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Hajime Hayashi

Jay B. Hunter

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Hajime Hayashi, PhD* and Jay B. Hunter, BS*

The effect of HLA matching and other aspects of histocompatibility on renal graft survival were analyzed in 144 patients who received renal transplants at Henry Ford Hospital during the past ten years. Living related transplants with one haplotype match or better demonstrated a higher graft survival rate (at least 88%) at one year than the better matched cadaveric transplant. In cadaveric transplants, the group with fewer than two mismatched antigens appeared to have a higher graft survival rate than those with more than two. The patient population was not large enough to determine the effect of preformed antibodies, ABO grouping, or other recipient factors on graft survival.

MORE than ten years ago, human leukocyte (HLA) typing was introduced for the prospective matching of donor and recipient in organ transplantation. Yet the significance of histocompatibility testing is still controversial, even though many HLA antigens (tissue antigens) have been characterized.[1-7]

HLA antigens are glycoproteins expressed on the surface of all nucleated cells of human tissue. These determinants are coded by the major histocompatibility complex (MHC) located on chromosome 6 at four loci.[8] The inheritance of HLA antigens is exemplified in Figure 1. In obedience to Mendelian law, an individual can receive only two codominantly expressed antigens for each locus, one from each parental haplotype. The HLA-A, -B, -C, -D antigens comprise a highly polymorphic system with over 60 well-defined specificities. Table I lists the HLA antigens and workshop (W) antigens presently recognized by the WHO Nomenclature Committee.[9,10]

In the laboratory, antibody dependent procedures[11] identify serological determinants (SD) or antigens in HLA-A, -B, -C loci. Mixed lymphocyte culture (MLC) distinguishes lymphocyte determinants (LD) or antigens in HLA-D locus.[12] The mechanisms of recognition in LD are cellular while those in SD are humoral. Because the antigens in HLA-A and -B loci are more clearly characterized, most transplant centers report the data of HLA matching only for these antigens.

Since 1968, histocompatibility testing has been used in 149 renal transplants performed at Henry Ford Hospital. This is a preliminary report of histocompatibility testing and graft survival involving these patients.

Materials and Methods

Patients

The total number of renal transplants (149) at Henry Ford Hospital from 1968 to 1977 is summarized in Table II. Of these, 126 were cadaveric and 23 were living related. Five

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*Department of Pathology, Henry Ford Hospital

Address reprint requests to Dr. Hayashi, Henry Ford Hospital, 2799 W Grand Blvd, Detroit, MI 48202

	Father		Mother	
a	A1, B5, CW1, DW5	c	A28, B12, CW3, DW7	
b	A2, B7, CW2, DW6	d	A29, B13, CW4, DW8	

Offspring

1.	a	A1, B5, CW1, DW5
	c	A28, B12, CW3, DW7
2.*	a	A1, B5, CW1, DW5
	d	A29, B13, CW4, DW8
3.	b	A2, B7, CW2, DW6
	c	A28, B12, CW3, DW7
4.	b	A2, B7, CW2, DW6
	d	A29, B13, CW4, DW8
5.*	a	A1, B5, CW1, DW5
	d	A29, B13, CW4, DW8

* Identical

Fig. 1

were not included in this study because only crossmatch (without HLA typing) was performed.

HLA typing and crossmatch

These procedures were carried out by a standard micro-lymphocytotoxicity method modified from Terasaki.[13] Briefly, lymphocytes were separated from heparinized blood by either nylon column or Ficol-Hypaque procedures (specific gravity: 1.076-1.077) and were adjusted to 1.5-2.0 million cells/ml.

For HLA typing, two or three micro-trays with a panel of known antisera were used. A lymphocyte suspension of 0.001 ml was added to each well of typing trays containing 0.001 ml of antiserum and incubated for 30 minutes at room temperature, then incubated for an additional 60 minutes with 0.05 ml of rabbit complement at room temperature. At the end of incubation, 0.005 ml of 5% aqueous eosin was added, followed by 0.005 ml of formalin. The lymphocyte reaction with antisera was read on an inverted phase microscope.

For crossmatching, the most recent specimen and most reactive sera from preformed antibody screening were included in the following dilutions made up in duplicate:

1:1.3	0.003 ml of undiluted serum
1:2	0.001 ml of undiluted serum
1:4	0.001 ml of 1:2 diluted serum

The incubation time of crossmatching was extended to 45 minutes after the addition of lymphocytes to the patient sera

TABLE I

NOMENCLATURE FOR FACTORS OF THE HLA SYSTEMS—1977

Locus A	Locus B	Locus C	Locus D	Locus DR
A1	B5	CW1	DW1	DRW1
A2	B7	CW2	DW2	DRW2
A3	B8	CW3	DW3	DRW3
A9	B12	CW4	DW4	DRW4
A10	B13	CW5	DW5	DRW5
A11	B14	CW6	DW6	DRW6
A25	B15		DW7	DRW7
A26	B17		DW8	DRW8
A28	B18		DW9	
A29	B27		DW10	
*AW19	B37		DW11	
AW23	B40			
AW24	BW16			
AW30	BW21			
AW31	BW22			
AW32	BW35			
AW34	BW38			
AW36	BW39			
AW43	BW41			
	BW42			
	BW44			
	BW45			
	BW46			
	BW47			
	BW48			
	BW49			
	BW50			
	BW51			
	BW52			
	BW53			
	BW54			
	BW4			
	BW6			

* W (workshop): Antigens in the process of characterization but not yet accepted by the WHO committee

TABLE II

RENAL TRANSPLANTS AT HENRY FORD HOSPITAL

	Cadaveric			Living Related			
Year	1st	2nd	3rd	1st	2nd	3rd	Total
1968	1			3			4
1969	4			0			4
1970	5	1		2			8
1971	4	2		0			6
1972	11			0			11
1973	6	1		2			9
1974	18	1		1			20
1975	25	2		4			31
1976	21	2		2			25
1977	14	7	1	6	1	2	31
Total	109	16	1	20	1	2	149

and 90 minutes after the addition of rabbit complement. The crossmatch between recipient's sera and donor's lymphocytes was negative in all cases. For the purpose of this analysis, the mismatched antigens in HLA-A and -B loci were grouped and compared accordingly.

Antibody screenings

These screenings were performed against a panel of 20 to 40 characterized cells selected to cover the majority of known HLA antigens at the time of screening. A specimen with 10% or greater presensitization against 20 to 40 cells was considered positive.

Results

HLA matching and renal survival rate of cadaveric transplants

Table III shows the renal graft survival rate at 6 and 12 months of 102 patients with a primary cadaveric transplant. The overall survival rate was 45% at 6 months and 38% at 12 months. The patient group with fewer mismatched antigens (two or less) demonstrated a higher survival rate, except those with one mismatched antigen at 12 months. Although these numbers are not large, it appears that the more closely HLA matched groups demonstrated greater graft survival rates.

TABLE III

HLA MATCHING AND RENAL GRAFT SURVIVAL
(First Cadaveric Transplant)

No. of Mismatched HLA Antigens	Graft Survival 6 Months			12 Months		
		%			%	
0	1/1	(100)		1/1	(100)	
1	5/10	(50)	20/34 (59%)	3/9	(33)	16/32 (50%)
2	14/23	(61)		12/22	(55)	
3	18/49	(37)	26/68 (38%)	17/49	(35)	22/67 (33%)
4	8/19	(42)		5/18	(28)	
	46/102 (45%)			38/99*	(38%)	

* Three of the patients had good kidney function but had not reached the 12-month follow-up.

Table IV summarizes the HLA matching and graft survival of 20 patients receiving a second and third cadaveric transplant. In this small group there is no correlation between HLA matching and renal graft survival rates. Even those patients with three HLA mismatched antigens showed a greater than 60% graft survival rate at 6 and 12 months.

TABLE IV

HLA MATCHING AND RENAL GRAFT SURVIVAL
(Second and Third Cadaveric Transplants)

No. of Mismatched HLA Antigens	Graft Survival 6 Months		12 Months	
		%		%
0	0/2	(0)	—	
1	3/4	(75)	2/3	(67)
2	1/3	(33)	1/3	(33)
3	4/6	(67)	3/5	(60)
4	2/5	(40)	0/5	(0)
	10/20 (50%)		6/16* (38%)	

* Four patients had good kidney function but had not reached the 12-month follow-up.

HLA matching and renal graft survival rate of living related transplants*

Table V summarizes the results of a group made up of sibling-to-sibling or parent-to-child transplants with at least one matching haplotype. Most patients in this group had no mismatched antigens. Patients with both first and subsequent transplants from living related donors demonstrated a high graft survival rate (more than 88%).

TABLE V

HLA MATCHING AND RENAL GRAFT SURVIVAL

	No. of Mismatched HLA Antigens	6 Months		12 Months	
			%		%
Living related 1st transplant	0	13/14	(93)	11/12	(92)
	1	4/4	(100)	4/4	(100)
	2	0/1	(0)	0/1	(0)
		17/19	(89.5)	15/17*	(88)
Living related 2nd and 3rd transplant	0	2/2	(100)	2/2	(100)
	1	1/1	(100)	1/1	(100)
		3/3	(100)	3/3	(100)

* Two patients had good kidney function but had not reached the 12-month follow-up.

HLA matching in ABO group on renal graft survival rate

Eighty-nine pairs (donor and recipient) were available for ABO group analysis at 12 months (Table VI). There is no significant difference in graft survival of O donor-to-O recipient and A donor-to-A recipient. In both groups, the value of HLA matching is evident. For the remaining 13 patients with other ABO groups, the number is not sufficient to draw any conclusion.

* In 1976, the MLC test was added to related donor screening but data are insufficient to include in this study.

TABLE VI

HLA MATCHING ABO BLOOD GROUP AND RENAL GRAFT SURVIVAL AT 12 MONTHS

No. of Mismatched HLA Antigens	Donor to Recipient 0-0	A-A
	%	%
0	—	—
1	2/5 (40)	0/2 (0)
2	7/13 (54)	3/6 (50)
3	6/19 (32)	6/17 (35)
4	1/6 (17)	1/8 (13)
	16/43 (37)	10/33 (30)

O-A 1 patient
B-B 10 patients
B-AB 2 patients

Preformed antibodies and renal graft survival rate

In 1974, preformed antibody screening was established in our laboratory. Seventy-eight patients with a first transplant were available for this study (Table VII). Among these, 13 demonstrated HLA cytotoxic antibodies before transplantation. Seven of the 13 showed a good graft survival rate (54%) in spite of the presence of preformed antibodies.

TABLE VII

PREFORMED CYTOTOXIC ANTIBODY AND RENAL GRAFT SURVIVAL AT 12 MONTHS

No. of Mismatched HLA Antigens	Graft Survival Rate: Antibody Positive*	Antibody Negative
	%	%
0	—	1/1 (100)
1	1/1 (100)	1/6 (17)
2	—	10/15 (67)
3	4/9 (44)	11/29 (38)
4	2/3 (67)	3/14 (21)
	7/13 (54)	26/65 (40)

* 10% or greater presensitization against 20 to 40 cells

Antibody screening of dialysis and transplant patients

At Henry Ford Hospital, all patients on dialysis (potential transplant recipients) or those already transplanted are screened monthly for cytotoxic antibodies to HLA antigens. As shown in Table VIII, 17 of 108 dialysis patients (16%) demonstrated preformed antibodies. On the other hand, 16 of 57 (28%) transplanted patients with functional kidneys and 11 of 17 (63%) who underwent nephrectomy of the transplanted kidney showed cytotoxic antibodies.

TABLE VIII

CYTOTOXIC ANTIBODY SCREENING

Patient Category	Number of Patients Antibody: Positive/Tested
	%
Dialysis	17/108 (16)
Functional transplanted kidney	16/57 (28)
Nephrectomy of transplanted kidney	11/17 (65)

Discussion

The Renal Transplant Registry[14] and others [15-17] report that living related transplants have a much higher graft survival rate than cadaveric transplants; also, that a good HLA match is correlated with good graft survival in the living related transplant. Our results support this view. However, HLA identical sibling transplants are not always successful. Opelz and Terasaki[15] reported that in their analysis of 3,171 related transplants HLA identical siblings demonstrated 85% graft survival at one year regardless of whether two, three, or four HLA antigens were identified. They estimated a 15% non-HLA factor, which is the difference between the graft survival rate in transplants of these HLA identical siblings and identical twins. Cheigh et al[16] also pointed out that genetic determinants other than HLA play an important role in the fate of grafts.

The questions arise whether to use a mismatched related kidney or a well matched cadaveric kidney; and if a mismatched related kidney is used, what antigen match would be acceptable. Our study of living related transplants does not answer these questions. The graft survival rate in related transplants (1st and 2nd) was extremely high because there was more than one haplotype match in most cases. Simmons et al[17] reported that the graft survival rate of mismatched related transplants, especially those with more than one haplotype mismatch, was no better than the well matched cadeveric transplants. More recently, Cerilli et al[18] and Cochrum et al[19] emphasized the importance of the MLC test in the living related transplant. Based on the significantly low graft survival, they warned against the use of a related donor with a high MLC response, regardless of the HLA match. Therefore, it is logical to use a living related kidney only when one or more HLA haplotypes match and the MLC response is low.

The significance of HLA matching in cadaveric kidney transplants has been emphasized again in the past three to four years. In 1974, Dausset et al[3] reported for the first time that the correlation between matched HLA antigens and graft survival was found to be significant in a series of 918 cadaveric kidney grafts performed within the France and London Transplant Group Network. The survival rate was 34% at two years when there was only one or no matched

antigen; whereas the survival rate was over 70% when the donor and recipient were serologically indistinguishable at the two HLA loci. Similarly favorable results were reported by various centers in Europe[4,5] and the United States.[6,7] Although the number of patients in our study was small, the results were better with matching HLA antigens on first cadaveric transplants. Fewer than two HLA mismatched groups demonstrated better survival rates at the one year follow-up.

The greatest overall success of the cadaveric transplant depends on the use of a well-matched kidney, although it is not always possible to achieve this goal. HLA matched pairs are rarely encountered among unrelated donor and recipient. Moreover, due to the shortage of cadaveric kidneys, all available kidneys are usually transplanted, regardless of HLA matching. Matching can be improved, however, by increasing the size of the recipient pool. In Michigan, an organ-sharing program[20,21] established through the Transplantation Society of Michigan has been active since March 1975. Sera for crossmatching are collected from all potential recipients throughout the entire state on a monthly basis. Crossmatch trays are sent to each of the four participating histocompatibility centers. When a cadaveric donor becomes available, the on-call laboratory performs HLA typing and crossmatching with recipients' sera to obtain the best match.

Our data as well as that of others[22] indicate there is no direct influence of preformed antibodies to HLA antigens on graft survival if the HLA crossmatch was negative at the time of transplant. These conflict with the report of the Renal Transplant Registry.[14] However, it is essential to screen for cytotoxic antibody on all recipients, including dialysis patients and patients who suffered the loss of a transplanted kidney. The latter must be closely followed because two thirds of these patients (11 of 17) demonstrated antibodies after nephrectomy of the transplanted kidney. The screening should be done once a month and more frequently after the recipient receives blood transfusions. To avoid secondary immune response, the potential recipient who has positive antibodies at any time before transplantation should not receive a kidney from a donor with corresponding antigens.

Other factors which affect graft survival, such as blood transfusion, age, sex, and the ABO group, have been reported on by many investigators.[11,22,24-26,28,29] Our data do not indicate whether age, sex, and the ABO group have any effect on graft survival. The beneficial effect of blood transfusions has been emphasized recently.[24-29]

Recently, an additional test procedure to identify the antigens on B lymphocytes was introduced.[11,30-32] B lymphocytes possess a separate series of polymorphic antigens (HLA-DR) that appear to be specificities of HLA-D locus. Antibodies to B lymphocyte antigens are believed to play an important role in graft survival. Hyperacute rejection did not occur in patients in whom renal transplants were performed against a positive crossmatch to B lymphocytes but negative to T lymphocytes.[33,34] More recently, these antibodies have been classified into two categories based on test temperatures: 1) autoantibodies, or cold cytotoxins, which may enhance graft survival; and 2) alloantibodies, or warm cytotoxins, which were involved in graft rejection.[33,34] Therefore, it becomes necessary to perform a crossmatch against B lymphocyte populations at 15°C and 37°C. According to Persigi et al,[35] the matching of antigens in HLA-DR locus also promises some improvement in renal graft survival.

The histocompatibility testing laboratory can contribute to many aspects of renal transplant.

Donor selection

1 Search for related donor among family members with the following considerations: a) compatible ABO group; b) at least one HLA haplotype match; c) crossmatch negative; d) MLC negative or low reactivity; e) at least two MLC tests as well as family studies, including parents.

2. If a suitable donor is not available among family members, then proceed with the well-matched cadaveric transplant.

Care of potential recipient

1. HLA typing on the recipient is required every six months, especially when all antigens are not identified. It is not only helpful in matching recipient and donor but also aids in identifying undesirable antigens in the donor. If a second or third donor has the same mismatched antigens as the previous donor, the recipient is more likely to develop antibodies to these antigens. This likelihood can be avoided by proper HLA typing of the recipient and donor.

2. HLA antibody screening on a monthly basis is required for all transplant candidates. If a recipient demonstrates antibodies to HLA, the strength and specificity of antibodies should be characterized. This screening procedure helps to identify an undesirable donor who has antigens corresponding to the antibodies of the recipient.

Typing and crossmatching B lymphocytes

Procedures have been under investigation and are available on a limited basis.

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