those patients with such a mismatch may have already died. Of equal importance, however, was the detection of weak antigens most apparent in those mismatched recipients with prolonged allograft survival. This latter group of patients has accounted for the majority of discrepancies between tissue typing and clinical status.¹

Patients and methods

Patients. Of 31 patients at the Cleveland Clinic Hospital who each received a renal allograft from a living related donor, maintained allograft function for from three months to six years, and were alive as of June 1, 1969,

This work was supported by United States Health, Education, and Welfare Contract No. PH 43-68-1298.

** Technician, Division of Research.*

Table 1.—Short-term renal allograft recipients with living-related donors

Histocompatibility typing	Patient, no.	Donor	Allograft-survival, months	Clinical grade*
Matched	1	Sibling	11	A
	2	Parent	8	B
	3	Parent	4	B
Mismatched	4	Sibling	11	D†
	5	Parent	11	A
	6	Parent	11	D†
	7	Parent	11	A
	8	Parent	10	B
	9	Parent	8	B
	10	Sibling	6	C
	11	Parent	6	B
	12	Parent	6	B
	13	Parent	3	C
	14	Parent	3	A

* Clinical grade as of June 1, 1969 (see text for explanation).

† Patient recently died from allograft rejection.

30 were tested with their respective donors for 13 histocompatibility antigens. Twenty-two donors were parents and eight were siblings. In 14 recipients the duration of allograft survival was from three to 12 months (short-term) (*Table 1*); in 16 recipients the range of survival was from one to six years (long-term) (*Table 2*). The clinical status of each recipient was graded according to the following criteria:

A. Serum creatinine less than 1.6 mg per 100 ml; 24-hour urine protein excretion less than 300 mg; and diastolic blood pressure less than 90 mm Hg without therapy.

B. Presence of one or more of the following: serum creatinine concentration between 1.7 and 2.4 mg per 100 ml; 24-hour urine protein excretion between 300 and 1000 mg; diastolic blood pressure between 90 and 100 mm Hg with therapy.

C. Two or more of the following factors not responsive to immunosuppressive therapy: serum creatinine concentration between 2.5 and 3.2 mg per 100 ml; 24-hour urine protein excretion between 1000 and 3500 mg; diastolic blood pressure between 100 and 120 mm Hg with therapy.

D. Two or more of the following factors not responsive to immunosuppressive therapy: serum creatinine concentration more than 3.2 mg per 100 ml; 24-hour urine protein excretion more than 3500 mg; diastolic blood pressure higher than 120 mm Hg with therapy.

F. Allograft rejection causing death, requiring removal of the allograft, or chronic hemodialysis.

Table 2.—Long-term renal allograft recipients with living-related donors

Histocompatibility typing	Patient, no.	Donor	Allograft-survival, months	Clinical grade*
Matched	15	Sibling	71	A
	16	Parent	45	A
	17	Parent	34	B†
	18	Parent	33	B
	19	Parent	21	A
	20	Sibling	17	A
	21	Parent	13	A
Mismatched	22	Parent	71	A
	23	Parent	60	B
	24	Sibling	50	A
	25	Parent	46	A
	26	Sibling	42	D
	27	Parent	21	F
	28	Sibling	17	B
	29	Parent	15	F
	30	Parent	12	D‡

* Clinical grade as of June 1, 1969 (see text for explanation).

† Patient's status recently changed to grade D.

‡ Patient now requires chronic hemodialysis.

In all cases immunosuppressive therapy basically consisted of azathioprine and prednisone. A subtotal thymectomy and splenectomy were performed before transplantation in one matched (patient 16) and four mismatched patients (patients 23, 24, 25, and 26). The two patients who survived longest (patients 15 and 22) each had undergone subtotal thymectomy. All of the short-term allograft recipients as well as two long-term allograft recipients (patients 20 and 21) underwent splenectomy. Seven patients (patients 17, 18, 19, 27, 28, 29, and 30) did not undergo thymectomy or splenectomy. Within the last 18 months, six matched patients (by clinical criteria, 4 A's and 2 B's), and 15 mismatched recipients (by clinical criteria, 3 A's, 5 B's, 2 C's, 3 D's,* and 2 F's) received antilymphocyte globulin (ALG).

Methods

Histocompatibility testing was performed according to the microcytotoxicity technic.⁴ The defined leukocyte antisera were provided through the courtesy of Dr. Paul I. Terasaki from the National Institutes of Health Serum Bank. These antisera were capable of detecting 13 leukocyte antigens listed according to the two proposed HL-A subloci:⁵⁻⁸ LA sublocus—HL-A1, HL-A2, HL-A3, B4, B12, B13; 4 sublocus—HL-A5, HL-A7,

* Two of these patients recently died from allograft rejection, and the third has required chronic hemodialysis.

HL-A8, B6, B9, B10, B11. Of the 60 typings done in this study, 52 were performed on blood drawn less than one hour before, and eight on specimens taken from 24 to 48 hours before testing and mailed to our laboratory.

Matched recipients were those with no donor antigen mismatched but up to three recipient antigens mismatched. Mismatched recipients had at least one donor antigen and up to three recipient antigens mismatched.

Results

Matched group. In each of 10 recipients there was no mismatched donor antigen. As of June 1, 1969, all 10 patients had intact renal allografts with an A or B clinical status. Seven allografts were functioning for from one to six years after transplantation, and three for from four to 11 months. However, after 34 months in a grade B clinical status, one patient's condition (patient 17) recently deteriorated to grade D. There was no mismatched donor or recipient HL-A antigen in this case.

Mismatched group. Twenty recipients had from one to three mismatched donor antigens. In two of these patients the allografts failed after 15 and 21 months; four patients were in grade D at 11,* 11,* 12† and 42 months; and two patients were in grade C at three and six months. In these latter eight patients (patients 4, 6, 10, 13, 26, 27, 29, and 30) (subgroup 1) mismatched donor antigens were: HL-A7 (3), HL-A1 (2), B4 (2) and one each of HL-A2, HL-A3, B12, and B13. Mismatched recipient antigens were: HL-A2 (3), B10 (3), B12 (2), and one each of HL-A1, HL-A3, HL-A5, B4, B11, and B13. However, five patients (patients 22, 23, 24, 25, and 28) (subgroup 2) surviving for from 17 to 71 months (mean 48.8 months) have good to excellent function (3 A's and 2 B's). In these latter five patients mismatched donor antigens were: HL-A7 (4), HL-A1 (2), HL-A3 (2), B4 (2), and one each of HL-A8, B10, and B12. Mismatched recipient antigens were one each of HL-A1, HL-A2, HL-A8, B4, B6, B9, B12, and B13.

The other seven mismatched recipients did not warrant inclusion in either subgroup because their allografts had neither failed nor had a prolonged duration of good function.

Whether considered separately or together⁶ mismatched donor and recipient antigens in the eight failing (subgroup 1) and five long-surviving (subgroup 2) allografts showed no strong or weak single antigen or antigen pair, but suggested a difference in the strength of the two HL-A subloci.⁵⁻⁸ In subgroup 1 there were five instances of donor antigen mismatches exclusively in the LA sublocus, one in the 4 sublocus alone, and two in both subloci. Subgroup 2 had no mismatches exclusively in the LA sublocus, one in the 4 sublocus only, and four in both subloci.

* The patient subsequently died from allograft rejection.

† The patient required chronic hemodialysis because of virtually no allograft function.

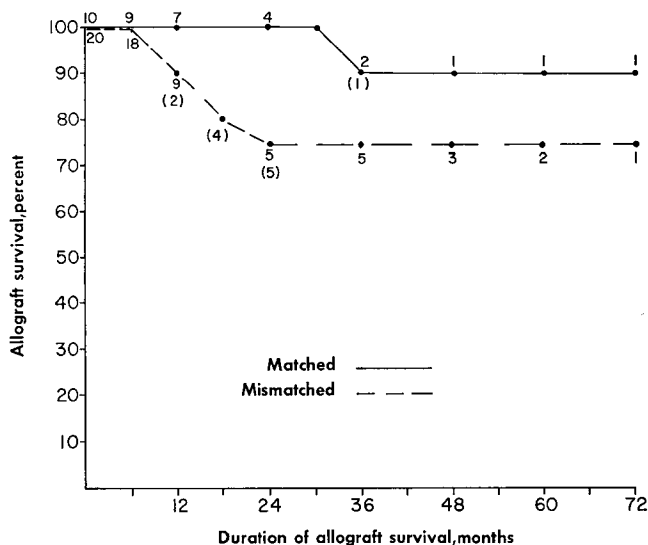


Fig. 1. Survival curves of 10 matched and 20 mismatched living-related renal allografts showed that allograft failure usually occurred within the first two years. The numbers along the survival curves represent the patients at risk at that time. The numbers in parentheses indicate the number of allografts lost up to that time.

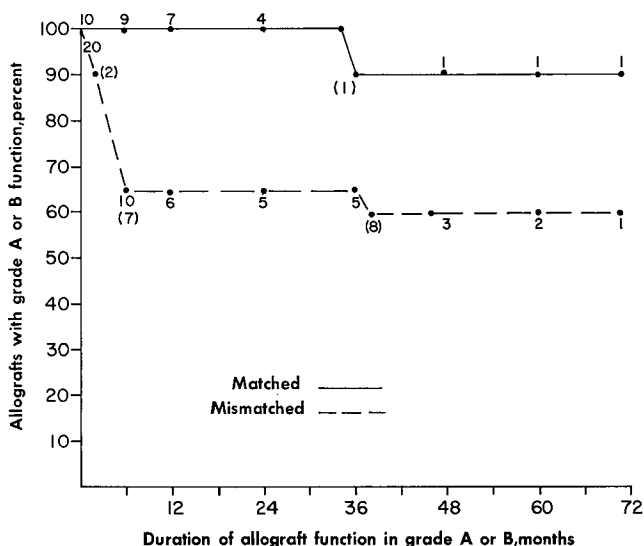


Fig. 2. Allograft function in 10 matched and 20 mismatched living-related renal allografts showed that when deterioration occurred it did so by six months in the majority of cases. Those that showed deterioration at six months included all those that failed within two years (Fig. 1). The numbers along the curves represent the patients at risk at that time. The numbers in parentheses indicate the patients whose function had deteriorated up to that time.

This difference was statistically significant ($p < 0.03$) (*Table 3*) and suggested that antigens of the LA sublocus exerted a stronger effect on allograft failure than did those of the 4 sublocus.

In evaluating the possible existence of a weak HL-A site it was found that each patient in subgroup 2 had one or two donor antigens mismatched in the 4 sublocus, whereas only three of eight patients in subgroup 1 had a single such mismatch. This was significant when viewed as a ratio of mismatched-to-matched 4 sublocus antigens ($p < 0.05$) (*Table 4*) in the two subgroups. It is possible that the 4 sublocus antigens by themselves may be weak or may modify strong LA sublocus antigens.

A comparison of survivals between matched and mismatched recipients revealed that allograft failure occurred within 24 months of transplantation (*Fig. 1*). A deterioration of function by six months heralded the eventual failure of these same allografts (*Fig. 2*). Although the survival curves in *Figure 1* appeared significantly different, they failed to show a statistically significant difference. Here, too, the retrospective nature of the study may have lessened the difference between the two groups by the exclusion of those mismatched recipients who had already died.

Discussion

Evidence to permit the distinction between strong and weak histocompatibility antigens in the cases of HL-A mismatches would clearly have an important bearing on donor selection, intensity of immunosuppressive therapy, and the survival of human allografts. Thus far, histocompatibility antigens in man have been graded as intermediate in strength, since efforts to detect a strong histocompatibility antigen in man similar to the H-2 in mice or Ag-B in rats have been unrewarding.¹

Other factors being equal, mismatched recipients losing an allograft because of rejection should have been exposed to strong histocompatibility antigens, whereas mismatched recipients with well-functioning allografts for prolonged periods should have had weak antigenic exposures. In the present study no individual or pair of mismatched HL-A antigens, when examined separately or together in the donor and in the recipient, appeared stronger than another. The capability of a retrospective study to assess a factor contributing to mortality was obviously impaired by the exclusion of recipients who had succumbed to that factor.

However, when donor antigen mismatches were grouped according to the proposed subloci of the HL-A locus, the 4 sublocus and the LA sublocus,⁵⁻⁸ there was evidence that antigenic mismatches in the LA sublocus presented a greater hazard to allograft survival than did those of the 4 sublocus (*Table 3*). This finding agreed with results obtained in both skin⁸ and renal allograft⁷ studies in man. Complementing this was evidence that the 4-sublocus antigens may have modified strong LA antigens or been weak antigens themselves (*Table 4*). Information concerning these two pro-

Table 3.—Distribution of mismatched histocompatibility antigens according to HL-A subloci in allograft failures and successes

Result	LA Sublocus	4 Sublocus	LA + 4 Subloci
8 failures (subgroup 1*)	5	1	2
5 successes (subgroup 2*)	0	1	4

The occurrence of five allograft failures with donor antigens of only the LA sublocus mismatched, and the absence of exclusive LA mismatches in long-term successes suggested that the LA sublocus was a stronger histocompatibility site than the 4 sublocus ($p < 0.03$).

* See text for definition of subgroups.

Table 4.—Proportion of mismatched to matched antigens of the 4 sublocus in allograft failures and successes

Result	Number of antigens		
	Mis-matched	Matched	Total
8 failures (subgroup 1*)	3	53	56
5 successes (subgroup 2*)	6	29	35

The higher proportion of mismatched 4 sublocus donor antigens that were formed in long-term successes (6 of 35) as compared to failures (3 of 56) suggested that the 4 sublocus was a weaker histocompatibility site than the LA sublocus ($p < 0.05$).

* See text for definition of subgroups.

posed subloci has been too incomplete to provide any substantive reason for such a difference. The prevalence of the HL-A7 antigen, though, in 4-sublocus mismatches of both subgroups still indicated the importance of individual host response.

The finding of such a group difference has not excluded the possibility that certain individual antigens may yet be stronger than others. Basic variability in host responsiveness to the same antigen, when altered further by antecedent uremia and subsequent immunosuppressive agents, may well have obscured the hierarchy of histocompatibility antigens. Although total thymectomy of adult mice has been associated with a decline in immunologic capacity after from six to nine months,⁹ the possible role of splenectomy and subtotal thymectomy in the long-term survival of four mismatched allograft recipients was extremely difficult to assess, particularly since long survivals of similarly mismatched recipients without such treatment have been reported.¹⁰ Use of ALG in our recipients also ap-

peared to have had no clearly defined effect on host response to allograft antigens.

The successful outcome of HL-A matched allograft recipients with living related donors was again apparent in this series (*Fig. 1 and 2*). In contrast to the allograft failure reported by Singal, Mickey, and Terasaki,¹ the failures in our series of patients occurred within the first 24 months and showed little attrition thereafter (*Fig. 1*). Eventual loss of the allograft was predictable at six months, when function had already deteriorated in these same allografts (*Fig. 2*). The single case of delayed allograft failure in which none of 13 HL-A antigens was mismatched,* raised the possibility of the existence of another important histocompatibility locus independent of the HL-A locus.¹¹

Summary

Tissue typing for 13 HL-A histocompatibility antigens was performed retrospectively on 30 renal allograft recipients who had living related donors and whose allografts functioned for from three months to six years after transplantation. Ten matched patients each had grade A or B clinical status, whereas of 20 mismatched recipients eight had failing allografts (grade C, D, or F). Although no individual HL-A antigen or antigen pair appeared stronger than any other, there was evidence to suggest that antigens of the LA sublocus were strong and those of the 4 sublocus weak. In addition to different strengths of HL-A histocompatibility antigens, other factors such as variable host response to an antigen and the possible existence of other histocompatibility loci may have affected allograft survival.

Acknowledgments

We are indebted to Professor J. N. Berretoni, of Case Western Reserve University, for statistical advice; to Dr. Satoru Nakamoto and Dr. Kathryn L. Popowniak, of the Department of Hemodialysis, Cleveland Clinic, for their cooperation in this study; and to Mr. Daniel Grecek, of our Division, for technical assistance.

References

1. Singal, D. P.; Mickey, M. R., and Terasaki, P. I.: Serotyping for homotransplantation. XXIII. Analysis of kidney transplants from parental versus sibling donors. *Transplantation* 7: 246-258, 1969.
2. Payne, R.; Perkins, H. A., and Najarian, J. S.: Compatibility for Seven Leukocyte Antigens in Renal Homografts: Utilization of a Micro-Agglutination Test With Few Sera, p. 237-245, in Curtoni, E. S.; Mattiuz, P. L., and Tosi, R. M. (editors): *Histocompatibility Testing 1967*. Baltimore: The Williams & Wilkins Co., 458 p.
3. Rapaport, F. T., and others: Serologic factors in human transplantation. *Ann. Surg.* 166: 596-608, 1967.

* *This patient died recently. Histologically the allograft showed thickening of glomerular basement membranes, intimal hyperplasia of arterioles, and interstitial fibrosis.*

4. Mittal, K. K., and others: Serotyping for homotransplantation. XVIII. Refinement of microdroplet lymphocyte cytotoxicity test. *Transplantation* **6**: 913-927, 1968.
5. Singal, D. P., and others: Serotyping for homotransplantation. XVII. Preliminary studies of HL-A subunits and alleles. *Transplantation* **6**: 904-912, 1968.
6. van Rood, J. J., and others: Histocompatibility Testing for Kidney Transplantation, p. 106-107, in *Abstracts, General Sessions—Symposia IVth International Congress of Nephrology*, Stockholm, Sweden, June 22-27, 1969.
7. Terasaki, P. I., and others: Serotyping for homotransplantation. XXI. The role of the Mac (LA 2) antigen in kidney transplantation. *Transplantation*: In press.
8. Dausset, J., and others: The HL-A system subloci and their importance in transplantation. *Transplant. Proc.* **1**: (pt. 2) 331-338, 1969.
9. Miller, J. F. A. P.: The Thymus in Relation to the Development of Immunological Capacity, p. 153-180, in *Wolstenholme, G. F. W., and Porter, R. (editors): The Thymus: Experimental and Clinical Studies*. Boston: Little, Brown and Co., 538 p.
10. Terasaki, P. I.; Vredevoe, D. L., and Mickey, M. R.: Serotyping for homotransplantation. X. Survival of 196 grafted kidneys subsequent to typing. *Transplantation* **5**: (pt. 2) 1057-1070, 1967.
11. van Rood, J. J.: Tissue typing and organ transplantation. *Lancet* **I**: 1142-1146, 1969.