Histocompatibility Testing

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Sero-Typing of Human Lymphocyte Antigens: Preliminary Trials on Long-Term Kidney Homograft Survivors*

Paul I. Terasaki, Thomas L. Marchioro, and Thomas E. Starzl

Departments of Surgery
University of California School of Medicine, Los Angeles
University of Colorado School of Medicine
Denver Veterans Hospital

The rationale behind attempts to select proper donors for homotransplantation has been aptly given recently by many authors (Hamburger *et al.*, 1962; Brent and Medawar, 1963; Rogers, 1963; Wilson, Henry, and Merrill, 1963; Marchioro *et al.*, 1964; Starzl *et al.*, 1964a). How this might be accomplished is the immediate concern of this conference. In this preliminary study we have investigated the possibility of using allogenic antisera derived from women with multiple abortions or pregnancies as reagents to detect transplantation antigens presumably possessed by indicator blood lymphocytes. That the resulting serologic reaction would reveal the major transplantation antigens is the prime premise of this approach. Although experiments with mice have provided evidence that strong transplantation antigens are demonstrable serologically (Gorer, 1956; Brent, Medawar, and Ruszkiewicz, 1961), and that the reaction against minor antigens can be suppressed (Shapiro *et al.*, 1961; McKhann, 1962; Medawar, 1963), there is no evidence that an analogous situation exists in man.

As the initial step in testing the efficacy of the lymphocyte cytotoxic reaction as a typing procedure for homotransplantation, long-term kidney homograft survivors and their donors were tested for compatibility. If fortuitous matching of transplantation antigens had been principally responsible for the long survival of renal transplants in these selected patients, a reasonable degree of compatibility should be evident from the typing studies. Precise correlation of survival time with typing methods cannot be expected, since the method of immunosuppression was regulated by the clinical course of individual patients. However, if the matching of antigens, particularly by the device of obtaining grafts from related donors, had played an important role in long-term survival, a distinct trend in matching should be evident. In the present provisional trials, such a trend was obtained.

Methods

The methods of transplantation in adults and children (Starzl *et al.*, in press, a, b), as well as techniques of nephrectomy (Marchioro *et al.*, 1964) and perfusion (Starzl *et al.*, 1963), have been reported earlier. A synopsis of the case histories of 12 patients from the Denver Veterans Hospital and the University of Colorado School of Medicine is given in Table 1. Summaries of two long-term survivors of kidney transplants at the UCLA Medical Center (Goldman *et al.*, submitted for publication) are shown in Table 2.

Heparinized blood (20 to 50 units/ml) obtained from kidney donors and recipients in Denver was flown to Los Angeles. Viable lymphocytes were isolated within 12 hr from the time of bleeding. The method of isolation consisted of differential adherence of granulocytes on a polystyrene surface followed by centrifugation in capillary tubes to eliminate most of the erythrocytes (Terasaki *et al.*, in press; Terasaki and McClelland, submitted for publication). Lymphocyte antigens were identified by cytotoxicity of 50 different human antisera. The sera were obtained mainly from women with multiple abortions and pregnancies; a few were obtained from multiple transfusions (#2, 6, and 15), and two from persons immunized by

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TABLE 1

Summarized Case Histories of 12 Renal Transplant Patients from the Univ. of Colorado School of Medicine and Denver V.A. Hospital

Recip.	Donor Relation	Time since Transpl.	+ or − days of Transpl.		Ischemia Time, min	Reject., Days P.O.	Therapy for Reversal*	Current	
			Splenect.	Nephrect.				BUN	Creat. Clear.
••1	Mother	18 mo.	−20	−20	34	12	Azathiop., Pred., Act. C	15	91.3
2	Brother	13 mo.	−26	0, +7	35	18	Pred., Act. C	16	114
4	Brother	10 mo.	0	0	22	1½	Pred., Act. C	33	86.8
5	Mother	9 mo.	−21	−21	37	21	Pred., Act. C	28	76
6	Mother	7 mo.	0	0	42	5	Pred., Act. C	22	51.6
7	Wife	7 mo.	0	0	38	1	Pred., Act. C	16	101
9	Mother	6 mo.	0	0	18	10	Pred., Act. C	21	77.6
10	Mother	6 mo.	0	0	27	12	Pred., Act. C	19	61.1
11	Father	6 mo.	0	0	26	4	Pred., Act. C	21	101.5
•••12	Aunt	3 mo.	0	0	25	30	Local X-ray, Act. C	40	96.5
•••13	Father	3 mo.	0	0	27	3	Local X-ray, Act. C	32	112
•••14	Mother	3 mo.	0	0	30	2	Local X-ray, Act. C	19	114

* Azathiop. = azathioprine; Pred. = prednisone; Act. = actinomycin.

Patients 1 and 2 were thymectomized 1 mo. before transplantation.

All patients except 1 and 13 were treated with azathioprine for 10 days preoperatively in doses of 1 to 3 (mg/kg)/day. Postoperatively, azathioprine was continued in doses of 2 to 6 (mg/kg)/day, the exact dose being determined by the peripheral white cell count on the day of drug administration. Patient 13 was preforcated for 1 day only.

•• Immunosuppression consisted of 300 r total body radiation preoperatively and 100 r total body radiation postoperatively, administered in divided doses. With the onset of rejection, azathioprine was added to the regimen and continued in doses of 2.5 to 4 (mg/kg)/day.

••• These three patients received 150 to 200 mg of prednisone per day preoperatively for 1 to 2 days in addition to pretreatment with azathioprine. Both drugs were continued postoperatively. Prednisone dosage has been systematically decreased in each case in accordance with renal function. Local irradiation, 150 r to the transplant, was used to reverse rejection crises. It was delivered on 3 alternate days for a total dose of 450 r per course of therapy. The last two patients received two full courses of X-ray. The patient 12 has had only one course. Prednisone is no longer taken by 1 or 2.

TABLE 2

Summarized Case Histories of Two Renal Transplant Patients from UCLA (Drs. Goodwin, Martin, Kaufman, and Goldman)

Recip.	Donor Relation	Time since Transpl.	+ or − days of Transpl.		Ischemia Time, min	Reject., Days P.O.	Therapy for Reversal*	Current	
			Splenect.	Nephrect.				BUN	Creat. Clear.
3	non-related	12 mo.	not done	0	45	10	Azathiop., Pred., Act. D	19	43
8	Mother	6 mo.	0	0	40	40	Azathiop., Pred., Act. C	15	41

* Azathiop. = azathioprine; Pred. = prednisone; Act. = actinomycin

three or four consecutive skin homografts (#22 and 24).* Antisera #15, 27, and 14 were from habitual aborters.**

The microdroplet oil chamber method of testing has been described (Terasaki and McClelland, submitted for publication). Essentially, mixtures of 2,000 lymphocytes, 0.003 ml of absorbed rabbit complement, and antibody dilutions by thirds from 0.003 to 0.00003 ml were incubated for 4 hr at room temperature. The microreactions were accomplished in an oil chamber and assayed by counting the percentage of viable cells under phase-contrast microscopy at 620 × or 820 × magnification.

Erythrocytes were typed with standard commercial antisera, using the Coulter Counter to measure agglutination (Terasaki *et al.*, in press).

Results

Red-cell antigens. Many of the long-term surviving kidney homograft patients have survived despite one to three "minor" erythrocyte-group incompatibilities with their respective donors (Fig. 1). Most erythrocyte groups seemed innocuous, for incompatibilities were noted with E, Fy^a, M, C, D, s, and c. Among the limited numbers of survivors tested, no incompatibilities were found for A, B, N, or e. The fact that eight out of 11 of the 6-months-or-longer group possessed one incompatibility or none may suggest, however, that multiple incompatibilities may be deleterious. On the other hand, since two of the longest survivors had two or three incompatibilities, mismatched erythrocyte antigens may not be a critical factor to survival of the graft. In patient #4, in whom there was a C and D mismatch, 10 months after transplantation antibodies were detected against donor erythrocytes. If it is assumed that no transfusions of Rh^+ blood were given, these antibodies may have been elicited by antigens in the donor kidney. The fact that the kidney is functioning 10 months after transplantation demonstrates that the presence of such antibodies is not clinically deleterious to the kidney graft. Similar tests on papainized erythrocytes of the donor with the plasma of recipients #1, 2, 12, 14, 13, and 10 were negative. In these

* Kindly contributed by Dr. Roy Walford; #22 and 24 are designated Bu and Mo, respectively, in Walford, Gallagher, and Sjaarda (1964).

** Generously contributed by Dr. Phillip Levine.

patients an indirect Coombs test using the donor erythrocytes was also negative.

Lymphocyte typing. Cytotoxicity of antisera dilutions were recorded as approximate percentages of cells killed and plotted for each donor and recipient pair as shown in Fig. 2. This example illustrates the reactions of the donor (mother) and recipient #9. By comparison of the cytotoxicity of each antiserum horizontally between the donor and recipient, it can be seen that antigens 5, 17, 22, 25, 41, 44, 45, 48, and 53 are present in the donor but lacking in the recipient. These antigens may be considered to be foreign to the host, and therefore immunogenic. Although identity tests of these antisera are not yet complete, it would appear that incompatibilities detected by these eight antisera are not completely deleterious, for with the immunosuppressive treatments used (Table 1), the patient is alive and well 6 months postoperatively.

One example in which the donor antigens were quite dissimiliar to the recipient's antigens but were in almost all instances *less* than the recipient's is given in Fig. 3. In this pair, the donor and recipient were *not* related (wife to patient #7). According to the lymphotoxicity reactions, only one incompatibility with antiserum 16 was noted.

Results of titrations as given in Figs. 2 and 3 are summarized in Fig. 4 by plotting only those antigens that were incompatible. A "unit" was taken as the difference in dilution "droplet" at which comparable reactions were obtained. One unit difference therefore corresponds to a three-fold disparity in volume of antisera necessary to kill, since the antisera were diluted by 1:3. The maximum of five units corresponds to a 243-fold antiserum volume difference. Since earlier unpublished work on inbred mice has indicated that quantitative differences obtained with presumably polyvalent unabsorbed antisera may reveal qualitative variances, some attempt was made to indicate the extent of incompatibility if the host possessed measurable antigens. Thus a distinction is made between a complete incompatibility in which the recipient lacks all detectable antigens (indicated by the solid bars) and partial incompatibility in which the recipient possesses some antigenic activity but at a level lower than the donor (indicated by the striped bars). Complete incompatibilities, which would be deemed detrimental to a graft, were found to be slightly more predominant in the recipients who are living 3 to 6 months than in those living 6 to 18 months. Partial incompatibilities were more randomly distributed. Perhaps significantly, no complete incompatibility was found in patient #1, who has lived 18 months after the operation. Of the patients tested, the number of mismatched antigens was the largest in one 6-month survivor (#9) and one 3-month survivor (#12).

As an initial attempt to devise criteria by which degrees of incompatibility can be assessed, the following test was applied. Taking four long-term survivors as recipients, 16 individuals who were kidney donors or recipients were tested as hypothetical donors. Every combination of donor-to-recipient was examined for lymphocyte antigen incompatibility and plotted by four methods (Tables 3 and 4). In Table 3, the actual *number of* antigens which were mismatched was totaled. "Complete" incompatibilities, as defined earlier, and "partial" incompatibilities were added separately and noted; the trial donors with the lowest number of incompatibilities appear toward the left. Significantly, the actual donors used, 1D, 2D, 6D, and 7D, fell within the first two columns, whereas many other possible donors possessed six or more incompatible antigens. Individual #14 can be seen to be the worst possible donor for all the recipients, whereas individual 7D (shown in Fig. 3) would have been a universal donor according to this scheme. If dissimilarities of up to five antigens can be suppressed with pharmacotherapy, the actual donor (1D—mother) or 7D could have been used as a donor for recipient #1 and 15 other persons could have been unsuitable. For recipient #2, out of 16 possible donors, 11 might have been used. Summation of partial incompatibilities yielded somewhat similar results in showing certain individuals to be better donors than others.

If the difference in *units* of mismatch are totaled as in Table 4, a greater spread in the range of incompatibility is obtained. Increased specificity in selection of donor seems to be gained, for the actual donors 1D, 2D, 7D, and 8D can be seen to be good donors for some recipients, but excessive-antigen donors for others. For example donor 2D possessed 13 excess units (u) for #1, 5u for #2 (the actual recipient), 6u for #7, and 9u for #8.

In the course of these studies, an interesting observation was made on one patient (of Drs. Goodwin, Martin, Kaufman, and Goldman) who suffered an "immediate rejection" of a kidney transplant. The kidney, trans-

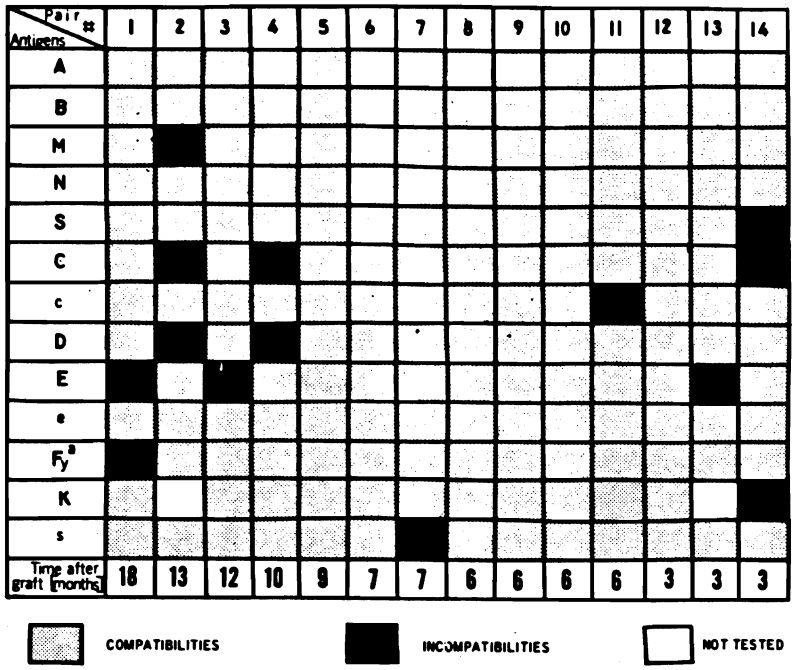

Figure 1.—Erythrocyte-group incompatibilities between kidney homograft donors and their recipients are shown in the dark bars.

planted from a brother to sister, remained anuric for 2 weeks, after which it was removed. Tests of the recipient's serum *before* grafting revealed cytotoxic antibodies against the donor's lymphocytes. Serum taken on the day of transplantation just before grafting was cytotoxic to 70 percent of cells (using 0.003 ml of serum, 0.005 ml of C′, and 2,000 lymphocytes) and became slightly less toxic 2 and 6 days after grafting. These antibodies could have been elicited by transfusions and may be suspected of being responsible for the immediate rejection.

Discussion

The preliminary results described here suggest that lymphocyte cytotoxicity may be used to detect and match transplantation antigens. The fact that relatively few incompatibilities were found between the lymphocyte antigens of the donor and of the kidney-graft recipients who have survived for 6 to 18 months (Fig. 4) may be taken to indicate that this underlying tissue compatibility was an important contributory factor in the successful outcome. Obviously, matching alone is not a *sufficient* condition, for numerous factors, such as low ischemia times and proper diagnosis and therapy of "rejection" crises, are essential (Starzl *et al.*, 1964b). A lesser degree of matching presumably would have led to rapid rejection. The possibility that the apparent correspondence was merely the result of similar but nonspecific reactions of the antisera was tested by comparing the typing reactions of random hypothetical donors with the donor actually used. In most instances, the actual donor was found to be one of the better donors with few incompatibilities, whereas many other donors possessed numerous antigens in excess of the recipient (Table 3). Of considerable significance is the fact that for some recipients a nonrelated individual was an even more suitable donor than the close relative used. Persons who lacked many antigens, such as the wife of recipient #7, may be almost universal donors. Others, such as individual #14, would have been undesirable donors for all the recipients tested.

An apparent degree of matching may also have been produced by the action of numerous antibodies of varying specificities present in a reagent antiserum. Thus two cells possessing different antigens may be killed by the same antiserum (containing several antibodies) and therefore may appear as cells

Figure 2.—Lymphocyte-cytotoxicity titration of the donor (mother) and recipient (#9). The antisera are listed on the left and volumes of the antisera tested X 10^{-3} ml are given in the top row of figures. The approximate extent of cytotoxicity at each dilution is denoted by the height of the dark bars. Incompatibilities are present wherever the donor (shown on the left) reacts with a serum and the recipient does not.

having identical antigens. This difficulty can be circumvented, at least theoretically, by absorbing the sera with recipient cells and testing with the donor cells (Becker and Terasaki, submitted for publication). In a few instances in which this procedure was adopted for all reactions for which the donor and recipient were positive, no new specificity could be uncovered. As a temporary expedient until "purified" antisera are produced, it was also anticipated that, by testing with numerous antisera, differences not revealed by one "mixture" may be detected by another.

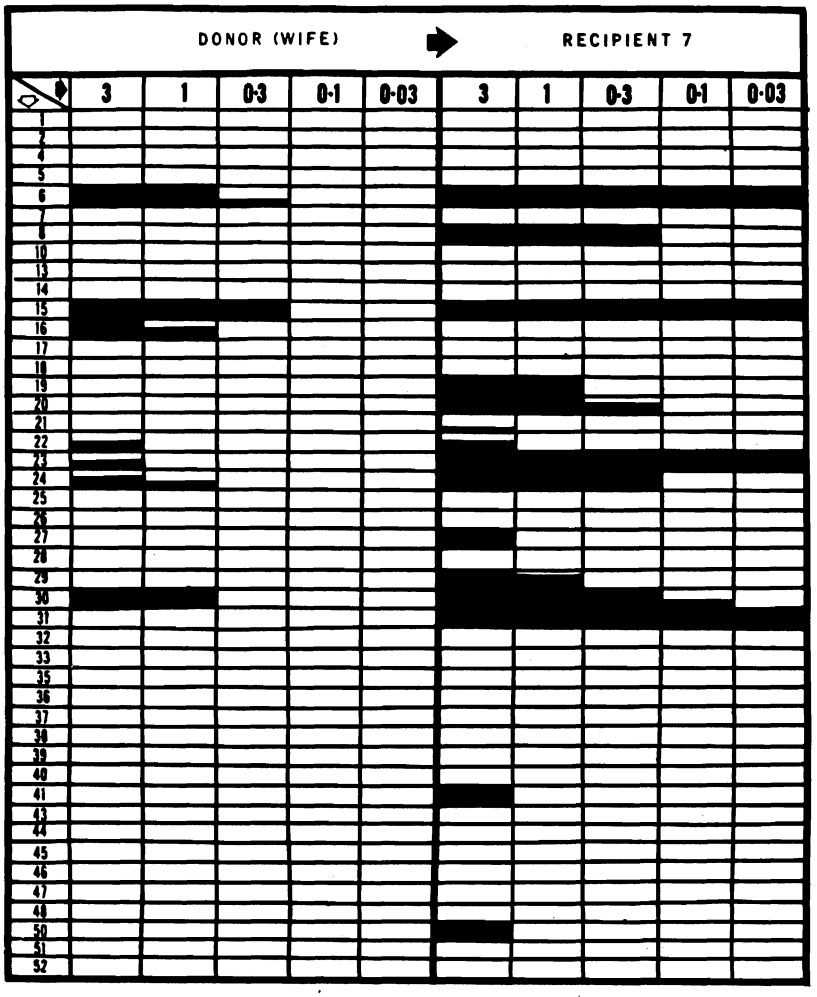

Figure 3.—Lymphocyte-cytotoxicity titration of a donor (wife) and unrelated recipient (#7). The results are plotted as in Fig. 2. Of particular interest is the fact that the donor possessed only one antigen that the recipient did not, whereas a graft in the opposite direction would have been incompatible for 8 antigens.

An incompatibility in which the donor reacts with a given antiserum and the recipient does not is not open to the same polyvalent-antiserum criticism. Cells of the donor may react on the basis of A, B, C, . . . Z antigens, whereas the recipient can be taken to lack all these antigens from a negative reaction. This type of incompatibility, however, does suffer from another difficulty, in that a non-reactive cell may be capable at times of absorbing out cytotoxicity (Becker and Terasaki, submitted for publication; Terasaki *et al.*, in press). Van Rood has earlier encountered a similar phenomenon with leuko-agglutinins which he termed agglutination-negative-absorption-positive reaction van Rood and van Leeuwen, 1963), and similar reactions have been found with erythrocytes (Race and Sanger, 1962). Absorption experiments may therefore be essential to prove that an antigen is lacking, for the amount of antigen present may be sufficient to absorb

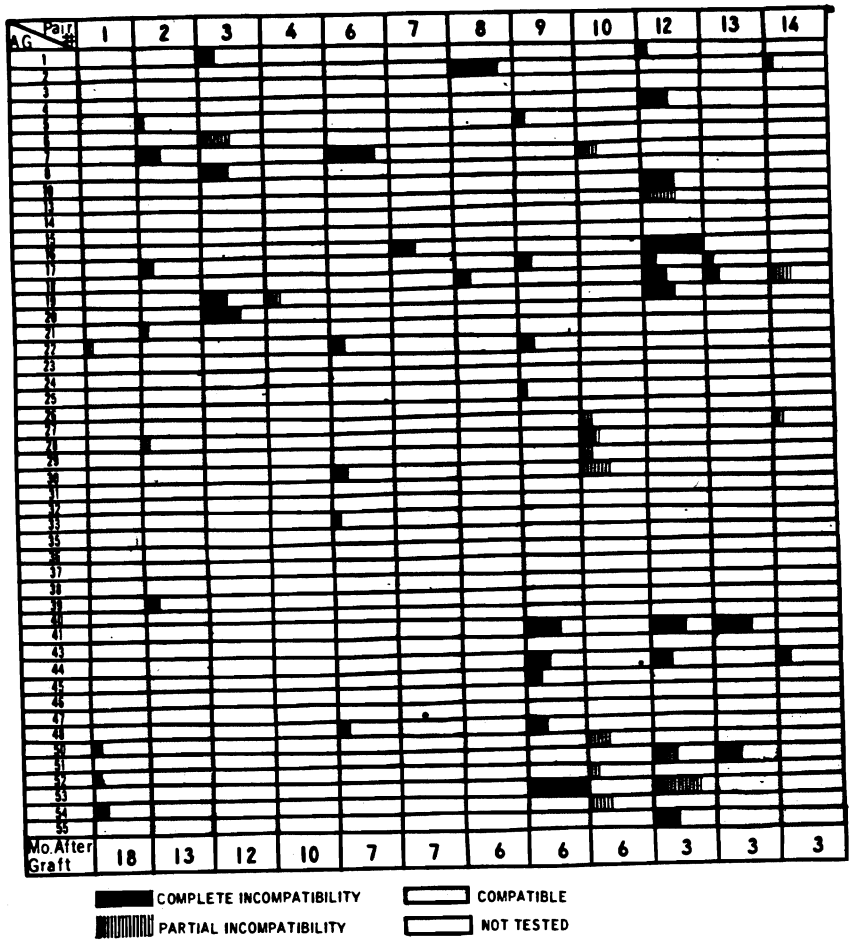

Figure 4.—Summary of the incompatibilities found in kidney homograft survivors. From comparisons of each donor-and-recipient pair as shown in Figs. 2 and 3, the incompatible antigens were determined and plotted. A solid black bar indicates a "complete" incompatibility in which no detectable cytotoxicity was found with recipient cells. A striped bar denotes "partial" incompatibility in which the recipient possesses some antigens, but fewer than the donor. The length of the bars expresses the "units" of difference as defined above ("Results"); a full bar is equivalent to a 5-unit difference.

but insufficient to act as a cytotoxinogen or leukoagglutinogen site. Alternatively, the absorption results may be misleading, for antibodies of varying specificities could be fixed nonspecifically. Pirofsky, Cordova, and Imel (1962) demonstrated that anti-D and normal human globulins fix in equal numbers upon erythrocyte surfaces independently of the antigenic nature of the cell. Nonimmunologic absorption of normal globulins, however, did not lead to agglutination with rabbit anti-human globulin serum. Hence it can be speculated that absorption of antisera may be *less* specific than absorption plus a second reaction (such as antiglobulin absorption or complement fixation).

The premise that quantitative differences in level of reactivity may expose *qualitative* disparities is based on the following reasoning. In any mixed antiserum, not all antibodies would be expected to occur in equal concentrations or reactivity. Antibodies against the stronger antigens may be the most active, and others distributed normally around them. Upon dilution, the mixed antibodies are cut off at different levels, so that at the highest dilution, only the strongest anti-

TABLE 3

Number of incompatibilities of 16 hypothetical donors compared with those obtained with the actual donors. The possible donors were classified according to the total *number* of sera which showed incompatibilities. Complete incompatibilities are defined as those in which the recipient possessed no detectable cytotoxinogens. Partial incompatibilities are those in which the recipient possessed *fewer* antigens than the donor. Numbers correspond to donors (D) and recipients listed in Table 1. • = Actual donor used.

Recipient Number	*NUMBER OF COMPLETE INCOMPATIBILITIES*								
	0–2	*3–5*	*6–8*	*9–11*	*12–14*	*15–17*	*18–20*	*21–23*	*24–26*
# 1	7D 1D•		10D, 7 10 15	2D, 6 15D 6D, 2	13D 9D 9	14D 13			14
# 2	7D 7 6	15D 10D 1, 1D 5, 6D 2D• 10	9 13D	13	14D 9D	14			
# 6	7D 10, 7 10D	1, 2 6D• 2D	15 13D 15D	9 1D 13	9D 14D		14		
# 7	7D•	10D 6, 6D 2, 1 1D	10 13D	2D 15D 15, 9 9D, 10		14D			14
Recipient Number	*NUMBER OF PARTIAL INCOMPATIBILITIES*								
	0–2	*3–5*	*6–8*	*9–11*	*12–14*	*15–17*	*18–20*	*21–23*	*24–26*
# 1	7D	6D 1D• 7	9D 6 15D	13D 2D, 10 2, 14D 13	15 10D 14 9				
# 2	7, 7D 6, 6D 9D, 1 2D•	1D 15D 10 13D	14D 10D 13, 9 15		14				
# 6	1, 7D 9, 7 6D• 9D	14D 15D	2D 13D	10 13, 2 10D 15	9	14			
# 7	7D• 6D	9D, 1 1D, 6 15D	2 14D 13 2D	13D 10 9 15 10D	14				

bodies may remain, since the weaker ones have been diluted to subthreshold levels. Taking the difference in reactivity of the donor and recipient as immunogenic factors, it was shown here that in four long-term survivors, the actual donor used was among the best donors out of 16 hypothetical donors (Table 4). Although this result may be attributable to inadequate numbers of patients tested thus far, it is a surprisingly encouraging result, for this provisional method of adding incompatibilities does not take into account the possibilities that (1) some antisera may have identical specificities, (2) certain antigens may be more potent transplantation antigens than others, and (3)

TABLE 4

Units of incompatibilities of 16 hypothetical donors compared with those obtained with the actual donors. Classification is according to the *units* of mismatch. Unit of difference = difference in number of dilution tubes (1:3) which produced equivalent degrees of cytotoxicity. Complete incompatibilities are defined as those in which the recipient possesses no detectable cytotoxinogens. Partial incompatibilities are those in which the recipient possessed *fewer* antigens than the donor. Numbers correspond to donors (D) and recipients used in Table 1. • = Actual donor used.

Recipient Number	*UNITS OF COMPLETE INCOMPATIBILITIES*								
	0–3	*4–6*	*7–10*	*11–15*	*16–20*	*21–25*	*26–30*	*31–40*	*41–56*
# 1	7D 1D•			2D 15D	6D, 7 6, 10D 10	2, 9D 15	14D	13D, 9 13	14
# 2	7D, 6 15D, 7	1 2D• 10D 1D, 15 6D	10		13D	9D 9 13 14D		14	
# 6	7D 6D• 7, 2	10, 1 10D 2D	15D 1D 15		13D 13	9D 14D 9		14	
# 7	7D•	6 2 6D	10D 10 2D 15D	15	13D 9D	9 13		14D	14

Recipient Number	*UNITS OF PARTIAL INCOMPATIBILITIES*								
	0–3	*4–6*	*7–10*	*11–15*	*16–20*	*21–25*	*26–30*	*31–40*	*41–56*
# 1	7D 1D•		6D 2D 9D 7	10 2, 6 13D 15D 13	14D 10D	15 14	9		
# 2	7, 7D 2D• 6, 6D 10	15D 1, 1D 13D 9D	10D 14D 13 15	9 14					
# 6	7D, 1 1D, 7	9D 6D• 2D	15D 2, 13D 10 14D	15 10D 13 9		14			
# 7	7D•	1D 6D 1, 6	9D 15D 2D 2	14D 15 13 10	13D 10D 9	14			

antibodies of numerous specificities may be acting synergistically (Möller and Möller, 1962; Becker and Terasaki, submitted for publication) by complex interactions at each dilution level.

Despite these current problems, certain long-term advantages of a serological approach to typing over other proposed methods can be noted. One important factor is that it does not depend on the physiological functioning of the lymphocyte, as does the lymphocyte-transfer test of Brent and Medawar (1963) and the tissue-culture reactivity tests of Bain, Vas, and Lowenstein (1964) and Bach and

Hirschhorn (1964). Lymphocytes of uremic patients were shown by Bridges and co-workers (1964) to be less capable of giving an intracutaneous reaction than lymphocytes of normal individuals. Too, since physiological activity is imperative for the transfer and tissue-culture tests, the conditions under which the cells are isolated and used are more exacting than in serological methods. The time taken for the reactions to develop precludes the use of nonliving donors (which certainly must be considered the ultimate source of donors). The distinct advantage of sero-typing in being able to mass-type a large battery of donors and recipients at any time and use them in any combination, as is done with blood transfusions, is evident.

The particular serological reaction employed, that of lymphocyte cytotoxicity, appears to have several advantages over the other kinds of reactions previously tested. The method does not suffer from the troublesome difficulties of non-specific agglutination found with leukoagglutination tests (van Rood, van Leeuwen, and Bosch, 1962; Dausset, 1962). The present microdroplet technique permits performance of numerous tests with extremely small quantities of rare human antisera. Moreover, since lymphocytes are often difficult to obtain in large numbers from patients undergoing immunosuppressive therapy, a microtest such as that described is indispensable. For example, from 10 ml of the blood of a kidney-graft recipient, the yield of purified lymphocytes has often been on the order of 400,000 cells, from which 200 separate droplets can be tested in a typical antibody-titration experiment. Nevertheless, for routine large-scale typing, a more quantitative and rapid method of assaying cytotoxicity on an equivalent microlevel would be more desirable. A microcomplement-fixation test was not as sensitive in our hands and entailed the unnecessary introduction of an extra step, as does globulin consumption. Lympho-agglutination, which circumvents the introduction of complement to the antibody-antigen mixture, was found to be less sensitive than the cytotoxicity test by a factor of 10 or more.

The lymphotoxicity reaction was found to be of potential value in avoiding early renal homotransplant failure. In one patient who suffered an "immediate rejection," cytotoxins against lymphocytes of the kidney donor were shown in a sample of serum obtained *before* grafting. Pre-existence of humoral factors has been suspected as being the cause of an immediate reaction by Hamburger *et al.* (1962), but no direct test of pregraft serum with the donor cells was performed. Although the possible pre-existence of antibodies against transplantation antigens was considered by Dempster (1963) in his recent analysis of the anuria phenomenon, it was dismissed as unlikely. From Dempster's review, it appears that as many as 50 percent of clinical renal transplants are anuric for more than 48 hr, though such a high incidence does not occur with canine renal transplants. A point of particular difference between the "first set" reaction of humans and dogs which is not considered by Dempster is the fact that, in contrast to dogs, most uremic patients have usually been transfused prior to grafting. Blood transfusions could lead to formation of antibodies again leukocytes which could immediately cross-react with kidney cells (Terasaki and McClelland, 1963). The "cross match" test in which the serum of the prospective recipient is tested with the donor's cells *before* transplantation is done easily and may be important in preventing a certain number of early rejections. This consideration, however, does not detract from the importance of technical factors, such as minimal ischemia time, which have been shown to play a major role in securing immediate renal function (Starzl *et al.*, 1964b).

With respect to the role of the ABO red-cell antigens, it appears that the A and B incompatibilities should be avoided in general (Starzl *et al.*, 1964a). However, that A and B antigens function as transplantation antigens, or are present on vital sites of a kidney graft, has not been established. Some reason to suspect the above conjectures is the finding that a kidney from a type B person survived in a type A recipient (Starzl *et al.*, 1964a) and is still providing normal renal function after 17 months.

Whether the "minor" erythrocyte antigens are at least of secondary importance has not yet been settled. Woodruff and Allen have reported (1953) an instance in which, in spite of complete matching for 10 antigens, exchange skin homografts were rejected within 3 weeks. On the other hand, in an interesting case described by Peer and co-workers (1960), in which a mother-to-son skin homograft survived for 238 days, only K was incompatible out of 13 antigens tested. A brother's graft also survived for 56 days; the only incompatibility was with Fy^a. Further indication for the influence

of "minor" erythrocyte groups in transplantation is given by Rogers (1963). From the present initial trials, survival for more than 6 months was obtained in spite of incompatibilities with E, Fy^a, M, C, D, s, and c (Fig. 1). Perhaps some significance could be attached to the fact that seven out of 10 long-term survivors (10 to 18 months) possessed two or three antigen incompatibilities. Moreover, in one patient, anti-CD antibodies were found together with good renal function 10 months after grafting. The conclusion which might be indicated is that matching of "minor" erythrocyte antigens may increase the chance of success, though complete matching may not be obligatory.

Summary

A microdroplet lymphocyte-cytotoxicity reaction was tested as a means of predicting histocompatibility matching by titrations of 50 different allogenic human antisera. As the trial system, 11 kidney homograft recipients who have survived 6 to 18 months following transplantation were tested for compatibility with their respective donors. The degree of incompatibility expressed in terms of numbers or units of mismatched antigens was relatively small when compared with that of hypothetical grafts from unrelated donors typed by the same methods. Though many improvements in the typing system remain to be made, it is suggested that with further experience, this method of histocompatibility typing may be practicable in uncovering major transplantation "types."

Acknowledgments

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Discussion

DR. CEPPELLINI: What is the reproducibility of your test?

DR. TERASAKI: Four persons were typed with 50 different antibodies on three separate days and an average of less than three-tenths of a tube difference was obtained. The percent of living cells at each dilution may vary somewhat from day to day, as with most serological reactions.

DR. METZGAR: I would like to point out that Dr. Terasaki is dealing with 50 antisera, and that one may have a good duplication of the antibodies in these various sera, so that the same antibody could conceivably be found in perhaps 10 sera and would pick out by this method, 10 incompatibilities. In reality, we are really measuring incompatibilities for one antigen. In addition, even when these multispecific antisera react with both the donor and recipient cells they may not be detecting the same antigen, so that by your classification two compatibilities may indeed be incompatibilities. This is the danger of using antisera containing mixtures of antibodies *vs.* monospecific antisera. I would also like you to comment on the rather long-term survivor that had many incompatibilities with the donor lymphocytes by your typing method.

DR. TERASAKI: Your last question goes back to what you said earlier: that it is possible that some of these antisera contain similar antibodies and it's not quite clear what proportion of duplicated antibodies one is dealing with if one counts up the number of these matches by this kind of system. We have only done this as a tentative sort of thing to see if, by doing so, we come out with some sort of order in the long-term kidney-homograft survivors. Certainly, we would feel very strongly that it is much better to end up with pure antibodies than mixed antibodies, and we would like to accomplish this. We have used the method described only as a first trial, rather than spending time trying to purify the antisera, because we did experience difficulty in absorption of antibodies. If it should turn out that each antiserum contains as many as 50 separate antibodies, many years of work would be required to obtain "unit" reagents.

DR. WALFORD: We are also of the persuasion, as I believe Dr. Terasaki to be, that

for serologic typing of human transplantation antigens, the lymphocyte offers a great deal of promise. Rather than using sera of multiparous women, we have investigated lymphocyte cytotoxicity of human sera following actual skin grafts, and after intradermal leukocyte injections. Table 1 shows the titers of such sera in relation to the time of grafting or injection in one of our donor-recipient pairs. All grafts in this table were from this same donor to the same recipient. The donor's lymphocytes were used as the test cell in the cytotoxicity test. The numbers refer to the percent of lymphocytes killed by the serum at each dilution. It is clear that antilymphocyte antibodies were first demonstrable following the second-set graft, and that they increased mildly in strength or avidity with successive grafts. Injection of leukocytes at a much later date (210 days after the fourth-set skin graft) caused a marked increase in the titer of the antibodies. We have also studied these antisera by alcohol fractionation and find that the great majority, and perhaps all, of the antibody activity resides in the gamma-globulin fraction.

I believe our results may strengthen Dr. Terasaki's very important contribution, for they prove that the lymphocyte cytotoxicity phenomenon which he finds with post-pregnancy serum can also be demonstrated after skin grafting *per se*.

TABLE 1

Lymphocyte Cytotoxicity in Relation to Time of Skin Grafting and Leukocyte Injection. Skin Grafts at Approximately 36-day Intervals; Leukocyte Injection 210 Days After Last Graft.

Serum Specimen	*Titer of Recipient Serum vs. Donor Lymphocytes*					
	1:2	*1:8*	*1:32*	*1:128*	*1:512*	*1:2048*
Pregraft	0	1	—	—	—	—
14 days after 1st set	1	5	—	—	—	—
14 days after 2nd set	43	34	9	—	—	—
15 days after 3rd set	80	50	14	4	—	—
12 days after 4th set	86	70	14	0	—	—
7 days after leukocyte injection	95	61	58	67	51	12