Antibodies and Human Transplant Rejection

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Recent studies in "hyperacute rejection" have stimulated interest in the role of antibody in human allograft rejection. This dramatic form of graft rejection was reported to occur in recipients who prior to transplantation had serum antibodies reactive to the histocompatibility antigens of the donor.[4, 14, 19] Experience has shown that not all recipients having cytotoxic antibodies to the donor's lymphocytes have violent rejections,[9] and further some recipients having early intravascular coagulation in the allograft did not have detectable antibodies to the donor's lymphocytes.[12, 15] These observations have raised clinically significant questions. First, is the cross-match between the recipient's serum and the donor's lymphocytes a reliable means for excluding the presence of antibodies having pathogenic effects upon the graft? If not, what technics are available for the performance of a valid cross-match? Second, if high levels of circulating antibody were acutely toxic to the graft, what is the effect of lower levels of the same antibody or of antibodies reacting with less accessible antigens? Finally, what is the extent of the clinical problem posed by patients rejecting multiple transplants? The studies to be reported here represent attempts to answer these questions based on correlations between the clinical course and serological findings accompanying 21 human allografts.

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Material and Methods

The patients reported in this study were hospitalized at the Clinical Transplantation Center at the Medical College of Virginia with one exception, patient D. L. Patient D. L. received a kidney from a cadaver in Richmond and underwent transplantation in Washington, D. C. by the Transplantation Service at Georgetown University. The patients reported are those whose serological response to the transplant could be monitored by reactions with cultured kidney cells from the organ donor. The group includes all those patients who rejected a kidney transplant that could be successfully cultured, and all who received a cadaveric organ where one kidney from the cadaver was not transplanted but placed into tissue culture. The technics of transplantation and of immunosuppression that were used were the same as those reported previously.[3] A small group of the patients received intramuscular injections of horse antilymphocytic globulin prepared by Monaco.[6] The serological and tissue culture technics used are identical to those described previously.[15, 16, 18]

TABLE 1. *The Time Distribution of the Numbers and of Types of Renal Allografts Performed at the Medical College of Virginia*

Period	No. of Transplants		
	All Primary	Secondary Cadaver	Functional Secondary Cadaver
1963–66	102	11	11
1967	11	7	3
1968	11	5	1
1969 (Jan–May)	4	4	2

TABLE 2. *Hospital Time and Success Rate of Secondary Renal Allografts*

Total number of patients	23
Total patient-months of hospitalization to date	154
Number of patients with currently functioning allografts	9
Number of patients whose allografts have functioned more than 1 year	7
Number of patients whose allografts could have functioned more than 1 year	19
Mean patient-months of hospitalization required per successful secondary allograft at 1 year	20.3

Results

The Clinical Problem

During the first 4 years of the renal transplantation program at the Medical College of Virginia 102 primary and 11 secondary kidney allografts were done (Table 1). Ninety-seven of the 102 primary and all of the secondary transplants functioned. This rate of patient turnover stopped in 1967. Only 11 new patients received kidney grafts in 1967 and 1968. Twelve secondary transplants were done of which only four had any significant period of function.

The time, effort and expense of caring for patients with allograft rejection was considerable (Table 2). Seven patients had good renal function one year following secondary transplantation out of a total of 19 patients who could have had successful function at 1 year. This group of 19 patients undergoing secondary transplantation required a sum total of 142 patient-months of hospitalization. Six patients accumulated 102 patient-months of hospitalization during which time they received nine kidney grafts which never functioned. Only one of these patients is still alive. The mean number of patient-months of hospitalization expended to produce one graft successful at one year was 20.3. Bed space on the Transplantation Unit was pre-empted by patients whose grafts had failed and who continued to reject additional grafts.

In order to define the reason for the high failure rate of secondary transplants occurring after 1966, a search was first made for possible changes in technic. An analysis of the success rate of primary and secondary transplants from cadaveric donors revealed that there was no comparable decline in the success rate for primary grafts. Functional grafts were obtained in 39 of 43 primary transplants, but in only 17 of 27 secondary transplants. Technical factors complicating transplantation were clearly present in three of four primary transplants that failed. However, no apparent technical faults contributed to the failure of the ten secondary grafts. Early biopsies taken from nine secondary transplants revealed large numbers of polymorphonuclear leukocytes in the renal capillaries on eight occasions. Thus other than the small sample size, there was no obvious explanation why secondary transplants functioned uniformly well prior to 1967 but failed to have any period of function in over one half of the transplants carried out after 1967. Paradoxically, prior to 1967 the recipients of second transplants were matched with their donors for ABO compatibility only.

Rejection and Preformed Antibodies

Early in our experience five kidney grafts were placed into recipients whose serum

showed cytotoxicity to the donor's lymphocytes. Three of the five kidneys were removed at the time of insertion on the basis of extreme cyanosis and dramatic reductions in blood flow. The two kidneys which were not removed at transplantation never functioned (Table 4).

A negative cytotoxicity reaction between the recipient's serum drawn within 2 weeks of transplantation and the donor's lymphocytes formed the basis for the selection of donors for 11 transplants. Despite this precaution, six kidney grafts failed to function, although none of these reactions were sufficiently violent to require resection of the graft at the time of transplantation. One liver recipient and one heart transplant recipient experienced transient function of their grafts, but both died from rejection 7 days after transplantation. All of the recipients were found to have antibody detectable in reactions with the cultured kidney cells of the donor (Table 4).

The serum from three recipients failed to react with either the lymphocytes or the kidney cells of the donor and their grafts functioned normally (Table 4). Two of these patients had previously rejected one kidney transplant and had demonstrable cytotoxic antibodies to other potential donors. Three other patients are enjoying normal renal function 12, 5, and 2 months following transplantation despite the presence of pre-existing serum antibodies reactive against donor kidney cells. The antibody titers were low in two of these recipients

TABLE 3. *The Frequency of Non-functional Renal Allografts in Primary and Secondary Cadaveric Donor Grafts*

Category	Function of Cadaveric Kidneys	
	Present	Absent
1st transplants	39	4*
2nd transplants	17	10**

* Three known technical failures.
** No known technical failures.
Two additional secondary transplants were rejected on A-V shunts.

TABLE 4. *Correlations between Preformed Antibody Activity and the Clinical Outcome of Human Allografts*

Organ	Patient & # Transplant	Technic for Detecting Pre-existing Antibody against Antigens			Clinical Outcome			
		Lympho-Cyto-toxicity	Antibody Titer Mixed Agglut. vs. Kidney Cells	Antibody Titer Imm. Adher vs. Kidney Cells	Graft Removed at Transpl. Oper.	Non-Functional	Transient Function	Functional
Kidney	S. S. #3	+	1/90	1/64	+			
	W. J. #2	+	1/90	1/32	+			
	M. D. #2	+	1/810		+			
	M. D. #3	+				+		
	S. G. #2	+	1/810			+		
	S. S. #2	−	1/90			+		
	P. S. #3	−	1/90			+		
	H. F. #1	−	1/30					+
	E. R. #3	−		1/30		+		
	P. S. #4	−	1/200	1/8		+		
	C. R. #2	−		1/30		+		
	D. P. #1	−		1/2		+		
	D. P. #2	−		1/30				+
	D. L. #1	−		1/2				+
Heart	P. J.	−		1/16			+	
Liver	A. H.	−		1/30			+	
Totals					3	8	2	3

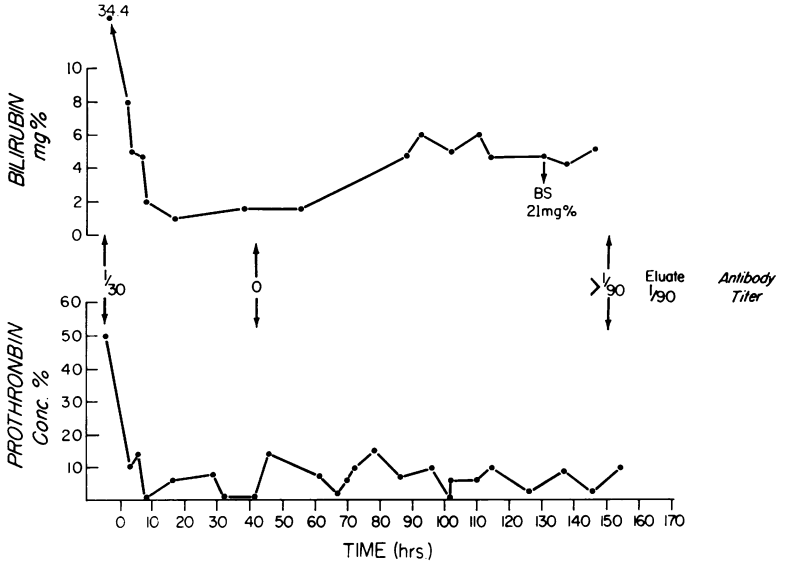

FIG. 1. The postoperative course of A. H., recipient of a liver transplant. Despite daily exchange transfusions the prothrombin concentration remained low and antibody reappeared in the circulation.

(H. F., D. L.) but the third patients (D. P. #2) had a relatively high titer of antibody to donor kidney cells by the immune adherence test (Table 4).

In addition to the 16 grafts listed in Table 4, two kidneys developed extensive accumulations of polymorphonuclear leukocytes in the renal capillaries while being perfused through the recipient's A.V. shunt. Cytotoxic antibodies were present against donor lymphocytes in one instance. In the other, cytotoxic antibodies were not present, but antibodies were detected by immune adherence in reactions with donor kidney cells. Including these latter two kidneys, serum antibody detected in reactions with donor cells was present prior to transplantation on 18 occasions. Thirteen kidney grafts never functioned; three functioned well, and one liver and one heart transplant were rejected at one week. No case of early dramatic failure occurred in which pre-existing antibodies to donor antigens were not detected. Systemic heparinization and massive steroid treatment were ineffective in reversing these fulminating rejections.

The two patients who rejected non-renal transplants acutely are of particular interest. Both recipients were Negroes and both received Negro organs. Neither of the patients had any history of blood transfusions or pregnancies. The course following heart transplantation in P. J. has been described in greater detail elsewhere.[18] The heart functioned well for 4½ days, but at that time uncontrollable rejection occurred. The heart at autopsy was edematous and hemorrhagic. The cellular infiltrate was a mixture of mononuclear and polymorphonuclear leukocytes. The clinical course of the liver transplant recipient was characterized by more rapid graft failure.

Case Report

A. H., a 2-year-old Negro boy with biliary atresia was admitted to the Pediatric Service of the Medical College of Virginia because of deterioration of liver function and ascites.

Eight potential donors were screened over a 2-month period, and, in three instances, the patient's serum produced cytotoxic activity against the potential donor's lymphocytes. The eventual donor of the liver transplant was a 3-year-old child who was accidently shot in the head. Leukocyte typing revealed donor-recipient incompatibilities at HLA-1 and HLA-3. A freshly drawn serum sample from the patient failed to react with the lymphocytes of the donor by cytotoxicity. The decision to transplant the liver from this donor

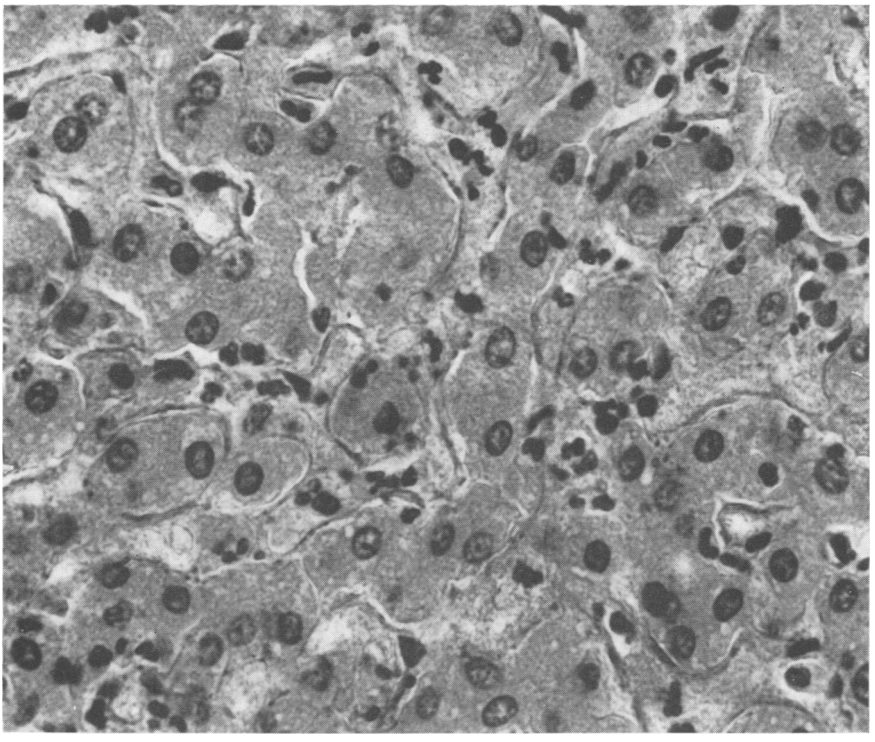

FIG. 2. Liver biopsy from A. H., 10 hours post-transplantation. Polymorphonuclear leukocytes in large numbers were present in the sinusoids.

was difficult in view of the typing results. However, transplantation was undertaken because of the unavailability of small donors and the grave clinical condition of the patient.

The liver transplant was conducted under optimal conditions. The liver donor fulfilled the criteria of brain death and there was no period of hypotension. The total cold ischemia time before restoration of portal circulation was 40 min. There were no arterial anomalies and the liver seemed to perfuse extremely well following revascularization.

The postoperative events are illustrated in Figure 1. There was a rapid fall in serum bilirubin from preoperative values in excess of 30 mg./100 ml. to levels of 1–2 mg./100 ml. The child awakened normally from anesthesia without evidence of abnormal bleeding. However, 4 hours after transplantation the patient developed abdominal distention and had a sudden respiratory arrest. He was resuscitated quickly but required respiratory assistance. Ten hours after transplantation the patient was re-explored because of a further increase in the abdominal girth. At operation diffuse bleeding was encountered, the liver was pale and mottled, and there was a mass in the left lobe. A liver biopsy revealed the accumulation of large numbers of polymorphonuclear leukocytes in the portal areas and sinusoids of the liver (Fig. 2).

A liver scan carried out on the second postoperative day revealed normal gold uptake in the right lobe and no uptake in the left lobe. The patient was treated with Solu-Medrol 1 Gm./day, antilymphocytic globulin 10 cc./day, azathioprine 50 mg./day and daily exchange transfusions. Despite this regimen deepening coma occurred. Hypoglycemia episodes occurred on the 6th day following transplantation and cardiac arrest occurred on the 7th post-transplantation day.

At autopsy the main branches of the portal vein and hepatic artery were patent. The left hepatic artery was dissected distally as far as possible and no thrombi were encountered. Histologically the liver showed evidence of extensive necrosis more marked at the periphery of the liver. Acute and chronic inflammatory cells were present throughout the liver but were more concentrated in the portal areas.

The serological studies demonstrated unmistakable antibody activity to donor kidney cells in the pre-transplantation serum (Fig. 1). One day following transplantation, antibody was not detectable in the circulation, but at the time of demise antibody with a titer in excess of 1–90 was present. The heat eluate prepared from the liver removed at autopsy also contained antibodies reactive against donor kidney cells.

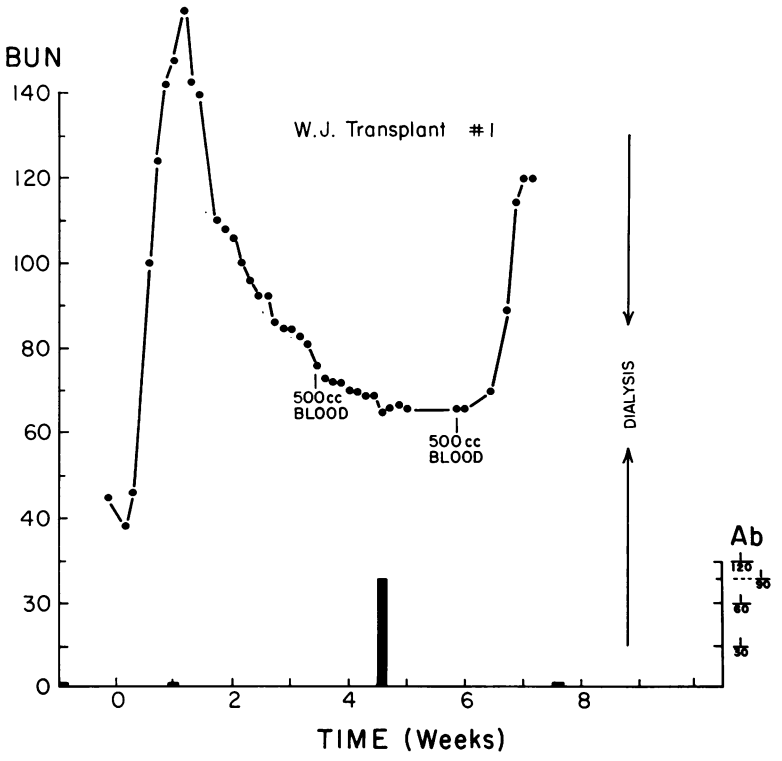

FIG. 3. The relationship between the detection of donor-kidney-cell-reactive antibody and the clinical course of patient W. J. The patient received two units of whole blood and renal function deteriorated 5 days after the second transfusion.

The Detection of Serum Antibody Following Transplantation

The ability of the patients receiving prednisone and azathioprine to synthesize antibody was studied during the course of eight allografts which were eventually rejected. Samples of serum drawn at 2 to 6-month intervals post-transplantation and stored at −70° C. were reacted with kidney cells cultured from the rejected organ. In six instances antibodies reacting with kidney cells from the grafts were detected only following removal of the grafts or at the terminal stages of chronic rejection when the grafts were non-functional.

A significant level of antibody reacting with donor kidney cells was present during the course of two allografts. The clinical course of one of these patients, W. J., is depicted in Figure 3. Here antibody was detected during an interval of stable renal function but following an earlier severe rejection reaction and 13 days prior to the onset of an irreversible rejection crisis. This patient had two whole blood transfusions which have interesting temporal relationships with the time antibody was detected and the onset of fulminating rejection.

On a second occasion the recipient of a heart transplant developed detectable levels of serum antibody active against donor antigens. This patient died from cardiac failure, 6 days after antibody was detected in the circulation.

It was possible to follow the concentration of antibody in eight patients who had antibodies reactive to donor kidney cells prior to transplantation. The antibody titer declined during the first week following transplantation in six of eight instances. On two occasions antibody concentrations rose progressively. One of these renal grafts (D. P. #1) developed stenosis of the renal artery anastomosis. Following correction of the stenosis the antibody concentration

continued to increase. A second patient (P. S. #4) received a graft which never functioned and which was necrotic on removal 13 days following transplantation.

Serum antibody reacting with cultured donor kidney cells was never demonstrated in one case of graft failure. (E. R. #2). This patient developed proteinuria, and the transplant had linear deposits of IgG and BIC globulin along the glomerular basement membrane. Serum drawn prior to this transplant produced significant proteinuria in squirrel monkeys.[11]

The Detection of Antibody in Heat Eluates Prepared from Rejected Allografts

Heating homogenized rejected allografts to 56° C. released antibodies reactive to cultured cells from donor kidneys in 13 of 14 instances (Table 5). Antibody was not recovered from the graft of E. R. #2, nor, as mentioned above could isoantibody be detected following removal of this transplant.

Donor reactive antibody was detected in eluates prepared from organs rejected by hyperacute, acute, or chronic mechanisms. Antibody was recovered from two kidneys removed for hyperacute rejection 2 hours after the initiation of blood flow to the graft. Antibodies were also present in all of the eluates prepared from five kidneys undergoing fulminating early rejection but removed at 1 to 9 weeks following transplantation. The eluates prepared from three chronically rejected kidneys removed at 2, 2 and 3 years were also positive. In each instance the titer of antibody relative to the concentration of IgG present in the eluate was higher than the corresponding values in the serum, suggesting that our positive results could not have been due to contamination of the hemogenates by antibody in the serum.

TABLE 5. *The Detection of Donor-kidney-cell-reactive Antibody in Eluates of Rejected Organs*

Organ	Clinical Type of Rejection	No.	No. with Antibody
Kidney	Chronic	4	3
	Acute	1	1
	Hyperacute	6	6
Heart	Acute	2	2
Liver	Hyperacute	1	1
		14	13

Discussion

During the past 2½ years, 10 of 16 secondary renal allografts performed at the Medical College of Virginia did not function. This experience has been disconcerting particularly since 11 of the secondary grafts carried out prior to 1967 functioned (Table 1). During the past 2 years bed space and medical attention has shifted from the care of postoperative patients to the care of dibilitated "rejectors." In this setting of clinical concern and frustration methods were developed and applied in the hope of distinguishing the critical factors related to the success and failure of secondary allografts.

An early case of "hyperacute rejection" influenced the direction for our studies. In this case, studies carried out in collaboration with Milgrom and Kano[15] demonstrated that, while the recipient's pre-transplantation serum failed to react with the lymphocytes of the donor, there was a clear reaction with the donor's kidney cells. That cells from different tissues contained important differences in antigen concentration was also suggested by the distribution of A and B blood group antigens of recognized importance in human transplantation. These antigens known to be in high concentration on erythrocytes and endothelial cells[13] are demonstrated to be on lymphocytes by extremely sensitive technics[2] and not by lymphocytotoxicity.

Systematic studies comparing the spectrum of activity of an individual's lymphocytes and kidney cells to characterized

sera disclosed significant differences in typing patterns. Discrepancies were noted when kidney cells were typed by mixed agglutination and lymphocytes by cytotoxicity,[16] and discrepancies were also noted when the lymphocytes and kidney cells were typed by a common technic, immune adherence.[1] The latter experiments comparing the reactivity between kidney cells and lymphocytes from the same individual to a panel of 17 well characterized typing sera disclosed discrepancies in over ⅓ of 234 comparisons. Absorption experiments suggested that the differences in typing patterns represented quantitative rather than qualitative differences in the antigen composition of these two cells. For example, sera recognized HLA 7 reacted to kidney cells more frequently than lymphocytes. In two individuals the activity to kidney cells was substantially reduced by absorptions with the negatively reacting lymphocyte from the same individuals. In contrast to the discrepancies found when the two cell types were characterized by identical technics, there was close agreement in the typing patterns of lymphocytes using immune adherence and cytotoxicity technics. Under these circumstances discrepancies occurred in just 4% of the reactions, emphasizing that the significant differences found in the typing pattern of the two cell types were not related to methodology but to differences in antigen composition.

Antigens which are poorly detected on lymphocytes but well detected on kidney cells may be important clinically, as the available evidence supports the view that antibody to these antigens may mediate hyperacute rejection. In the present report, six renal allografts failed to function despite negative lymphocytotoxic cross-matches. In early biopsies all but one of these grafts had the characteristic histological features of hyperacute rejection. In all of these patients donor-kidney-cell-reactive-antibody was present prior to transplantation (Table 4). In a recent communication, Patel and Terasaki[9] reported nonfunction of allografts in 4% of immunized recipients and in 15% of immunized recipients despite the fact that their serum failed to react to the donor's lymphocytes prior to transplantation. It is possible, as in the present series, that the high incidence of nonfunctional allografts in the immunized recipients was associated with antibodies not detectable by the lymphocytotoxic cross-match.

Studies on the recipients of one liver and one heart transplant provided additional insight into the biological properties of antigen on kidney cells and their corresponding antibodies detected by immune adherence. In each of these cases pretransplantation serum contained antibody detected in reaction with the donor's kidney cells but not with the donor's lymphocytes. In each case, serum antibody levels to the donor's kidney cells declined following transplantation, but could be recovered in the eluates prepared from the rejected organs. An early biopsy of the heart transplant was not available, but biopsy of the liver graft at 10 hours disclosed evidence of hyperacute rejection. There were polymorphonuclear leukocytes in the portal veins, and large numbers of these cells were present in the liver sinusoids (Fig. 2). These findings are analogous to the histological features of hyperacute rejection in kidney transplants.[15] It is noteworthy that the antibodies associated with the fulminating rejection of these *non-kidney* allografts were still detected in reactions with *kidney cells* of the organ donor. This suggests clearly that the antigens detected on the kidney cells are individual antigens (iso-antigens) and are not tissue specific or kidney antigens. It may be just fortuitous that kidney cells were selected as target cells for monitoring these isoimmune reactions.

The grafts placed into sensitized recipients displayed different degrees of function. Three out of the total of 16 grafts studied were clearly non-viable at one hour; eight others had sufficient perfusion to risk leaving the graft in place, and five grafts appeared to be functioning well at the conclusion of the operative procedure. Three of these five grafts continue to function well. Quantitative considerations might explain why two of these three grafts functioned despite the presence of antibody (Table 4), but the third patient had titers of antibody to donor kidney cells associated with violent rejections in other patients. It is evident that antibody is just one of several factors important in immediate graft failure. The co-existence of "blocking" or "enhancing" antibodies, and defects in the compliment and coagulaiton system might explain why these three grafts functioned well despite evidence of pre-existing immunity. This situation appears analogous to the success of some transplants mismatched for major blood group antigens.

It is likely that antibody synthesized after transplantation also produces variable effects on the graft. There is increasing experimental evidence recently reviewed by Najarian [7] that antibodies can be toxic to allografts, and the present studies leave little doubt about the ability of even immunosuppressed human recipients to synthesize antibody to their grafts. At some time following transplantation donor-kidney-cell-reactive-antibodies were detected in the circulation of 15 of 16 patients and antibodies were recovered in eluates from 13 of 14 rejected grafts. The single patient (E. R. #2) not demonstrating antibody in either the serum or eluate probably lost the function of her graft on the basis of recurrent glomerulonephritis. Attempts to monitor the rejection process by measuring the serum levels of the donor-kidney-reactive-antibody are hampered by the absorption of this antibody by the graft. Donor-kidney-cell-antibody was detected in most instances at times when the graft was clearly functioning poorly. Only two patients had detectable levels of serum antibody during intervals of clinically stable graft function. However, here the detection of antibody preceded fulminating graft rejections unresponsive to steriods. These findings support the view advanced previously that antibodies are important mediators of allograft rejection, and further, that detection of donor-reactive antibody in the circulation represents a situation where the rate of antibody synthesis exceeds the rate of absorption by the graft. For this to occur either antibody synthesis must be very rapid or circulation to the graft must be poor, or both. Under any of these circumstances the prognosis is gloomy for the continued function of the graft.

In the future it seems evident that, in addition to means for better donor organ procurement and better immunosuppression, better methods are required for performing a prospective donor-recipient cross-match to exclude pre-sensitization. Current immunosuppressive technics have been applied extensively to our immunized prospective recipients without affecting the titers of circulating isoantibodies.[10] At present it appears most likely that the problem of pre-immunization must be solved by proper donor selection rather than relying on more potent immunosuppressive agents. Our experience using donor kidney cells as targets for detecting pre-immunization has been rewarding, but these technics are certainly not ideal. A prospective cross-match using donor kidney cells is not possible with mixed agglutination and is impracticable with immune adherence especially in living donors. This defect was manifested in our present studies where we lacked the opportunity to correlate the absence of preformed donor-kidney-cell reac-

tive antibodies with good graft function. Donor kidney cells were available for culture in only three instances in which there was no pre-existing antibody. All of these grafts functioned well but further studies are required to establish whether the absence of antibody activity to donor kidney cells is invariably associated with a functional graft.

Leukoagglutination cross-matches have been studied recently but this does not appear to be a promising technic for excluding pre-sensitization. One case of hyperacute rejection has occurred when the leukoagglutination (EDTA method) cross-match was negative.[17] The decided advantage to date of the donor-kidney-cell-reactive antibody test rests in the absence of the dangerous false negative reactions common to the other tests mentioned. The use of the immune adherence technic with other target cells may prove rewarding, for the immune adherence reaction mimics the hyperacute rejection phenomenon. The positive reaction is caused by the ability of antibody to combine with the target cell and fix certain components of compliment.[8] This complex reacts with receptor substances present on primate erythrocytes but especially granulocytes[5] causing these cells to adhere to the target cell. Similarly, hyperacute rejection is distinguished histologically by the adherence of polymorphonuclear leukocytes to the endothelial cells of the graft.[15] One may further speculate that optimal correlations between *in vitro* serological activity and *in vivo* results may be obtained when technics are developed which permit the rapid assay of antibody to donor endothelial cells. Intuitively, it is this cell type that is exposed to the highest concentration of toxic humoral factors, and early graft function depends upon its integrity. Until a reliable cross-match is developed for organ transplantation, donor selection resembles the transfusion of blood matched for major blood groups but not carefully cross-matched. The organ grafts like erythrocytes are tolerated in the majority of cases but reactions of variable severity are certain to occur in others.

Summary

1. A negative lymphocytotoxic cross-match between the organ donor and the recipient does not exclude the possibility of hyperacute rejection.

2. Methods have been studied which detect antibody not detectable by lymphocytotoxicity. These antibodies reacting to antigens on donor kidney cells were present prior to transplantation on 11 occasions: Six kidney grafts never functioned; one liver and one heart transplant functioned transiently; three kidney grafts remain functional. Donor-kidney-cell-reactive antibody was not present prior to transplanting one primary and two secondary grafts. These grafts remain functional.

3. Kidney - cell - reactive - antibody was present in the eluates from 13/14 rejected organs and in the serum prior to, during, or following transplantation in 15 of 16 instances. The only patient failing to synthesize detectable serum or kidney bound antibody had evidence of recurrent glomerulonephritis.

4. The methods developed in these studies for detecting antibodies are useful chiefly for retrospective studies. A rapid, simple cross-match technic is badly needed to prevent catastrophic early rejection which not only may kill the recipient or immunize him further, but also deprive other potential recipients of functioning transplants.

Acknowledgments

Drs. F. Milgrom and V. Eijsvoogel were instrumental in inspiring and guiding the technical and theoretical aspects of this report. The faults are ours not theirs. We acknowledge gratefully the able technical assistance of C. Alvarez, G. Hanscom, A. Siegel and M. Millington.

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Discussion

Dr. Watts R. Webb (Dallas): I think Dr. Williams and his coworkers have presented some very important studies. Histocompatibility, however, is far from the total answer in the rejection phenomenon and just as important is the presence of preformed antibodies directed specifically against the organ that is being transplanted.

We have had two patients with heart transplants that have undergone hyperacute rejection and [slide] with immunofluorescent technics. The papillary muscle when stained with antiglobulin, as you see, takes on the green fluorescent stain indicating gamma globulin is deposited there.

[Slide] The next slide shows a similar response when the papillary muscle is stained with beta I complement. Complement is utilized in this re-