

Comparison of the HL-A Phenotypes of Lymphocytes and Kidney Cells Determined by the Fluorochromasia Cytotoxicity Assay

KATHRYN S. DOUGLAS, HERBERT A. PERKINS, KENT COCHRUM, and SAMUEL L. KOUNTZ

From the Irwin Memorial Blood Bank of the San Francisco Medical Society, San Francisco, California 94118, and the Section of Hematology and Immunology, Department of Medicine and the Department of Surgery, University of California School of Medicine, San Francisco, California 94122

ABSTRACT Correlation of leukocyte typing with homograft survival suggests that HL-A typing of white blood cells reflects the histocompatibility factors of the kidney, yet some apparently well-matched kidneys are rejected. The latter results may, in part, reflect inadequacies of typing techniques, incomplete expression of HL-A factors on white blood cells as compared with the cells of the rejected organ, or isoantigens not shared with leukocytes.

In this study the kidney cells and lymphocytes (from blood or nodes) of 14 individuals were typed for HL-A factors 1, 2, 3, 5, 7, 8, 9, and 12, and factors 4a and 4b by fluorochromasia cytotoxicity. Biopsied kidney cells were prepared with 0.25% trypsin and typed fresh, after varying periods in monolayer culture or after storage in liquid nitrogen, in all cases resulting in cells which were pleomorphic but uniform in reactivity.

Reproducibility of lymphocyte typing was 99%, and of kidney cell typing, 93%. The 4a factor was detected on the lymphocytes but not the kidney cells of four individuals. HL-A7 and HL-A8, in contrast, were detected on kidney cells and not lymphocytes of four and three individuals, respectively. Results were consistent within the groups of individual sera used to detect each factor. The HL-A factors detected on both kidney cells and lymphocytes never resulted in more than two alleles at each genetic sublocus.

Several examples of post-rejection sera have reacted with donor kidney cells but not with lymphocytes. Kid-

ney cells may thus be useful in compatibility tests to aid in selection of donors for a retransplant.

The ability to store donor kidneys by perfusion provides time to employ kidney cells for typing and in compatibility tests, and the use of a standard cytotoxic assay makes their routine use practical. Typing kidney cells as well as lymphocytes thus offers an approach to more complete and accurate HL-A phenotyping.

INTRODUCTION

There is little doubt as to the importance of HL-A matching of leukocytes and the success of organ transplantation, as shown by this (2), and other laboratories (3-7). Some well-matched recipients, however, have rejected kidneys, whereas other poorly-matched recipients have experienced no or only minor rejections. With the availability of kidney cells from pretransplant biopsies and the ability to maintain these cells in monolayer culture, the phenotyping of kidney cells in a routine cytotoxicity test seemed feasible as a possible means of improving donor-recipient tissue matching.

Detection of HL-A antigens in short and long term cell cultures derived from various human tissues and using several different techniques has been reported by a number of investigators (8-11). Thus far, the techniques used to phenotype kidney cells have been lengthy and complex. Most of the comparative studies of lymphocytes and kidney cells have involved the use of different techniques, varying in sensitivity, to test each cell type (12-15). The present study was undertaken in an attempt to determine whether the HL-A phenotype of kidney cells was, in fact, identical with that of the lymphocytes of the same individual, and ultimately, using post-

A preliminary report on this work was presented at the Fourth International Histocompatibility Workshop, Los Angeles, California, 29 January 1970 (1).

Received for publication 14 April 1970 and in revised form 30 July 1970.

rejection antisera, to investigate the presence and role of kidney specific antigens. The HL-A phenotypes, determined by the fluorochromasia cytotoxicity assay (16), of lymphocytes and kidney cells from 14 individuals are reported.

METHODS

Antisera

The antisera used in this study were obtained from multiparous women and volunteer donors (immunized with human skin grafts and/or leukocytes). A minimum of 44 antisera, containing the specificities for antigens HL-A1, HL-A2, HL-A3, HL-A5, HL-A7, HL-A8, HL-A9, HL-A12, 4a, and 4b, were used. From three to eight sera of known specificity were tested for each antigen. Characterization of the cytotoxic specificities of some of these antisera has been reported previously (17-19). Phenotypes were assigned taking into account the results reported at the Fourth International Histocompatibility Workshop (Los Angeles, Calif., 1970). 12 of the antisera were produced in this laboratory. Most of the others were generously supplied by Dr. Rose Payne, and the remainder were supplied by the Serum Bank of the National Institute of Allergy and Infectious Diseases, Transplantation Immunology Branch.

Cells

Lymphocytes and kidney cells were obtained from 14 individuals: 12 kidney donors (three living, nine cadaver) and two prospective kidney recipients. Lymphocytes were obtained from mesenteric lymph nodes (removed at the time of transplant and/or nephrectomy) of the recipients and the cadaver donors and from the peripheral blood of the living donors. Kidney cells were prepared from a wedge biopsy, through the renal cortex, taken at the time of transplant or at subsequent removal of a rejected kidney. Biopsies of the recipients' own kidneys were taken at nephrectomy.

Cell preparation

Lymphocytes. Peripheral blood lymphocytes were separated from defibrinated blood and purified on nylon wool columns, as described by Bodmer, Tripp, and Bodmer (16). Lymph nodes were excised and immediately placed in cold McCoy's medium. The nodes were refrigerated until lymphocytes could be prepared (usually within 2 hr but occasionally as long as 12 hr). The nodes were dissected free of the surrounding connective tissue and fat, were washed with Hanks' balanced salt solution and were placed on a fine mesh stainless steel screen and cut into small pieces. A blunt-ended instrument was used to free the lymphocytes from surrounding tissue; occasional rinsing with a few drops of Hanks' solution facilitated drainage of the cells through the screen. The resulting suspension was counted (counts ranged from 20 to $60 \times 10^6/\text{mm}^3$ in approximately 5 ml Hanks' solution) and the appropriate dilution of lymphocytes was made (see below). The cells were then treated in the same manner as lymphocytes prepared from the peripheral blood.

Kidney cells. Kidney cells were prepared by gentle teasing of the material obtained at biopsy and by incubation with 0.25% trypsin in Hanks for 30 min at 37°C. The cells were then washed three times in Hanks, and approximately 0.5×10^6 cells were placed in Spinner salt solution-Eagle 1X (Grand Island Biological Company, Berkeley, Calif.) con-

taining 20% fetal calf serum (FCS) in a siliconized tube. These cells were incubated for 1 hr at 37°C, treated with fluorescein diacetate (FDA) (see below), and tested in the cytotoxicity assay. The remainder of the kidney cells were suspended in MEM containing 20% FCS and maintained in culture as a monolayer. The cultures were fed every 3-5 days and subcultured when necessary. The cultured cells were prepared for cytotoxicity by treatment of the monolayer with 0.25% trypsin and 0.02% versene in Hanks for 10 min. The cells were washed and treated in the same manner as those prepared from a fresh biopsy. (In the initial experiments, the cultured cells were treated only with trypsin [0.25%] in Hanks for 30 min, and were washed and incubated overnight. However, these cells occasionally resulted in 95-100% lysis with normal serum in the cytotoxicity assay.) The method, utilizing a trypsin-versene mixture with short treatment and incubation periods, produced cells which were stable in the test system.

Fluorochromasia cytotoxicity test

The fluorochromasia cytotoxicity assay was employed in the same manner for the testing of both lymphocytes and kidney cells. The method of Bodmer et al. (16) was used with several modifications as described.

Preparation of fluorescent cells. A stock solution of FDA in acetone (5 mg/ml) was diluted 1:50 in Hanks and further diluted 1:10 in the cell suspension. The cells were incubated with FDA at room temperature for 30 min, washed in Hanks, and centrifuged. The supernatant was discarded, and the resulting cell pellet was resuspended in Hanks containing 3 mg/100 ml of bovine serum albumin (BSA) to a concentration of approximately $5 \times 10^6/\text{mm}^3$.

Assay. A 2 wk supply of phenotyping plates was prepared at one time and kept frozen at -60°C. Plates for testing were thawed for 30 min at room temperature. 1 μl of FDA treated cells ($5000 \text{ cells}/\mu\text{l}$) was added to each serum droplet (2 μl). The plates were manually rotated for a few seconds, covered with an opaque cover, and incubated at room temperature for $\frac{1}{2}$ hr. 4 μl of rabbit complement (Hyland Laboratories, Los Angeles, Calif.) was then added to each droplet; the plates were again rotated, covered, and incubated at room temperature for 2 hr. In addition to the nonantibody containing serum control, with which all other sera were compared, droplets of FDA treated cells plus complement and FDA treated cells alone were dispensed. As the kidney cells were more susceptible than lymphocytes to nonspecific cytotoxic effects of the rabbit complement, the cells were tested, in initial experiments, with several lots of complement. One lot was found to be less damaging to the cells than others tested, and it was used, undiluted, with both kidney cells and lymphocytes for most of the study. If other lots were used, a dilution of 1:2 produced satisfactory results. Freshly prepared rabbit serum which was absorbed with human kidney cells was not significantly better than the commercially prepared complement. The test droplets were read and scored from 1 to 4 (16). The readings did not change when the plates were stored in the refrigerator for several hours following the 2 hr incubation at room temperature; this, however, was not a routine procedure.

¹Lymphocytes which were to be frozen, as described (16), were resuspended to a concentration of $20 \times 10^6/\text{mm}^3$ in Hanks-BSA containing 10% dimethyl sulfoxide (DMSO) and were thawed and diluted to the appropriate concentration in Hanks-BSA immediately before testing.

TABLE I
Reproducibility of Phenotyping

Antigen	Lymphocytes, % reproducibility*	Kidney cells, % reproducibility†
HL-A1	98	100
HL-A2	100	91
HL-A3	100	86
HL-A9	100	94
HL-A5	100	97
HL-A7	100	86
HL-A8	100	91
HL-A12	100	94
4a	98	97
4b	100	94

* Based on 43 phenotypings of 14 individuals.

† Based on 35 phenotypings of 14 individuals.

RESULTS

Lymphocytes and kidney cells from 14 individuals were tested for 10 HL-A antigens. The lymphocytes were obtained and tested at various times before kidney transplant. Kidney cells were tested on the day of biopsy and/or after monolayer culture (ranging from 6 to 43 days of culture and primary to five subcultures). The kidney cells demonstrated a lower percentage of reproducibility on repetitive typing (93%) as compared with the reproducibility of the lymphocytes (99%) from the same individuals (Table I). This somewhat lower percentage was not restricted to antisera of a given specificity but was found with almost all of the test sera in reactions with both cultured and noncultured kidney cells. No pattern of antigenic loss or gain with increasing number of subcultures or day in culture was observed in the cultured kidney cells. In general, however, culturing longer than 7-8 wk or subculturing more than nine times did not yield a sufficient number of cells for testing due to their decreased viability, a greater proportion of clumped cells, and an increased susceptibility to nonspecific lysis. Therefore, whenever possible, 2 to 6-wk cultures were harvested, and the cells were frozen in liquid nitrogen. These cells were then available for repeat testing, either immediately upon thawing or after reculturing.

Previous reports of HL-A antigens on renal cells have not included morphologic examination. In the present study, cell suspensions were examined by light and electron microscopy. The kidney cells were generally pleomorphic and varied in tinctorial properties with Wright's stain; however, they could be divided into three groups on the basis of size. Occasional multinucleate giant cells were present, and some mitotic figures were observed in the cultured cells. Preliminary electron microscopic stud-

ies² of the kidney cell cultures showed many of the cells to resemble early forms with prominent nucleoli and loose euchromatic nuclei. Collagen formation was not observed. To date it has not been possible to relate the varying sized cells to specific kinds of kidney cells. The inability to classify these cells, however, does not impair the ability to phenotype them with cytotoxic antisera. Cells of all sizes were labeled with the same intensity by the fluorochromatic reagent, and lost label (were killed) to an equal extent when exposed to appropriate typing sera; they retained label to an equal extent when exposed to typing sera with which they did not react.

A comparison of the lymphocyte and kidney cell phenotypes of the 14 individuals studied is shown in Table II. The over-all agreement of lymphocytes vs. kidney cells for 10 antigens was 86% based on two or more phenotypings of each cell, as indicated. Two of the 14 lymphocyte-kidney cell pairs agreed for 10/10 antigens, seven agreed for 9/10 antigens, four agreed for 8/10 antigens, and one agreed for 7/10 antigens. No significant differences were observed in comparing lymphocytes to rejected kidney or to nontransplanted kidney (Table III); the comparisons between lymphocytes and kidney cells are based on all of the kidney cells, irrespective of origin, except where indicated otherwise. As shown in Table III, agreement between lymphocytes and kidney cells was 100% for antigens HL-A1, HL-A2, HL-A5, and HL-A9. Antigens which showed the greatest discrepancies were 4a, HL-A7, and HL-A8. The 4a factor was present on the lymphocytes and was not detected on the kidney cells of four individuals. HL-A7 was present on the kidney cells and not on the lymphocytes of four individuals, and HL-A8, of three. These discrepancies were found in seven different individuals, including one who was both HL-A7 lymphocyte-negative, kidney cell-positive, and HL-A8 lymphocyte-positive, kidney cell-negative.

The discrepancies between lymphocytes and kidney cells did not appear to be biased in favor of individual antisera which may be particularly strong or weak but were consistently observed with all of the antisera of a given specificity. This is illustrated in Table IV which shows the frequency of reactivity, per cent reproducibility, per cent agreement, and the discrepancies for the individual antisera of the specificities which showed the greatest disagreement between lymphocytes and kidney cells, 4a, HL-A7, and HL-A8.

DISCUSSION

The present investigation is the first comparison, to our knowledge, of human lymphocyte and kidney cell phenotypes determined in an identical cytotoxicity test system.

² Performed by Dr. Steven D. Douglas, Mt. Sinai School of Medicine, New York.

This method, the fluorochromasia cytotoxicity assay (16) provides a rapid, efficient, and highly reproducible test which requires only a small number of cells and

2 μ l of test serum. Nonetheless, there are factors related to the assay of kidney cells which deserve further consideration before considering the discrepancies be-

TABLE II
Comparative Phenotypes of Lymphocytes and Kidney Cells

Patient	Status	Cell type	Cell source	Number of days in culture	Number of times sub-cultured	Number of times tested	HL-A								4a	4b
							1	2	3	9	5	7	8	12		
A. H.	PR	Ly	Bld and LN			3	-	-	+	-	+	-	-	-	-	+
		Ki	Bx	0	0	1	-	-	+	-	+	-	+	-	-	+
				12	1	1	-	-	+	-	+	-	+	-	-	+
J. McC., Jr.	PR	Ly	Bld and LN			3										
		Ki	Bx	6	1	1	+	+	-	-	-	-	+	-	-	+
				15	2	1	+	+	-	-	-	-	+	-	-	-
				19	2	1										
J. B.	CD	Ly	LN			3	-	+	+	-	-	-	-	-	-	-
		Ki	Bx	0	0	2	-	+	+	-	-	+	-	-	-	-
L. C.	LRD (son of W. C.)	Ly	Bld			4	-	+	-	-	-	-	-	+	+	+
		Ki	Bx	7	1	2	-	+	-	-	-	-	-	+	-	-
R. D.	CD	Ly	LN			3	-	+	+	-	-	+	-	-	+	+
		Ki	Bx	0	0	1	-	+	-	-	-	+	+	-	-	+
				15	1	1										
P. D.	CD	Ly	Bld and LN			4	-	+	+	-	-	-	-	-	-	+
		Ki	Bx	0	0	1	-	+	+	-	-	-	+	-	-	+
J. McC., Sr.	LRD			35	3	2	-	+	+	-	-	-	+	-	-	+
		Ly	Bld			2	+	+	-	-	-	+	+	-	-	+
		Ki	Bx	0	0	1	+	+	-	-	-	+	+	-	-	+
				9	1	1	+	+	-	-	-	+	+	-	-	+
D. McCo.	CD	Ly	Bld and LN			3	-	+	-	-	-	-	-	+	+	+
		Ki	Bx	0	0	1	-	+	-	-	-	-	-	+	+	+
				12	1	1	-	+	-	-	-	-	-	-	-	+
						4	-	-	+	-	-	-	-	+	+	-
R. O.	CD	Ly	LN			1 (fresh)	-	-	+	-	-	+	-	+	+	-
		Ki	Bx	14	2	1 (frozen)	-	-	+	-	-	+	-	+	+	-
				14	2		-	+	-	-	-	+	-	-	+	+
D. P.	CD	Ly	LN			2	-	+	-	-	-	+	-	-	+	+
		Ki	Bx	0	0	2	-	+	-	-	-	-	-	-	+	+
K. O.	CD	Ly	Bld and LN			3	-	-	-	+	-	-	+	-	-	+
		Ki	Bx	25	3	1	-	-	-	+	-	-	+	+	-	+
				35	5	1	-	-	-	+	-	-	+	+	-	+
		Ki	Rej	12	1	2	-	-	-	+	-	-	+	+	-	+
				27	2	1	-	-	-	+	-	-	+	+	-	+
W. C.	LRD (mother of L. C.)	Ly	Bld			3	-	+	-	-	-	-	-	+	+	+
		Ki	Rej	20	2	1	-	+	-	-	-	+	-	+	-	+
				27	4	1	-	+	-	-	-	+	-	+	-	+
T. L.	CD	Ly	Bld and LN			4	-	+	+	-	+	+	-	-	-	+
		Ki	Rej	27	2	1	-	+	+	-	+	+	-	-	-	+
K. S.	CD			43	3	1	-	+	+	-	+	+	-	-	-	+
		Ly	LN			3	+	+	-	-	-	-	+	-	-	+
		Ki	Rej	23	3	2	+	+	-	-	-	-	+	-	-	+
				39	5	1	+	+	-	-	-	+	-	-	-	+

PR, prospective recipient; CD, cadaver donor; LRD, living related donor.

Ly, lymphocyte; Ki, kidney cell.

Bld, peripheral blood; LN, lymph node; Bx, biopsy of nontransplanted kidney; Rej, biopsy of nephrectomized rejected kidney.

TABLE III
Comparison of HL-A Factors Between Lymphocytes
and Kidney Cells

Antigen	% Agreement, Ly vs. Ki		Discrepancies	
	Non-Tx*	Rej†	Ly+ Ki-	Ly- Ki+
HL-A1	100	100	0	0
HL-A2	100	100	0	0
HL-A3	90	100	1	0
HL-A9	100	100	0	0
HL-A5	100	100	0	0
HL-A7	70	50	1	4
HL-A8	70	75	1	3
HL-A12	90	75	1	1
4a	70	75	4	0
4b	80	100	2	0

Ly, lymphocyte; Ki, kidney cell; Non-Tx, nontransplanted kidney; Rej, rejected kidney.

* Based on 10 comparisons.

† Based on four comparisons.

tween lymphocyte and kidney cell phenotypes. These include the duration of tissue culture and the possible effects of trypsin and other agents necessary for the preparation of kidney cell suspensions.

Rogentine and Gerber have found lymphoid cell lines to be antigenically stable over a period of 1 yr (10) and more recently for as long as 3 yr (20) in a culture system which did not require the use of proteolytic enzymes for subculture. In the present study, both trypsinized, noncultured kidney cells and kidney cells prepared with a trypsin-versene mixture which were cultured as long as 43 days and subcultured as many as five times gave results in good agreement with the lymphocytes. The discrepancies observed were in the directions of both lymphocyte and kidney cell positivity and did not correlate with the length of culture or number of subcultures. This would appear to be evidence against a marked destructive or enhancing effect of trypsin on the HL-A antigens of cells prepared as described above and tested in the fluorochromatic assay. Additional evidence that short periods of incubation with 0.25% trypsin does not significantly alter the cell surface antigens was reported by dePanque, Williams, Siegel, and Alvarez (14). They found a less than 4% incidence of discrepancies in a comparative study of the reactions of 120 sera with trypsinized and nontrypsinized lymphocytes from five individuals. A 45 min microcytotoxicity test based on the use of enzyme-treated lymphocytes was recently reported by Mittal, Mickey, and Terasaki (21). They showed that treatment of lymphocytes with the proper concentration of ficin did not alter the typing for HL-A groups, while it did afford a three-fold reduction in the

incubation time required for the cytotoxic reaction to take place. Although the reactivity of a few sera was altered by the use of ficinized cells, none of the HL-A specificities was differentially affected.

The data presented here indicate that kidney cells can be successfully phenotyped by a routine cytotoxicity test and that, in general, the results agree well with lymphocyte phenotypes of the same individuals. However, a number of reproducible differences between the two cell types have been observed in several of the pairs tested. The 4a factor was not detected on the kidney cells of four individuals on whose lymphocytes it was present, whereas HL-A7 was found on the kidney cells of four individuals and HL-A8 on the kidney cells of three individuals on whose lymphocytes these antigens were not detected. Four of the 14 kidneys studied were from rejected renal allografts, which raises the possibility that antigens detected in the kidney cell preparation might be on invading host lymphocytes. This was fortunately eliminated from consideration by the fact that none of the antigens detected on kidney cells but not on lymphocytes of the organ donor were present on the lymphocytes of the graft recipient. Furthermore, the kidney cells of the two lymphocyte-kidney cell pairs which agreed for all 10 antigens were from rejected allografts. As all but one of the antisera of specificities 4a, HL-A7, and HL-A8 showed consistent reactivity with lymphocytes and kidney cells (Table IV), the possibility that the observed differences result from random chance is unlikely.

Phenotyping discrepancies similar to those presented here have previously been reported in studies of cultured cells. Rogentine and Gerber (10) reported particular difficulty in assigning the antigen 4a to lymphoid cells in long term tissue culture. In a preliminary study, Williams, Rolley, and Hume (13) observed the presence of antigen 7d (HL-A8) on the cultured kidney cells of six individuals as detected by mixed agglutination and did not detect it on the lymphocytes of these individuals by cytotoxicity. HL-A8 was the most frequently observed antigen discrepancy, although they reported similar differences for the other antigens. All eight antigens for which they tested were reported as lymphocyte-negative, kidney cell-positive in at least one individual, and only a partial discrepancy at HL-A1 was reported as lymphocyte-positive, kidney cell-negative. Metzgar, Zmijewski, Seigler, and Oleinick (12), using leukoagglutination for leukocytes and mixed agglutination for kidney (two individuals) and skin (three individuals) cell typing with 20 antisera, found five instances of leukocyte-positive, tissue cell-negative, and 10 instances of leukocyte-negative, tissue cell-positive. The above two reports differ from this one primarily in the detection of many more HL-A specificities on tissue cells as compared to lymphocytes (leukocytes) than we observed. In interpreting

TABLE IV
Reactions of Lymphocytes and Kidney Cells with Individual Antisera

Specificity	Frequency		% Reproducibility		% Agreement,* Ly vs. Ki	Discrepancies	
	% + Ly	% + Ki	Ly	Ki		Ly + Ki -	Ly - Ki +
HL-A7	29	50	100	86	64	1	4
Baker	21	50	98	82	71	0	4
McDonald	14	36	92	84	64	1	4
Walecka	36	100	100	100	50 (12)	0	6
Sutthoff	21	36	98	88	71	1	3
Cowen	36	43	95	85	93	0	1
Melnikoff	14	64	100	79	57	0	6
Dominquez	36	71	98	80	71	0	4
Cutten	27	21	98	84	86	1	1
						3	29
HL-A8	29	43	100	91	71	1	3
Willet	36	43	94	88	64	2	3
Chayra	29	52	100	77	57	1	5
D66-17910	33	33	100	81	63 (8)	1	2
						4	10
4a	43	14	98	97	71	4	0
Deely	71	0	100	88	33 (6)	4	0
Julian	43	21	98	93	79	3	0
Brown	43	21	89	91	64	4	1
Chamard	45	21	79	83	50 (10)	4	1
						15	2

Ly, lymphocyte; Ki, kidney cell.

* (), number of comparisons if fewer than 14.

their results, however, one must take into account the fact that comparisons were made of two cell types as tested by two different methods which varied in sensitivity. In contrast, dePlanque et al. (14) recently reported a comparative study of lymphocytes and kidney cells, both phenotyped by immune adherence, in which anti-HL-A7 sera and one anti-HL-A3 serum reacted with greater frequency and intensity to kidney cells and in which two 4b antisera accounted for eight of the less frequently observed discrepancies of lymphocyte-positive, kidney cell-negative. Their findings, with respect to HL-A7 and 4b, agree with those reported here. McKenzie and Morris (15), in a paper presented at the Fourth International Histocompatibility Workshop Conference (Los Angeles, Calif., 1970), reported HL-A7 lymphocyte-negative, kidney cell-positive in six of 17 individuals and HL-A8 lymphocyte-negative, kidney cell-positive in three of 17 individuals, as well as several other less frequently occurring discrepancies. Thus, frequent detection of HL-A7 and HL-A8 on kidney cells

but not on lymphocytes of the same individual has now been reported by three different laboratories, including this one.

One can rule out kidney specific antigens as an explanation for the discrepancies in typing results observed in the present study on the basis of the fact that the antisera used were produced through immunization of pregnancy, peripheral blood leukocytes, or skin grafts and would not be expected to contain antibodies specific for kidney tissue. A difference in reactivity observed in the same test system suggests that the discrepancies are cell-related rather than test-related. Berah, Hors, and Dausset (22) and Dausset (23) have shown different concentrations of antigen Mac (HL-A2) in various human organs, based on the amount of antibody which could be absorbed. Moreover, lymphocytes which have failed to react in a cytotoxicity test have, on occasion, demonstrated the capacity to absorb antibody from the same serum. Final proof that the discrepancies noted here between kidney cell and lymphocyte typing reflect

only a difference in the concentration or reactivity of HL-A factors will require absorption studies which have not yet been performed.

Further evidence that the discrepancies which we have observed between lymphocyte and kidney cell reactions are within the HL-A system is provided by the fact that all the antisera of a given HL-A specificity reacted similarly. Moreover, the total number of HL-A factors detected on lymphocytes and kidney cells together never exceeded the number dictated by genetic theory. Genetic control of HL-A factors has been clearly confirmed by the findings of the Fourth International Histocompatibility Workshop to reside in two closely linked subloci (or segregating series), each of which can result in the phenotypic expression of a maximum of two alleles. It should be noted, however, that the results of tests with 4a and 4b antisera frequently duplicate results of more specific antisera within the second segregating series because of the broad cross-reactivity of the 4a and 4b reagents. For this reason, results with anti-4a and 4b are not necessarily regarded as indicating additional alleles at this sublocus. In consideration of the discrepancies which are reported here, it is of note that if all of the antigens which showed discrepancies are included in determining the phenotype of a cell donor, in no case does this result in the finding of more than two alleles within the first segregating series (HL-A1, HL-A2, HL-A3, and HL-A9) or within the second (HL-A5, HL-A7, HL-A8, and HL-A12). As one would expect the genetic expression of both cell types to be the same within the HL-A system, our findings suggest that an antigen found on either lymphocytes or kidney cells may exist on both, and that the observed discrepancy is a result of failure to detect it in one of the cell types.

The discrepant results between kidney cell and lymphocyte reactions with HL-A typing sera have also been noted in compatibility tests employing serum of the prospective recipient and both lymphocytes and kidneys cells of the prospective donor. This may reflect differential reactivity of the HL-A factors of the two kinds of cells, as noted above. On the other hand, the occurrence of a reaction with donor kidney cells but not lymphocytes might have additional significance if the prospective recipient has already rejected one kidney. It is theoretically possible that the serum of such an individual could react with isoantigens on the kidney cells which are not shared with lymphocytes. The existence of such antibodies remains to be proven, but we have found several examples of post-transplant sera reacting with the kidney cells and not with the lymphocytes of the kidney donor, both by the techniques described here and by using a ^{51}Cr microcytotoxicity method (24).

The differences in detection of antigens on lymphocytes and kidney cells noted in this study may have practical

clinical significance in selecting donor-recipient pairs for organ transplantation. It appears to offer an approach to more complete and accurate HL-A typing and compatibility testing. Using the described techniques we have been able to phenotype both lymphocytes and kidney cells of prospective cadaver donors and to perform compatibility tests with both kinds of cells in little more time than it takes to test with lymphocytes alone (25). Temporary storage of the cadaver kidneys in a perfusion device (26) allows time for these procedures.

ACKNOWLEDGMENTS

We wish to acknowledge the advice and encouragement of Dr. Rose Payne and the excellent technical assistance of Mrs. Marianne Gumbert in the preparation of the kidney cells. We also wish to thank Mrs. Eleanor Howell for her helpful suggestions in the preparation of this manuscript.

This work was supported in part by contract PH 43-68-945 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, USPHS Research Grant AM 11290, and a grant from the Life Insurance Medical Research Fund.

REFERENCES

1. Perkins, H. A., K. S. Douglas, K. Cochrum, and S. Kountz. 1970. Direct typing of kidney cells by standard cytotoxic technics. *In* Histocompatibility Testing 1970. P. Terasaki, editor. Munksgaard, Copenhagen, Denmark. 583.
2. Payne, R., H. A. Perkins, and J. S. Najarian. 1967. Compatibility for seven leukocyte antigens in renal homografts: utilization of a micro-agglutination test with few sera. *In* Histocompatibility Testing 1967. E. S. Curtoni, P. L. Mattiuz, and R. M. Tosi, editors. Munksgaard, Copenhagen. 237.
3. Lee, H. M., D. M. Hume, D. L. Vredevoe, M. R. Mickey, and P. I. Terasaki. 1967. Serotyping for homotransplantation. IX. Evaluation of leukocyte antigen matching with the clinical course and rejection types. *Transplantation*. 5: 1040.
4. Dausset, J., and F. T. Rapaport. 1966. The role of blood group antigens in human histocompatibility. *Ann. N. Y. Acad. Sci.* 129: 408.
5. Rapaport, F. T., J. Dausset, J. Hamburger, D. M. Hume, K. Kano, G. M. Williams, and F. Milgrom. 1967. Serologic factors in human transplantation. *Ann. Surg.* 166: 596.
6. Starzl, T. E., C. G. Groth, L. Brettschneider, G. V. Smith, I. Penn, and N. Kashiwagi. 1969. Perspectives in organ transplantation. *Antibiot. Chemother.* 15: 349.
7. Stickel, D. L., D. B. Amos, C. M. Zmijewski, J. F. Glenn, and R. R. Robinson. 1967. Human renal transplantation with donor selection by leukocyte typing. *Transplantation*. 5: 1024.
8. Metzgar, R. S., J. F. Flanagan, and C. M. Zmijewski. 1965. Detection of tissue isoantigens on primary and serial heteroploid cell lines of human origin by the mixed agglutination technique. *J. Immunol.* 95: 494.
9. Milgrom, F., and K. Kano. 1966. Application of cell cultures for human tissue typing. *In* Histocompatibility Testing 1965. H. Balmer, F. J. Cleton, and J. G. Eernisse, editors. Munksgaard, Copenhagen, Denmark. 179.

10. Rogentine, G. N., Jr., and P. Gerber. 1969. HL-A antigens of human lymphoid cells in long-term tissue culture. *Transplantation*. 8: 28.
11. Rolley, R. T., G. M. Williams, and D. M. Hume. 1968. Distribution of leukocyte antigens in human tissues. *Surg. Forum*. 19: 241.
12. Metzgar, R. S., C. M. Zmijewski, H. F. Seigler, and S. R. Oleinick. 1968. A comparison of the leukocyte agglutination and mixed agglutination techniques for detecting human tissue isoantigens. *Transplantation*. 6: 84.
13. Williams, G. M., R. T. Rolley, and D. M. Hume. 1968. A comparison of lymphocyte and kidney cell typing in eleven patients. *Surg. Forum*. 19: 209.
14. DePlanque, B., G. M. Williams, A. Siegel, and C. Alvarez. 1969. Comparative typing of human kidney cells and lymphocytes by immune adherence. *Transplantation*. 8: 852.
15. McKenzie, I. F., and P. J. Morris. 1970. HL-A and ABO antigens of kidney cells in culture. In *Histocompatibility Testing 1970*. P. Terasaki, editor. Munksgaard, Copenhagen, Denmark. In press.
16. Bodmer, W., M. Tripp, and J. Bodmer. 1967. Application of a fluorochromatic cytotoxicity assay to human leukocyte typing. In *Histocompatibility Testing 1967*. E. S. Curtoni, P. L. Mattiuz, and R. M. Tosi, editors. Munksgaard, Copenhagen, Denmark. 341.
17. Payne, R., W. F. Bodmer, G. M. Troup, and R. L. Walford. 1967. Serologic activities and specificities of eleven human leukocyte antisera produced by planned immunization. *Transplantation*. 5: 597.
18. Payne, R., J. Bodmer, W. Bodmer, and E. Shanbrom. 1970. Production of defined human leukocyte typing sera. Proceedings of the International Congress on Blood Transfusion (Moscow, 1969). In press.
19. Shanbrom, E., E. Feingold, L. Shepherd, and R. L. Walford. 1968. Antisera for tissue-typing: the production of cytotoxic and agglutinating antibodies following intradermal leukocyte injections in man. *Blood*. 32: 402.
20. Rogentine, G. N., and P. Gerber. 1970. Qualitative and quantitative comparison of HL-A antigens on different lymphoid cell types from the same individuals. In *Histocompatibility Testing 1970*. P. Terasaki, editor. Munksgaard, Copenhagen, Denmark. 333.
21. Mittal, K. K., M. R. Mickey, and P. I. Terasaki. 1969. Serotyping for homotransplantation. XXXI. A 45-minute microcytotoxicity test. *Transplantation*. 8: 801.
22. Berah, M., J. Hors, and J. Dausset. 1968. Concentration of transplantation antigens in human organs. *Lancet*. 2: 106.
23. Dausset, J. 1969. Tissue typing and transplantation. *Vox Sang*. 16: 263.
24. Cochrum, K. C., and S. L. Kountz. 1969. Cytotoxic antibodies following human renal transplantation. *Surg. Forum*. 20: 302.
25. Kountz, S. L., K. C. Cockrum, H. A. Perkins, K. S. Douglas, and F. O. Belzer. 1970. Recipient selection by leukocyte and kidney cell phenotyping. *Surgery*. 68: 69.
26. Belzer, F. O., B. S. Ashby, P. F. Gulyassy, and M. Powell. 1968. Successful seventeen-hour preservation and transplantation of human-cadaver kidney. *N. Engl. J. Med*. 278: 608.