TRANSPLANTATION
Copyright © 1966 by The Williams & Wilkins Co.

Vol. 4, No. 6
Printed in U.S.A.

SEROTYPING FOR HOMOTRANSPLANTATION V. EVALUATION OF A MATCHING SCHEME[1]

P. I. TERASAKI, D. L. VREDEVOE, K. A. PORTER[2], M. R. MICKEY[3], T. L. MARCHIORO[4], T. D. FARIS[4], T. J. HERRMANN[4], AND T. E. STARZL[4]

Departments of Surgery and Preventive Medicine and Public Health, School of Medicine, University of California, Los Angeles, the Department of Pathology, St. Mary's Hospital, London, England, the Department of Surgery, School of Medicine, University of Colorado, and the Veterans Administration Hospital, Denver, Colorado

SUMMARY

An attempt was made to determine whether 36 long-term kidney homograft recipients and their donors were compatible for 7 major leukocyte groups. It was found that 21 of these recipients were surviving 2 to 3 years in spite of incompatibility for 1 or 2 major leukocyte antigens. Survival of mismatched grafts does not itself indicate that the antigens being measured are not transplantation antigens, for it was shown that the 15 recipients with no groups of mismatch were clinically superior to those with group incompatibilities. Moreover, histopathologic scores given to biopsy specimens taken 2 to 3 years after transplantation were significantly correlated with the number of group mismatches. Because the leukocyte groups were determined by cytotoxicity reactions of peripheral blood lymphocytes, the results may have been influenced considerably by chimerism in chronically dialyzed uremic patients or change in lymphocyte antigenicity or susceptibility to lysis upon prolonged immunosuppressive treatment. Although the possibility of these complications could not be ruled out in all instances, it was shown that 52 dialyzed uremic patients and 49 patients who had been treated with immunosuppression for over 1 year did not possess more or less antigens than a random population of normal individuals. It is concluded that: (1) the major leukocyte antigens are histocompatibility antigens, and (2) since survival can be attained at times despite mismatches for these groups, the antigens are of intermediate strength and kidney homograft rejection may occur if excessive numbers of antigens are incompatible or if particular combinations of antigens are mismatched.

INTRODUCTION

In the past year knowledge of tissue antigens represented on human leukocytes has accumulated rapidly, thus permitting for the first time a study of

[1] This investigation was supported in part by Research Grants AM 02375, AM 07513, AI 04444, AM 06283, AM 06344, HE 07735, AM 07772, AI 04152, FR 00051, FR 00069, AI 06091, AI 04606 and FR-3 from the National Institutes of Health, United States Public Health Service, and by grants from the University of California Cancer Research Coordinating Committee, and from the Medical Research Council of Great Britain.

[2] St. Mary's Hospital, London, England.

[3] School of Medicine, University of California, Los Angeles.

[4] School of Medicine, University of Colorado, and the Veterans Administration Hospital, Denver, Colorado.

the correlation of kidney transplant survival with compatibility of donor and recipient leukocyte antigens. If leukocyte antigens act as histocompatibility determinants, then these antigens would be expected to be well matched in human kidney transplant patients who have survived for long periods. Accordingly, as the test system, 36 patients who have survived 2 years or longer after renal transplantation were examined along with their respective donors. Compatibility for the 7 major antigenic groups described earlier (*20*) was tested by lymphocyte cytotoxicity tests. From the recent Histocompatibility Workshop (*3*) and studies resulting from the exchange of antisera (*23*) it appears that the 7 groups identified previously by cytotoxicity (*20*) are closely associated with the different antigenic groups of Amos (*1*), Dausset (*4*), Payne and Bodmer (*2*) Shulman (*14*), and van Rood (*22*). In spite of the initial seemingly insuperable complexities of leukocyte antigens, it is clear now that a relatively few serologically strong leukocyte antigens have been repeatedly detected by independent laboratories. Undoubtedly, further refinements will split off numerous subgroups, but it is of interest to examine the compatibility of long-term survivors in terms of the partially defined groups already known.

It will be shown here that contrary to expectations, many kidney transplant survivors were incompatible with their donors in 1 or sometimes 2 of the serologically strong leukocyte antigens. These serologically detected antigens are therefore probably not by themselves strong transplantation antigens in the sense that a mismatch would result in inevitable graft rejection in spite of current immunosuppressive therapy (*15*, *16*). However, they are likely to be at least intermediate strength histocompatibility determinants, for patients with no groups of incompatibilities were on the whole superior, both from the clinical standpoint and from biopsy findings, to those with 1 or more groups of incompatibilities.

MATERIALS AND METHODS

Subjects. Lymphocytes were isolated from the blood of 36 long-term kidney homograft survivors (1 to 2 years after transplantation) and their respective donors. Clinical information on these survivors from the University of Colorado and the Denver Veterans Administration Hospital can be obtained from previous publications (*17*, *18*) since the same identification numbers were used throughout. Thirty-one patients had received grafts from related donors and 5 from unrelated donors. An additional patient who has now survived 2½ years with a graft from a living unrelated donor (a patient of Drs. W. E. Goodwin, J. J. Kaufman, and D. C. Martin, University of California, Los Angeles) is included for analysis. All cytotoxicity tests were performed within 18 hours after venipuncture by methods described previously (*18*, *24*).

Antisera. The panel of allogeneic antisera employed for testing was obtained from multiparous women and volunteers immunized with cells. Many of the sera had been kindly given to us by Drs. D. B. Amos, R. Ceppellini, J. Dausset, P. Lalezari, R. Payne, J. J. van Rood, and R. L. Walford. Data on

TABLE

Summary of antigenic mismatches in 36 long-term kidney transplant survivors from Denver together with clinical rank and biopsy scores

LD No.	Donor relation	Duration of survival (days)[a]	Clinical rank[a]	Biopsy score[b]	Group mismatch determined by regression analysis	% sera mismatched
1	Mother	1248	I	10	0	1.4
2	Sister	1180	I	0	4	4.3
3	Frat. twin	1171	I	2	0	3.9
6	Brother	1104	I	0	0	4.4
12	Brother	1053	II	8	0	5.4
13	Mother	1027	II	9	1, 6	12.9
14	Brother	1025	I	5	0	7.3
15	Brother	1022	II	5	0	10.2
17	Mother	1011	I	4	1, 4	9.7
18	Sister	Died, 937	III	6	6	13.0
22	Mother	987	II	5	0	3.3
25	Brother	978	I	1	4	4.5
27	Unrelated	Removed, 953	IV	7	3	12.2
30	Unrelated	938	III	14	6	8.1
33	Mother	931	I	0	0	2.3
34	Mother	927	I	3	1	8.3
36	Unrelated	Died, 712	IV	13	6, 7	5.2
37	Mother	920	II	5	2	11.1
39	Mother	894	II	4	1	11.1
40	Mother	891	III	10	4, 6	9.7
41	Mother	884	III	8	2, 4	17.7
42	Father	880	I	4	0	6.9
44	Sister	870	II		0	5.6
45	Sister	867	III	10	2, 5	9.8
47	Brother	Died, 666	IV	9	6	10.6
48	Sister	836	III	4	4	6.6
49	Sister	783	I	2	0	3.0
50	Father	821	II	5	3	5.8
51	Aunt	805	II	7	6	12.5
52	Father	798	I	2	0	5.4
53	Uncle	793	I	3	0	21.3
54	Unrelated	791	IV	14	2, 5	18.7
55	Father	789	III	8	5	11.6
58	Sister	773	I	0	0	11.4
60	Cousin	769	II	8	1	8.8
63	Unrelated	759	II	10	0	6.2

[a] Survival and clinical rank are to 25 April 1966.

[b] As given in Methods section.

the strength, frequency and specificity of these antisera are given elsewhere (*20, 23*).

Matching of donors and recipients. Incompatibilities were expressed in the following ways (*5*)[5].

[5] Computing assistance was obtained from the University of California, Los Angeles, Health Sciences Computing Facility, sponsored by National Institutes of Health grant FR-3.

1. Raw mismatch with separate antisera: (a) *Percent sera mismatch.* Mismatches were considered when the donor cells reacted with a serum and the recipient cells failed to react. Matching with regard to any serum occurred whenever the donor cells did not react or whenever the recipient cells were killed by the serum. Thus it can be seen that a match may be tallied in some instances when a given serum was not tested with *both* the donor's and recipient's cells. The mismatched sera divided by the total mismatches and matches (as defined above) then resulted in the percent sera mismatch (Table 1). (b) *Percent total mismatched units.* The details and rationale involved in this method of expressing incompatibility are given in earlier publications (*24*). Briefly it approximates for any given serum the *extent* to which the donor's cells reacted in excess of the recipient's ce . The extent of cytotoxicity was expressed as "units" of dilution "tubes" at which complete killing took place. Fractions of a unit represented the cytotoxic index in the dilution at which only a portion of the cells were killed. Thus 1.5 units equals complete killing in the first dilution and half of the viable cells killed in the second 1:3 dilution. To obtain the units mismatch for a given serum, the units of reactivity of the recipient's cells were subtracted from the units of reactivity of the donor's lymphocytes. As explained earlier (*18, 24*), the summated total of units was corrected for the strength of the particular antiserum tested in common for each pair by expression as a percentage of the maximum mismatch score. A listing of the values of percent total units mismatched for each of the transplant pairs was given previously (*18*).

2. Group mismatches. Group mismatches were derived by matching of regression "types." These types for each cell were determined as described earlier (*20*) by analyzing the reactivities of each antiserum by regression analysis. Factor "loadings" for the 7 groups of antisera previously established by factor analysis were employed as the independent variable. This method of assignment of cells to the 7 recognized gro gs permits a correction for missing antisera in a given test and elimination of a few inconsistent reactions. Most importantly, reactions with antisera composed of several factors are considered to "belong" to certain groups depending on the best "fit" with reactions of other antisera.

Determination of the biopsy scores. Approximately 2 years after transplantation, a large wedge biopsy was obtained at open operation from each renal allograft. The material was serially sectioned and the presence or absence of 7 major histopathological features determined. These were: (1) fibrous thickening of the intima of the interlobular arteries; (2) hyalinization of the walls of the arterioles; (3) thickening of the glomerular capillary basement membranes; (4) generalized interstitial fibrosis; (5) superficial subcapsular interstitial fibrosis; (6) cellular infiltration; (7) tubular atrophy. If present, the lesions were arbitrarily graded in severity from 1 to 3. The grades were then added up giving a final score for each biopsy. In this way a renal transplant showing all 7 lesions in maximal degree would be scored as 21, while a graft displaying none of these changes would be scored as 0.

RESULTS

Raw mismatch scores. If mismatches in sera or units are considered without regard to groups, it was readily evident that most (28 out of 36) of the survivors had mismatches which were significantly greater than the variability expected in the reproducibility of the test (*24*). On the whole those recipients with few mismatched sera or units tended to be in the clinically superior categories (Table 1, and see (*18*)). Well matched patients may be expected to have few mismatches with any large panel of sera regardless of the purity of the antisera or replication of similar specificities. As the number of mismatches increases, the total percentage of sera which mismatch is influenced by the composition of the serum panel. Thus if many sera of one type are present in the panel, one incompatibility may be counted repeatedly. Although this measure of mismatch is obviously a crude one, it has the advantage over grouped mismatches in detecting incompatibilities in yet unrecognized groups.

The raw percent mismatch scores can be readily compared with those which occur in random populations. To determine whether the level of mismatches found in these patients was significantly less than would be expected from an unselected population, 81 randomly selected persons were matched with each other with the aid of computers to form 6480 random matches. The mean mismatch in the random population was 16.0% total mismatched units with a range of 0.3–40%. This background population of unrelated donors and recipients can then be compared to the mismatches of 6 surviving recipients who received kidneys from living unrelated donors. The mean mismatch of the survivors was 11.2 ± 4.2% total mismatch units[6]. The difference between the survivors with unrelated donors and the random unrelated population was statistically significant ($0.01 < P < 0.05$). Thus, although only a relatively few survivors with kidneys from living unrelated donors were available, their mismatches were significantly less than random matchings, and the highest mismatch was less than the mean for the random group.

Incompatibilities of recipients receiving kidneys from related donors can be compared to those which may occur among random related transplants. Broken down further, the mean mismatch between 14 surviving recipients with a sibling donor was 8.2 ± 4.5% total units compared to 11.0 ± 6.9 for the 114 random sibling matches tested. If the random matchings among siblings are averaged for each family to correct for excessive numbers of matchings among sibs of large families, a mean of 10.8 ± 3.5 was obtained. This mean value, compared with the mean for the transplanted population was not statistically different by Student's *t*-tests. Adjustment for skewness in the distribution by transformation into logarithmic or square root scales also did not show a significant difference. Fourteen transplants from parent to child were mismatched by a mean of 9.8 ± 4.0% total units whereas 25 random parent to child combinations were mismatched by 11.3 ± 6.0%. The mean for

[6] The indicated interval is the 95% confidence interval $\bar{x} = ts_{\bar{x}}$, in which $\bar{x}$ denotes the mean, $s_{\bar{x}}$ denotes the standard error of the mean and t is the percentage point of the Student t distribution. This notation is used throughout the test.

the transplanted group of parent to child was less than the random group as in the above sibling comparison, but the difference was again not statistically significant.

The lower mean mismatch of 8.2% among sibs versus 13% among parent to child matchings is in keeping with theoretical expectations (*11*, *15*, *16*) and the survival data of kidney transplantation (*10*).

Regression group mismatches. Two of the objections to merely totaling up all differences as done above are that: (1) if sera which tend to react alike are present in the panel, one difference is counted repeatedly, and (2) certain determinants may be more important histocompatibility antigens than others. Grouping of associated antisera by factor analysis (*20*) permitted correction for duplication of antisera. Since certain sera possessed 2 or more specificities, regression analysis was employed to determine whether any given cell belonged to each of the 7 recognized groups. Groups present on donor cells and not on recipient cells were considered to be the incompatibilities. Fifteen of the 36 survivors possessed no groups of mismatch, 14 had one mismatched group and 7 had two mismatched groups (Table 1). No survivor with 3 or more mismatched groups was found. Group 1 (LA 1, 7d, Dauset 8) was mismatched in 5 recipients, group 2 (Dausset 1, Shulman B1, LA 2, 8a and Amos II) in 4 recipients, group 3 (4a, Dausset 3) in 2, group 4 (6a ?) in 5, group 5 (6b, 4d, Dausset 9, Amos II) in 3, group 6 (4c, 9a) in 7 and group 7 (4b, Dausset 7) in 1 recipient. Thus no single antigenic group appears to be of sufficient strength that no recipient could survive while being mismatched in that group.

Correlation of clinical outcome with groups of mismatch. Clinical ranking of kidney transplant recipients was extremely difficult. The influence of factors such as lowered immunologic competence associated with uremia (*9*, *26*) could not be accurately evaluated. Furthermore, the amount, timing and duration of immunosuppressive therapy were variable. Nevertheless on the basis of current renal function, the stability of this function during the late course, and the intensity of immunosuppressive therapy required to prevent late deterioration the patients were separated into 4 ranks for the analysis. None of the patients in rank I has had a rejection after the first 4 months; all have either had steroids completely stopped or are receiving 7.5 mg a day or less. Recipients in rank IV have all had late rejection with more or less severe long-term impairment of function; large doses of prednisone are required (20 to 45 mg per day) to prevent further renal damage. Ranks II and III have characteristics between those of the other 2 groups. The current ranks 1248 to 759 days after transplantation are given for each patient in Table 1.

The statistical significance of the indicated association (Table 2) can be evaluated by combining columns to form the table: clinical rank versus number of mismatched regression groups (i.e., disregarding which particular groups, if any, are mismatched). The value of χ^2 for the resulting 4 row, 3 column table is 15.5, 6 degrees of freedom, $P = 0.02$. The resulting P value can be regarded as understating the statistical significance of the association since the χ^2 statistic does not take account of ordering relations such as: rank

TABLE 2

Correlation of clinical rank with regression group mismatches

Clinical rank[a]	Mismatched regression groups[b]							
	None	One group						Two groups
		1	2	3	4	5	6	
I	1, 3, 6, 14, 33, 42, 49, 52, 53, 58	34			2, 25			17
II	12, 15, 22, 44, 63	39, 60	37	50			51	13
III					48	55	18, 30	40, 41, 45, 54
IV				27			47	36

[a] Clinical ranks as of 25 April 1966.

[b] Numbers in the body of the table are patient numbers (see (*17*, *18*)).

II is less satisfactory than rank I, etc. To account for the ordering, consider the 2 row-2 column table formed by combining rank I with rank II and rank III with rank IV and by representing mismatch as the dichotomy: no groups mismatched, one or more groups mismatched. The resulting single degree of freedom $\chi^2 = 8.98$ and the value of P (Fisher's exact test) for a single tail test is 0.0006. To make allowance for having selected the most significant of the relevant 2×2 tables, it is appropriate to multiply the obtained value of P by the number of 2×2 tables that preserve the ordering, 6 in the present case. The result is $P' = (6)\ (0.0006) = 0.004$, which is statistically highly significant.

Correlation of biopsy findings with 2-way regression group mismatches. Histopathologic findings on biopsies taken on 35 of these recipients about 2 years after transplantation have been described in detail by Porter, et al. (*13*). A statistically significant correlation was shown to exist between the biopsy rankings and regression group mismatches. For the present analysis, a score was derived for the pathologic changes found as described in the methods section. These scores were then compared to regression group mismatches in two directions—donor to recipient, and recipient to donor. If the phenomenon of syngeneic preference (*8*) occurs in human kidney transplantation, incompatibilities in the reverse direction could be expected to influence the quality of the transplant.

The correlation of biopsy findings with extent of mismatching is displayed in Table 3. Statistical significance of the association is established by analysis of variance and regression methods. If the data are combined into 3 groups according to the number of groups mismatched, donor to recipient, the among groups—within groups comparison by analysis of variance yields $F = 5.6$, 2 and 32 degrees of freedom, $P = 0.01$, and comparison of the mean score for two-groups mismatch with zero-groups mismatch yields $t = 3.1$, 32 degrees of freedom, $P = 0.002$. Table 3 conveys the impression that groups mismatched, recipient to donor, are also associated with biopsy results. A regression analysis, in which score is regressed on number of groups mismatched,

TABLE 3

Correlation of biopsy findings with mismatched groups[a]

Biopsy score	Patient numbers[b]								
No. of mismatched groups—donor to recipient	0	0	0	1	1	1	2	2	2
No. of mismatched groups—recipient to donor	0	1	2	0	1	2	0	1	2
0	58	33	6		2				
1						25			
2	3, 52	49							
3	53					34			
4	42			48	39		17		
5	15	14, 22			37, 50				
6				18					
7				27	51				
8	12					60, 55		41	
9					47				13
10		63	1					40, 45	
11									
12									
13									36
14						30			54
Av. score	3.3	4.4	5.0	5.7	5.0	6.8	4.0	9.3	12.0
No. patients	7	5	2	3	6	5	1	3	3

[a] Kidney biopsies taken approximately 2 years after transplantation were scored as given in the Methods section. The number of mismatched groups between the donor and recipient was determined after classification into regression "types." Incompatibility in the opposite direction from recipient to donor is also tabulated.

[b] See (*17*, *18*).

donor to recipient, and on number of groups mismatched recipient to donor, again shows that biopsy score is statistically significantly related to the donor to recipient mismatch ($t = 2.83$, $P = 0.008$), but that the suggested relation to recipient to donor mismatch is not quite statistically significant ($t = 1.55$, $P = 0.13$). This result indicates that although recipient to donor matching is less crucial than donor to recipient matching, recipient to donor matching should not be entirely neglected for more desirable long-term results.

Possibility of lymphocyte chimerism in uremics. Although no direct tests for chimerism could be executed, the following evidence suggests that foreign lymphocytes from transfusions did not materially affect these results. (1) In the course of selecting donors for transplantation 52 prospective uremic patients were typed. As a whole they did not react to a significantly greater proportion of the antiserum panel. This was tested by comparing the total percentage of mismatches which would occur if a random panel of 93 unrelated persons was used as donors to themselves or to the population of uremics. The mean total mismatch units of 18.3 ± 9.4% for the uremics was slightly more than the mean mismatch of 16.6 ± 8.1% for the random unrelated population. Thus uremics tend to have fewer, though not significantly fewer,

lymphocyte antigens than normal individuals. If their cells had consisted of 2 or more populations, a greater number of positive reactions and hence fewer mismatches would have been expected. (2) Nine of the prospective patients were typed on 2 different occasions with a number of transfusions occurring between the 2 tests. Analysis of the discrepant results between the 2 tests indicated that a slightly higher proportion of discordant results occurred with this group than with a comparable group of 8 normal persons, though the difference was not significant. One chronically dialyzed patient (of Drs. M. E. Rubini and R. Sneiweiss, Wadsworth Veterans Administration Hospital) received 29 units of transfused blood during the 5 month interval between 2 tests. Of the 89 antisera tested, 4 produced discrepant reactions of greater than 1 dilution magnitude. This was a greater, though not significantly greater, discrepancy than that found with normal lymphocytes.

Although these arguments are insufficient to exclude the possibility of the presence of small numbers of foreign cells, at least it can be concluded that, within the limits of sensitivity of these tests, complications in typing produced by chimerism were negligible.

Alteration of lymphocyte antigenicity by immunosuppression. If chronic immunosuppression alters the antigenic make-up of lymphocytes in some manner, retrospective typing of long-term survivors would be subject to question. On the whole, immunosuppression could not have caused loss in antigenicity, for the series of long-term survivors did not have fewer antigens than random individuals. This was tested by comparing the mismatches which would occur if 49 long-term recipients and 93 randomly selected individuals were considered as recipients for the same pool of 93 randomly selected donors. The mean total percentage of units mismatch was 17.9 ± 8.0 and 16.6 ± 8.1 respectively, which is not a significant difference.

If lymphocytes from recipients undergoing extensive immunosuppressive therapy are more susceptible to cytolysis by antibody and complement because of the drugs, an apparent gain in antigenicity may result. As noted above, the 49 patients who have undergone immunosuppressive treatment for 1 year (when they were tested) did not have, as a group, a significantly greater number of positive reactions than random persons. Also, 1 recipient tested before transplantation and then again 4 months after grafting while receiving drug therapy did not show significant alterations in his patterns of reactivity. Of the 52 sera tested in common, 2 gave discordant results of 1 dilution tube or more. The result of comparing the total antigenic profile of these 2 samples with respect to the comparison with 274 random individuals is given in (*20*) (Fig. 3, person 321). Only 2 random persons out of 274 were matched as well as the duplicate.

DISCUSSION

From the data presented here, it is obvious that many of the kidney transplant patients who survive more than 2 years were incompatible for each of the 6 antigenic groups of leukocytes. These leukocyte groups were serologically the strongest ones, for they had been independently detected by other workers

using various methods. Other designations for the mismatched groups according to recent correlations (*3, 23*) are: LA 1, LA 2, 4c, 4d, (*2*), 9a, 4a, 8a, 6b, (*22*), Dausset groups 1, 3, 7, 8, 9, (*4*), Amos group and II, (*1*), Shulman B1 (*14*). It is therefore apparent that these antigens not extremely strong transplantation antigens in the sense that a mismaten would lead inevitably to total immunologic rejection in spite of current immunosuppressive therapy.

Two obvious sources of error could have led to the above conclusion that the serologically strong antigens were mismatched in long-term survivors. First, since kidney transplant recipients often receive transfusions, it is possible that transfused lymphocytes multiply (*6, 12*) and survive in the recipient, thus leading to spurious typing results. This is particularly likely, for uremics are often immunologically crippled (*9, 26*) and are given immunosuppressive treatment with the transplant. If such chimerism were established within the host it would affect the results of the test only if the donor's cells comprised more than 10% of the total population. This level of contamination would be expected to lead to positive reactions with sera which were negative with the native cells. Thus such a chimeric population of cells would appear to possess more antigens than the recipient's own cells. In general, the group of 52 uremic patients did not tend to have a higher antigenic composition than cells from random normal persons. Moreover in 9 instances in which patients were tested before and several days after transfusion, no marked differences were noted.

A second source of erroneous typing is the possibility that the prolonged immunosuppressive therapy undergone by these recipients had altered either the antigenicity or susceptibility of lymphocytes to cytolysis. Although the reproducibility of the tests was lower among treated recipients than among normal subjects, 49 long-term survivors did not tend to have either more or fewer antigens than normal individuals.

If it is accepted that these leukocyte antigens are indeed mismatched in long-term survivors, it is of importance to question whether they are completely irrelevant to transplantation or whether they represent intermediate strength histocompatibility antigens. The response against such intermediate strength antigens could be variably suppressed with drug therapy and could be tolerated to varying degrees by different uremic patients. It is a common finding that individual differences exist in the immunologic response to foreign antigens, and that many individuals do not respond well to certain antigens. As an example, upon deliberate immunization of Rh-negative volunteers with Rh-positive red blood cells, if the ABO types were incompatible, as many as 28 out of 32 immunized persons failed to produce antibodies (*19*). Wiener, et al. (*25*) also could not produce red cell antibodies in all 18 individuals immunized against M, K, P, and S antigens despite immunizations over a period of 17 months. If this type of unresponsiveness to known allogeneic antigens in normal persons is added to now well established knowledge of the immunologic unresponsiveness of the uremic state (*9, 26*) it should be expected that some recipients may have survived in spite of mismatched antigens. Although this may account for some survivors with many incompatibilities,

immunologic unresponsiveness probably do[illegible] play a more dominant role than histocompatibility matching, for wit[illegible]valent immunosuppression a larger proportion of recipients who received [illegible]its from related donors survived than those who received grafts from unrelated [illegible]ors (*7, 10, 17*).

Since a high proportion of the long-term survivors (22 out of 36) were found to be incompatible for major leukocyte groups, the relevancy of leukocyte antigens in transplantation could be questioned. If any of these antigens had been strong antigens in the sense that an incompatibility would have led to certain homograft rejection, no 2-year survivors mismatched for these determinants should have been found. Evidence that leukocyte antigens represent *intermediate* strength histocompatilibity determinants comes from the fact that patients who survived despite incompatibilities represented significantly poorer results both from the clinical point of view and from evaluation of biopsies taken 2 years post-transplantation (Tables 2 and 3).. Thus for example patients mismatched for any single antigen such as group 2 (Dausset 1, LA 2, Shulman B1, 8a, Amos II) have survived, but generally with worse function and histological result than patients with no groups of incompatibility. The high degree of correlation with the histopathologic findings (*13*) and Table 3) indicate that mismatches for the major leukocyte types recognized here do manifest themselves in the 2-year biopsy. Because this extent of correlation was not as [illegible]lily evident in the clinical course within the first year (*21*), it could be suggested that early intensive immunosuppression can often mask incompatibilities which later become more apparent from histologic studies.

Although it is recognized that many more leukocyte groups exist which have not been detected in this study, it could be argued that the 7 groups which resulted from analysis of about 150 antisera constituted the greater part of the major groups. Further these groups, which were serologically the strongest (*20*), could also be the strongest with respect to transplantation, as has been true in the only systems (chicken and mouse) for which data on this correlation are available. The situation with regard to matching which emerges is then different from that envisioned by Simonsen (*15, 16*) in which one locus with 3 or 4 alleles are thought to determine the strong antigens of human kidney transplantation. The Simonsen hypothesis requires that mismatches in the strong antigens must lead to inevitable deaths within the 1st year. In contrast, the present study suggests that survival or nonsurvival may not be so precisely linked with mismatching. Thus, variable fractions of those mismatched may survive, for the unbreechable strong antigens of Simonsen may not exist. [illegible]her, intermediate strength antigens, the number of which cannot yet be estimated, may be involved to varying degrees.

The methods described and evaluated here have since been used to select donor and recipient combinations *before* transplantation. The results of this project are described elsewhere (*21*).

Acknowledgments. We would like to thank Mr. Edward Victoria, Jr., Mr. Terrell Rich,

and Mr. Charles Eversole for their excellent technical assistance. also acknowledge the help of United Airlines in expediting the shipment of the blood samples.

REFERENCES

1. Amos, D. B. 1965. p. 151. In *Histocompatibility testing, 1965*. Munksgaard, Copenhagen.
2. Bodmer, W. F.; Payne, R. 1965. p. 141. In *Histocompatibility testing, 1965*. Munksgaard, Copenhagen.
3. Bruning, J. W.; van Leeuwen, A.; van Rood, J. J. 1965. p. 275. In *Histocompatibility testing, 1965*. Munksgaard, Copenhagen.
4. Dausset, J.; Ivanyi, P.; Ivanyi, D. 1965. p. 51. In *Histocompatibility testing, 1965*. Munksgaard, Copenhagen.
5. Dixon, W. J. (ed.). 1964. *BMD biomedical computer programs*. Health Sciences Computing Facility, Dept. of Preventive Medicine and Public Health, University of California, Los Angeles.
6. Goodman, J. W.; Hodgson, G. S. 1962. Blood *19:* 702.
7. Hamburger, J.; Crosnier, J.; Dormant, J. 1965. Lancet *1:* 985.
8. [illegible]llström, K. E.; Möller, G. 1965. Progr. Allergy *9:* 158.
9. [illegible]rkpatrick, C. H.; Wilson, W. E. C.; Talmage, D. W. 1964. J. Exp. Med. *119:* 727.
10. Murray, J. E.; Gleason, R.; Bartholomay, A. 1965. Transplantation *3:* 684.
11. Newth, D. R. 1961. Transpl. Bull. *27:* 452.
12. Nowell, P. C. 1960. Cancer Res. *20:* 462.
13. Porter, K. A.; Rendall, J. M.; Stolinski, C.; Terasaki, P. I.; Marchioro, T. L.; Starzl, T. E. 1966. Ann. N. Y. Acad. Sci. (in press).
14. Shulman, N. R.; Marder, V. J.; Hiller, M. C.; Collier, E. M. 1964. Progr. Hematol. *4:* 222.
15. Simonsen, M. 1965. Lancet *1:* 415.
16. Simonsen, M. 1966. Information exchange group No. 5, memo 87.
17. Starzl, T. E. 1964. *Experience in renal transplantation*, Saunders, Philadelphia.
18. Starzl, T. E.; Marchioro, T. L.; Terasaki, P. I.; Porter, K. A.; Faris, T. D.; Herrmann, T. J.; Vredevoe, D. L.; Hutt, M. P.; Ogden, D. A.; Waddell, W. R. 1965. Ann. Surg. *162:* 749.
19. Stern, K.; Goodman, H. S.; Berger, M. 1961. J. Immunol. *87:* 189.
20. Terasaki, P. I.; Mickey, M. R.; Vredevoe, D. L.; Goyette, D. R. 1966. Vox Sang. *11:* 350.
21. Terasaki, P. I.; [illegible]rter, K. A.; Marchioro, T. L.; Mickey, M. R.; Vredevoe, D. L.; Faris, T. D.; St[illegible]zl, T. E. 1966. Ann. N. Y. Acad. Sci. (in press).
22. van Rood, J. J.; van Leeuwen, A.; Schippers, M. J.; Vooys, W. H.; Frederiks, E.; Balner, H.; Eernisse, J. G. 1955. p. 37. In *Histocompatibility testing, 1965*. Munksgaard, Copenhagen.
23. Vredevoe, D. L; Mickey, M. R.; Goyette, D. R.; Magnuson, N. S.; Terasaki, P. I. 1966. Ann. N. Y. Acad. Sci. (in press).
24. Vredevoe, D. L.; Terasaki, P. I.; Mickey, M. R.; Glassock, R.; Merrill, J. P.; Murray, J. E. 1965. p. 25. In *Histocompatibility testing, 1965*. Munksgaard, Copenhagen.
25. Wiener, G. S.; Samwick, A. A.; Morrison, H.; Cohen, L. 1955. Exp. Med. Surg. *13:* 347.
26. Wilson, W. E. C.; Kirkpatrick, C. H.; Talmage, D. W. 1964. J. Clin. Invest. *43:* 1881.

Received 29 October 1965.