The New England
Journal of Medicine

Volume 280 APRIL 3, 1969 Number 14

SIGNIFICANCE OF THE POSITIVE CROSSMATCH TEST IN KIDNEY TRANSPLANTATION*

RAMON PATEL, M.R.C.P., AND PAUL I. TERASAKI, PH.D.

Abstract Crossmatch tests of the prospective kidney-transplant donor's lymphocytes with the serum of the prospective recipient in 225 transplants showed that eight of 195 with negative crossmatch failed to function immediately, in contrast to 24 of 30 with positive crossmatch (p less than 0.001). Immediate failure occurred in significantly higher numbers among patients with a higher risk of having antibodies, such as multiparous females and patients receiving secondary transplants. The effect was not a nonspecific one, for more immediate failures occurred among transplants from unrelated than among those from related donors. The corresponding frequency of positive crossmatch was also lower among related donors. The presence of preformed cytotoxic antibodies against the donor appears to be a strong contraindication for transplantation.

PREFORMED allogeneic antibodies present in a recipient were first postulated as being responsible for immediate failure of a kidney transplant in 1964.[1] At that time it was suggested that a crossmatch test of the prospective recipient's serum against the donor's cells could be of importance. In the intervening four years, additional evidence[2-5] has confirmed the dramatic destruction of kidney grafts in recipients who have preformed antibodies. Immediate failure has also been observed when cytotoxic antibodies were not present,[4] and a case in which failure did not occur in spite of a positive crossmatch has been reported.[2]

Because prospective kidney-transplant patients are often sensitized, it is of critical importance to determine the clinical outcome when a sufficiently large number of these patients have received transplants. With the help of many transplant centers over the past four years, we have accumulated the results of 248 kidney transplants performed in 63 patients with preformed cytotoxic antibodies and 163 without. It is the purpose of this communication to document the high risk of immediate failure (43 per cent) when kidneys are transplanted into recipients with preformed antibodies and an even greater risk (80 per cent) when a direct positive crossmatch can be demonstrated.

*From the Department of Surgery, University of California, Los Angeles, School of Medicine (address reprint requests to Dr. Patel at the Department of Surgery, University of California Center for the Health Sciences, Los Angeles, Cal. 90024).

Supported in part by research grants (AM 02375, AM 07513 and AI 04444) from the National Institutes of Health, United States Public Health Service, and by a contract (PH 43 65 994) with the National Institute of Allergy and Infectious Diseases (computing assistance obtained from the Health Sciences Computing Facility, UCLA, sponsored by NIH Grant FR-3).

MATERIAL AND METHODS

The 226 kidney-transplant recipients were bled before transplantation, and the serums tested against lymphocytes of 10 to 40 randomly selected persons.[6] On the basis of these reactions, serums were divided into three groups: positive (those reacting with 20 per cent or more of cell samples of random persons); negative (those reacting with less than 10 per cent of cell samples of random persons); and doubtful (those reacting with 10 to 20 per cent of cell samples of random persons). Whether the antibodies detected against random lymphocytes were specific to the donor's lymphocytes was determined by crossmatching of the recipient's serum in three different volumes of 0.0005, 0.0015 and 0.0045 ml against the donor's lymphocytes. Clinical data pertaining to the patients were kindly supplied by the transplant centers concerned.

RESULTS

Occurrence of Preformed Cytotoxins

Preformed cytotoxic antibodies against lymphocytes of random persoms were present in 131 serum samples among 681 prospective recipients of first kidney transplants – a figure of 19.2 per cent (Table 1). This proportion is somewhat lower than that previously reported from our laboratory in 218 recipients[2] and could reflect improvements in typing technics.

The female recipients had a significantly higher

TABLE 1. *Frequency of Preformed Cytotoxic Antibodies in 681 Prospective Recipients of Kidney Transplants.*

SEX	NO. POSITIVE	NO. NEGATIVE	NO. DOUBTFUL	TOTALS
M	62 (15.0%)	319 (77.4%)	31 (7.5%)	412
F	69 (25.7%)	179 (66.5%)	21 (7.8%)	269
Totals	131 (19.2%)	498 (73.1%)	52 (7.6%)	681

proportion of antibodies (25.7 per cent) than the male recipients (15.0 per cent) (p less than 0.001). The possible influence of pregnancy was ascertained by a comparison of the frequency of cytotoxins in female recipients with and without previous pregnancies. A highly significant difference could be shown, for among prospective female recipients, 16 of 36 who had been pregnant (44.4 per cent) had cytotoxic antibodies and only three of 35 who had not been pregnant (8.6 per cent) had antibodies (p less than 0.005). Since cytotoxins have not been demonstrated in normal males, the figure of 15.0 per cent among male uremic recipients may be regarded as being due to immunization from the transfused blood, and it did not differ significantly from that in female recipients without pregnancies (p greater than 0.3 but less than 0.4).

Correlation of Immediate Graft Failures with the Results of the Crossmatch Test

Of the 226 recipients studied, 63 did, and 163 did not have preformed antibodies against lymphocytes of randomly selected persons before transplantation (Table 2). Thirty-eight of a total of 248 grafts failed immediately. They included grafts that never functioned and those that failed on the operating table or within a few hours of the completion of vascular anastomosis. Grafts that failed as a result of ABO incompatibility or technical problems were not considered. Thirty-four (42.5 per cent) of 80 grafts performed in recipients with preformed antibodies were rejected immediately in contrast to only four (2.4 per cent) of 168 grafts in recipients without preformed antibodies (p less than 0.001).

TABLE 2. *Classification of 248 Kidney Transplants Performed in 63 Recipients with and 163 without Preformed Antibodies According to the Duration of Graft Survival.*

GRAFT SURVIVAL	RECIPIENTS WITH ANTIBODIES			RECIPIENTS WITHOUT ANTIBODIES
	POSITIVE CROSSMATCH	NO CROSSMATCH	NEGATIVE CROSSMATCH	
Immediate failures	24 (80.0%)	6 (26.1%)	4 (14.8%)	4 (2.4%)
Failure within <3 mo	0	6	4	32
Failure after >3 mo	1	3	7	22
Survival for <3 mo	2	2	1	6
Survival after >3 mo	3	6	11	104
Totals	30	23	27	168

To study the effect of the crossmatch test, recipients with preformed antibodies were subdivided into three groups: those who gave a positive crossmatch test; those in whom the crossmatch test was not done; and those who gave a negative crossmatch test (Table 2). Of the 30 grafts performed in recipients with positive crossmatches, 24 (80 per cent) failed immediately, and one (3.3 per cent) was rejected at four months.

Two of the five grafts currently surviving were only transplanted two weeks and three months ago and could not be considered to have an adequate period of follow-up observation for assessment of the possible delayed influence of these donor-specific antibodies. Two other grafts, which have now survived 15 and 16 months, were tested only once against the donor's cells during an early period when our technical-error rate may have been higher. The recipient of the last graft in this group from a parental donor had a period of unexplained anuria of two and a half weeks, but the graft was functioning well six months later.

Six of 23 grafts (26.1 per cent) also failed immediately in recipients without crossmatches, as did four of 27 grafts (14.8 per cent) in recipients with negative crossmatches. In contrast, only four of 168 grafts (2.4 per cent) failed immediately in recipients who had no preformed antibodies in the serum and had negative crossmatches. The importance of a positive crossmatch test can be shown by a comparison of the frequency of immediate graft failures in recipients with positive and those with negative crossmatches. As shown in Table 2, 24 of 30 grafts (80 per cent) failed immediately when performed in the face of positive crossmatches. On the other hand, only eight of 195 (4.1 per cent) (27 with negative crossmatches, but with antibodies against random cells, plus 168 without antibodies against random cells) failed immediately in the group with negative crossmatches. This difference is statistically highly significant (p less than 0.001).

It may be noted from Table 2 that the immediate failures were considerably more frequent in recipients with preformed antibodies even if no crossmatches had been done (26.1 per cent) or a negative crossmatch had been obtained (14.8 per cent) than in those without preformed antibodies (2.4 per cent). Thus, patients with cytotoxic antibodies have a higher risk of immediate failure, as might be expected, if a transplant is done without a direct crossmatch test. The difference can most probably be explained by the occurrence of occasional false-negative crossmatch results or a failure of nonspecific cross stimulation.

It appears that a preformed antibody has its effect early, and does not necessarily affect the long-term outcome, for no statistically significant correlation was found in approximate clinical ranks of patients with or without preformed antibodies if immediate failures were excluded.

Donor Source and Immediate Graft Failures

The proportion of immediate failures among 247 related and 166 unrelated (143 cadaver and 23 living unrelated) donor transplants for which data on clinical outcome are available is shown in Table 3. Although the peculiar problems associated with the use of cadaver kidneys (such as ischemia) must undoubtedly contribute to the higher (15.4 per cent) rate of failure of cadaver donor transplants, the possible importance of the donor source is suggested by the fact that three of 23 grafts (13 per cent) from living unrelated donors also failed immediately. This difference is statistically significant for the related and cadaver donor transplants (p less than 0.005) and related and total unrelated donor transplants (p less than 0.005), but not for the related and living unrelated donor transplants. A reason for this may be the small number (23) of the latter available for study.

TABLE 3. *Immediate Graft Failures in 413 Kidney Transplants — Influence of Donor Source, Sex of Recipient and Graft Number.*

DATUM	TOTAL GRAFTS	IMMEDIATE FAILURES	PERCENTAGE	P VALUE
Related donor	247	13	5.3	
Unrelated donor:				<0.005
Cadaver	143	22	15.4	
Living	23	3	13.0	
Male recipient	256	16	6.3	
Female recipient	157	22	14.0	<0.01
1st graft	378	24	6.3	
2d graft	31	13	41.9	<0.001
3d graft	4	1	25.0	

Corresponding to this higher rate of immediate failures was a significantly higher proportion of positive crossmatches among recipients of unrelated donor transplants. Table 4 shows the occurrence of positive crossmatches among 318 recipients of potential related and 103 recipients of potential cadaver donor transplants. Positive crossmatches were nearly three times as frequent among recipients of unrelated donor (that is, cadaver) than among recipients of related donor grafts (p less than 0.005).

TABLE 4. *Rate of Positive Crossmatch in 421 Recipients against Potential Related and Unrelated Donors.*

SEX	RELATED DONORS		UNRELATED DONORS	
	POSITIVE CROSSMATCH	TOTALS	POSITIVE CROSSMATCH	TOTALS
M	10 (5.2%)	194	12 (20%)	60
F	12 (9.7%)	124	9 (20.9%)	43
Totals	22 (6.9%)	318	21 (20.4%)	103

Sex Distribution in Immediate Graft Failures

Since female recipients have a higher rate of preformed antibodies, immediate graft failure may be expected to occur more frequently among them. Among 413 transplants, the incidence of immediate failure occurred in 14 per cent of 157 transplants in females whereas the figure in 256 transplants in males was only 6.3 per cent (p less than 0.01) (Table 3). Of the 67 female recipients of first grafts for whom pregnancy data were available, in 35 who had one or more pregnancies before transplantation, there were eight immediate failures (22.9 per cent failure rate), whereas only two of 32 grafts (6.3 per cent) failed immediately in women who had never been pregnant. This difference fell short of statistical significance (p less than 0.05 but greater than 0.01), although the number of cases available for study is small.

Multiple Transplants and Immediate Graft Failures

Cytotoxic antibodies may develop as a result of homograft rejection and may be detected especially after transplant nephrectomy.[7] On this basis, immediate failures might be expected to be more frequent among second and subsequent transplants than among first transplants. The 413 grafts (38 immediate failures and 375 that survived the immediate postoperative period) were subdivided according to whether they were first, second or third transplants (Table 3), and from these data, the rate of immediate failures among first, second and third transplants was calculated. The figure for immediate failures among 31 second and four third transplants combined (40.0 per cent) was significantly higher than among 378 first transplants (6.3 per cent) (p less than 0.001).

DISCUSSION

According to Registry statistics,[8] 20 per cent cadaver, 13 per cent unrelated living, and 5 per cent related donor kidney transplants fail to function. These figures were almost exactly those found in the present study. Because large numbers of grafts from living, related donors are currently being performed, even a 5 per cent rate of acute rejection should no longer be acceptable. The association of preformed cytotoxic antibodies and the direct positive crossmatch test with immediate failure is now extremely high. From the data presented, if a prospective recipient has no preformed cytotoxic antibodies, his chance of an immediate failure is about only 2.4 per cent. Although some of the failures might be attributed to insensitivity of the crossmatch test, it seems more likely that this rate of failure might be expected for a variety of technical reasons. In contrast to this, if the recipient is known to have cytotoxic antibodies directed against lymphocytes from random persons, the risk of failure becomes significantly higher. By current means of testing, even if such a recipient has a negative crossmatch test, his chance of immediate failure becomes 14.8 per cent. Thus, it is likely that in about 10 per cent of these patients with antibodies a false-

negative crossmatch has been obtained. Some of this error is probably from technical causes, such as anticomplementariness of the uremic patient's serum. Another fraction may be accounted for by cross reactions, so that in presensitized persons, antigens that only weakly react with the antiserum may stimulate a high booster response within a few days. Recipients with antibodies in whom a crossmatch test was not done would be expected to have a failure rate between those giving positive and those giving negative crossmatches. As shown here, the risk to such recipients was 26 per cent, which was higher than the 14 per cent in recipients with negative crossmatches.

Among the key recipients in whom a positive crossmatch test could be shown, the failure rate was conspicuously different from that of the recipients of the categories discussed so far. Twenty-four of 30 (or 80 per cent) of kidneys transplanted across a positive crossmatch test had failed immediately. It might be questioned why the failure rate was not 100 per cent if the antibodies were directly responsible for the rejection. The possibility that at least some of the six grafts that escaped immediate destruction were in fact cases of false-positive reactions cannot be excluded with certainty, particularly since four of the six were from cadaver donors. Preparation of purified lymphocyte suspension is often difficult in cadaver donors, in whom contamination with high granulocyte count has been shown to give an excess of positive reactions – probably owing to the greater susceptibility of granulocytes to nonspecific cytotoxicity by rabbit complement. In addition, cadaver donors have often been transfused so that lymphocytes tested may not have been entirely of the donor origin. These technical errors are undoubtedly correctable; nevertheless, with the methods used whenever a positive crossmatch was found, 80 per cent of the kidneys had failed immediately. It may also be noted that one of the six grafts that survived the immediate post-transplant period was subsequently rejected at four months, and two were only recently transplanted (two weeks and three months ago). Thus, within limits of technical errors, true prolonged graft survival in the face of positive crossmatch must be considerably less common than the rate of 20 per cent suggested by the present data.

The conclusion that allogenic antibodies can produce rapid destruction of a kidney graft is supported by the fact that immediate rejection was shown to occur more frequently in patients with a greater tendency to have allogenic antibodies. Thus, immediate rejection was more common in females, in whom antibodies occurred nearly twice as often as in males. Likewise, patients who had rejected previous kidney transplants, and who are known to have a higher proportion of antibodies,[7] had more frequent immediate rejections than those receiving their first grafts. This also appeared to be true for patients receiving kidneys from unrelated donors, in whom positive crossmatches were likely to be encountered more often than in related donors. Results of animal experiments in which injury to the graft was produced by passive transfer of specific antibodies may be cited as additional evidence for the destructive role of allogenic antibodies.[9-12] The mechanisms by which this may occur have recently been discussed by Starzl and his associates[4] and Williams and his colleagues.[5]

In conclusion, the finding that 24 of 30 kidneys transplanted across a positive crossmatch failed immediately, in contrast to eight of 195 transplanted across a negative crossmatch, suggests that this test should be done routinely for all clinical transplants. The ethics of transplanting kidneys without the prior knowledge of the results of the crossmatch test, or across a known positive crossmatch result, can reasonably be expected to be questioned in the face of this evidence.

We are indebted to the following physicians for supplying the clinical data on patients: Drs. J. P. Merrill, J. E. Murray and C. B. Carpenter (Boston); Drs. T. E. Starzl, T. E. Marchioro, L. Brettschneider and D. Ogden (Denver); Drs. M. Rubini and M. Koppel (Wadsworth Veterans Administration Hospital); Drs. D. Martin, R. Goldman and H. Gonick (University of California, Los Angeles); Drs. S. Nakamoto and R. Straffon (Cleveland); Drs. D. Hume, M. Williams, H. M. Lee and R. Rolley (Richmond); Dr. J. Figueroa (New Orleans); Drs. J. Dossetor and J. Oh (Montreal); Dr. W. J. Flannigan (Little Rock); Dr. J. G. Turcotte (Ann Arbor); Drs. T. Berne and B. Barbour (Los Angeles County); Dr. J. Cerrili (Columbus); Drs. R. Fine and E. Lieberman (Childrens Hospital of Los Angeles); Drs. L. B. Berman and V. Vertes (Mount Sinai Hospital of Cleveland); and Drs. L. Stevens, R. Maddox and K. Reemstma (Salt Lake City).

REFERENCES

1. Terasaki, P. I., Marchioro, T. H., and Starzl, T. E. Sero-typing of human lymphocyte antigens: preliminary trials on long-term kidney homograft survivors. In Conference on Histocompatibility Testing, Washington, D.C., 1964. *Histocompatibility testing: Report of a conference and workshop sponsored by the Division of Medical Sciences, National Academy of Sciences, National Research Council, 7-12 June, 1964. Conference on Histocompatibility Testing.* Edited by P. S. Russell and H. J. Winn. *Workshop on Histocompatibility Testing.* Edited by D. B. Amos. Washington, D.C.: National Research Council, 1965. Pp. 83-95.
2. Terasaki, P. I., Thrasher, D. L., and Hauber, T. H. Serotyping for homotransplantation. XIII. Immediate kidney transplant rejection and associated preformed antibodies. In *Advance in Transplantation: Proceedings of the First International Congress of the Transplantation Society, Paris, 27-30 June, 1967.* Edited by J. Dausset et al. Copenhagen: Munksgaard, 1968. Pp. 225-229.
3. Kissmeyer-Nielsen, F., Olsen, S., Petersen, V. P., and Fjeldborg, O. Hyperacute rejection of kidney allografts associated with pre-existing humoral antibodies against donor cells. *Lancet* **1**:662-665, 1966.
4. Starzl, T. E., et al. Shwartzman reaction after human renal homotransplantation. *New Eng. J. Med.* **278**:642-648, 1968.
5. Williams, G. M., et al. "Hyperacute" renal-homograft rejection in man. *New Eng. J. Med.* **279**:611-618, 1968.
6. Terasaki, P. I., Vredevoe, D. L., and Mickey, M. R. Serotyping for homotransplantation. X. Survival of 196 grafted kidneys subsequent to typing. *Transplantation* **5**:1057-1070, 1967.
7. Morris, P. J., Williams, G. M., Hume, D. M., Mickey, M. R., and Terasaki, P. I. Serotyping for homotransplantation. XII. Occur-

rence of cytotoxic antibodies following kidney transplantation in man. *Transplantation* **6**:392-399, 1968.
8. Murray, J. E., and Barnes, B. A. World-wide status of kidney transplantation. In *Human Transplantation*. Edited by F. T. Rapaport and J. Dausset. New York: Grune, 1968. Pp. 45-60.
9. Terasaki, P. I., Akiyama, T., McClelland, J. D., and Cannon, J. A. Renal damage produced *in vivo* by homologous mouse antisera. *Ann. New York Acad. Sc.* **99**:645-656, 1962.
10. Altman, B. Tissue transplantation; circulating antibodies in homotransplantation of kidney and skin. *Ann. Roy. Coll. Surg., England* **33**:79-104, 1963.
11. Najarian, J. S., and Perper, R. J. Participation of humoral antibody in allogenic organ transplantation rejection. *Surgery* **62**:213-220, 1967.
12. Dubernard, J. M., Carpenter, C. B., Busch, G. J., Diethelm, A. G., and Murray, J. E. Rejection of canine renal allografts by passive transfer of sensitized serum. *Surgery* **64**:752-760, 1968.

HEMOGLOBIN SABINE BETA 91 (F 7) LEU ⟶ PRO*

An Unstable Variant Causing Severe Anemia with Inclusion Bodies

ROSE G. SCHNEIDER, PH.D., SATOSHI UEDA, M.D., JACK B. ALPERIN, M.D., BERNADINE BRIMHALL, PH.D., AND RICHARD T. JONES, M.D., PH.D.

Abstract Hemoglobin Sabine ($\alpha_2\beta_2$ 91 leu → pro) comprises 8 per cent of the hemoglobin of a 16-year-old Scotch-English-German girl who has suffered from hemolytic anemia since infancy. The spleen was removed at 18 months. She has about 9 gm of hemoglobin per 100 ml, of which 12 per cent is fetal, and a red-cell count of 2,500,000, with many reticulocytes and erythrocytic inclusions readily demonstrable on fluorescence microscopy. The erythrocyte half-time measured with ^{51}Cr is four days. Hemoglobin Sabine is deficient in heme-binding capacity, is easily converted to methemoglobin and precipitates readily on mild heating or storage. Replacement of the leucine by a proline residue at β 91 in the helical position F 7 evidently disrupts the helical sequence of the globin molecule at a point adjacent to its chief heme contact. Hemoglobin Sabine probably arose as a mutation in the proposita.

AFTER the description by Cathie,[1] in 1952, of an "apparent idiopathic Heinz body anaemia," a number of similar case histories were recorded,[2] and a recognizable syndrome has emerged. This is characterized by an unremitting hemolytic anemia of early onset, without specific erythrocyte enzyme deficiencies, but with numerous intraerythrocytic inclusion bodies, appearing after splenectomy or on in vitro incubation of the erythrocytes. In the hemolysate in one such case, Grimes and Meisler[3] found methemoglobin and a heat-precipitable fraction, and suggested that the inclusion bodies are denatured products of an unstable hemoglobin. Since then, several unstable hemoglobin variants have been implicated in hemolytic anemias of varying severity. We now report on hemoglobin (Hb) Sabine, β 91 (F7)† leu → pro, a new cause of unstable hemoglobin anemia, and on studies of its erythrocytic inclusions.

METHODS

Clinical and hematologic data were obtained by standard methods, and survival tests were performed on ^{51}Cr-labeled red cells.[5] Hemolysates were prepared from erythrocytes washed with 0.85 per cent sodium chloride and hemolyzed by the addition of 1 vol of water and 0.4 vol of toluene. Hemoglobin electrophoresis was performed on starch-gel and cellulose acetate, tris-ethylenediaminetetracetic acid boric acid (TEB) buffer, pH 8.4 to 8.6.[6] Quantitative evaluation of adult hemoglobins was obtained by chromatography on diethylaminoethyl (DEAE) Sephadex.[7] Fetal hemoglobin content was determined by the alkali denaturation method of Betke et al.[8] and by chromatography on CM Sephadex C50.[9]

Immunodiffusion was performed in agar, as previously described.[10] Methemoglobin was determined by the method of Evelyn and Malloy[11] on blood samples in Alsever's solution. Heme-protein ratios were obtained by comparison of optical densities of cyanmethemoglobin solutions at 540 and at 280 mμ, with the use of standard curves established with normal hemoglobin. The presence of a thermolabile hemoglobin fraction was determined by the method of Dacie et al.[12] Globin electrophoresis was performed in urea barbital buffer,[13] and inclusion bodies were extracted according to the procedure of Fessas, Loukopoulos and Kaltsoya.[14] Blood smears were examined for inclusion bodies after staining with brilliant cresyl blue, and they were stained for hemoglobin F by the method of Kleihauer and Betke.[15] For fluorescence microscopy unfixed, unstained smears were mounted in "entallen" and examined in a Wild Fluorescence Microscope with a dark-field condenser and a Mercury HB 200 bulb as light source. For ultraviolet fluorescence, the

*From the departments of Pediatrics and Medicine, University of Texas Medical Branch (address reprint requests to Dr. Schneider at the Department of Pediatrics, John Sealy Hospital, Galveston, Tex. 77550) (Dr. Ueda's present address is Yamaguchi University Medical College, Ubi, Yamaguchi Prefecture, Japan).

Supported in part by grants (AM 00780 and CA 07941) and a training grant (TI AM 5208) from the United States Public Health Service. Some of these studies were performed while patients were hospitalized in the Clinical Study Center, University of Texas Medical Branch, under support of a grant (FR-73) from the Division of Research Facilities and Resources, United States Public Health Service.

†The nomenclature is that of Perutz[4] with the eight helixes lettered A to H, and the amino acid residues numbered consecutively within each helix.