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HUMORAL ANTIBODIES IN PATIENTS AFTER RENAL HOMOTRANSPLANTATION[1]

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SUMMARY

Humoral antibodies have been demonstrated by antiglobulin consumption tests to be present in the serum of virtually all patients after renal homotransplantation. The most completely characterized was a γG immunoglobulin distinct from the Forssman antibody which reacts against sheep but not against human red cell antigens, and which absorbs selectively against panels of human liver, kidney or white blood cells. This antibody appeared within a few days or weeks after transplantation, usually shortly after a rejection episode, and was more or less continuously detectable thereafter. The antibody was found in all of 10 patients studied during the first 4 post-transplant months, and in 13 of 14 patients tested from 4 months to more than 2 years after operation. Preliminary observations are included on another humoral antibody which does not react with sheep RBC stromata, but which can also be measured with a modified antiglobulin consumption test. The latter antibody was less commonly demonstrable, but it also exhibited specific absorption characteristics when tested against a panel of leukocytes obtained from volunteers. The implications of these findings are discussed in relation to the possible value of such sera for histocompatibility typing, as well as the possible role of such antibodies in promoting homograft enhancement.

For a number of years there have been sporadic efforts to identify humoral antibodies in animals or humans after various kinds of homografting procedures. Although such circulating antibodies have been seen from time to time, their presence has been inconstant. Furthermore, the antibody systems studied have almost all been shown to have no relevance to rejection.

In the present report, two kinds of humoral antibody will be described which have been found in the sera of patients at varying times after renal homotransplantation and which were measured by their antiglobulin consumption (AGC). The most completely characterized of these antibodies is detectable by virtue of its reactivity with a heterogenetic antigen found in sheep red cell stromata, which remains in the sheep cell membrane after the isophile component is removed by boiling (*27*).

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METHODS

Case material. Ten patients were studied before and at intervals up to 4 months after renal homotransplantation. Eight of these recipients received their kidneys from healthy volunteers; 5 from blood relatives and 3 from nonrelated donors. These 8 cases were part of a series in which donors were selected prospectively by the Terasaki method on the basis of the best available match (*33*). The two others received cadaveric homografts in which it was not attempted to evaluate the biologic suitability of the donor in advance.

Fourteen patients were studied from 4 months to more than 2 years after transplantation. Three of these patients were from the above group. The other 11 were chronic survivors from an earlier series in which donor-recipient pairing was not based on attempted antigen matching (*28*).

The general methods of therapy have been fully described (*28*). Specifically, it should be noted that almost all the patients received azathioprine and prednisone from the day of operation onward. With rejection the steroid doses were elevated to as high as 400 mg per day, and in many cases local homograft irradiation and intermittent actinomycin C therapy were then instituted. A rejection episode was diagnosed principally on the basis of deteriorating renal function.

The sera of 33 control patients were also studied. Fourteen of these were healthy volunteers. Seventeen others had a major surgical procedure such as colon resection, herniorrhaphy, closure of perforated viscus, ileo-femoral thrombendarterectomy; the remaining 2 were recipients of identical twin transplants.

In both the control and test subjects blood was drawn and allowed to clot for 12 hr at 4 C and the serum was removed by centrifugation. The sera were inactivated by heating at 56 C for 30 min and then frozen at −20 C. In this way all of the antisera for any individual patient could be stored and analyzed at the same time.

Antiglobulin consumption (absorption-dilution) test. The human test serum (0.25 ml) was added to 0.25 ml boiled sheep red blood cell stromata suspension which was prepared by stepwise lysis of sheep RBC in decreasing strengths of phosphate buffer saline (*13*). The mixture was incubated at 37 C for 1 hr and at 4 C for an additional hour, and then centrifuged at 4000 rpm for 30 min. The sediment was resuspended in saline, recentrifuged in the cold 4 more times, and mixed with 0.05 ml of rabbit anti-human globulin (Coombs)[2] serum or rabbit anti-human γG globulin serum.[3] Before use, the Coombs serum was absorbed twice with 0.5 parts packed sheep RBC for 1 part of the serum.

The suspension was incubated at 37 C for 1 hr, for an additional hour at 4 C, and centrifuged in the cold for 30 min at 4000 rpm. The supernatant, which

[2] Ortho Pharmaceutical Corp., Raritan, New Jersey.

[3] Supplied by the Children's Asthma Institute, Denver, Colorado. Dr. K. Ishizaka of that institute has proved that this antiserum reacts only with γG globulin and that the content of antibody nitrogen is approximately 200 μg per ml. The titer of this substance judged by the ability to agglutinate latex coated by human globulin was 1:1024. For testing, it was therefore diluted 4 times to give a titer of 1:256, the same as that for the Coombs reagent.

contained the residual antiglobulin antibodies in quantities determined by the degree of antiglobulin consumption by the test antibodies under study, was pipetted off for subsequent testing. The residual antiglobulin content of the supernatant was measured by a latex agglutination test. A latex[4] suspension (0.15 ml) of uniform particles of 0.81 mμ was diluted in 3 cc glycine buffer saline. A 1% solution of human globulin[5] was added to the latex suspension and the 6.3 cc total incubated at 37 C for 40 min. The latex particles which were now coated with human globulin were washed 4 times with saline and resuspended in 6.3 ml of glycine saline buffer (pH 8.2).

The amount of residual antiglobulin in the supernatant was now measured by determining its ability to agglutinate the globulin-coated latex particles. The original supernatant was diluted with an equal volume of glycine saline buffer, and further two-fold serial dilutions prepared. One drop of the latex suspension was added to each dilution, the mixture was drawn into a capillary tube, and it was incubated first for one hour at 37 C and then for 30 min at room temperature. The presence or absence of agglutination was read by naked eye observation. For each experiment controls using normal serum were performed as well as controls using glycine saline buffer in place of serum. Control (absent) AGC was determined by the highest dilution at which both the normal serum and glycine saline buffer controls showed a positive agglutination when treated in exactly the same way as the test serum; both control systems invariably provided the same result. Thus if the highest agglutinating control system were 1:256, a test of 1:128 which indicates that half the antiglobulin was consumed was recorded as 1+; a test of 1:64 indicating 75% consumption was 2+; a test of 1:32 was 3+; and a test of 1:16 or 1:8 which indicate essentially complete consumption were read as 4+. If desired, these data can be converted into AGC units described by Dausset (8).

Absorption of recipient serum with homologous or heterologous red blood cells. Since a positive AGC test with the above technique depends upon antigen-antibody complexing of one of the antibodies under study to sheep RBC stromata, the specificity of this reaction was studied. Recipient serum (0.25 ml) was absorbed with whole sheep RBC, rabbit RBC, guinea pig kidney,[6] and homologous human RBC. The blood was drawn with heparin anticoagulation and centrifuged and washed 3–5 times (or until clear) with saline. The buffy coat and supernatant were discarded each time. The red cell pack (0.12 ml) was added to 0.25 ml test serum and incubated for 30 min at 37 C and for 5 hours at 4 C. The resultant material was then recentrifuged, the serum recollected, and exactly the same red cell absorption repeated 2 more times. AGC of the residual serum was then measured.

[4] Difco Laboratory, Detroit, Michigan.

[5] Two types of human globulin were used: (1) immune serum globulin (Cutter Laboratories, Berkeley, California), or (2) lyophilized human γ-globulin (Cohn fraction 2, Pentex Corp., Kankakee, Illinois). In the former solution γG globulin was the main component but immunoelectrophoresis also revealed small quantities of γA and γM; its use was therefore terminated. For most definitive tests the latter product was employed since it is γG globulin.

[6] Denver Serum Co., Denver, Colorado.

Absorption of recipient serum with donor red blood cells. To determine if the antibody under study represented an isoantibody response to red blood cells transplanted with the kidney, known positive sera were absorbed as described above with packed RBC prepared from the kidney donor. AGC was measured before and after absorption.

Absorption of recipient serum with donor white cells or kidney, and with recipient tissues. Three patient sera which exhibited 1–2+ AGC 9 to 34 months after transplantation were absorbed with white cell pack obtained from the renal donor by the technique to be described below for absorption with homologous white cells. AGC was then repeated. An additional patient (LD 9) with 2+ AGC had splenectomy, bilateral nephrectomy, and liver biopsy 57 days after cadaveric renal transplantation. His serum was absorbed with his own excised tissues, as well as with the donor's contralateral kidney which had been frozen in the interim. The absorption techniques employed are described below. After absorption AGC was repeated.

Further absorption studies to differentiate antibody from Forssman or Forssman-like antibody. Horse serum was diluted 4 times with saline and 0.25 ml added to 0.25 ml serum of 5 patients. The mixture was incubated for 30 min at 37 C and then for 12 hr at 4 C. Precipitation did not occur in any instance; the procedure was therefore repeated adding another 0.25 ml horse serum. AGC was then measured using the pooled human (0.25 ml) and horse (0.5 ml) serum. Absorption was also done with 10 mg guinea pig kidney powder[7] which was first washed with saline, and to which 0.6 ml recipient serum was then added. The mixture was then processed exactly as with the red cell absorption and the eventual supernatant tested for AGC.

Absorption of recipient sera with homologous liver and kidney. Sera of known positive AGC were tested from 7 patients by absorption against 3 different kidneys obtained from 3 cadavers; in addition the 7 sera were absorbed against 2 cadaveric livers which were pooled. The fresh post-mortem organs were perfused with cold lactated Ringer's solution, cut into small pieces, rewashed with cold saline until all blood was removed, and homogenized with an equal volume of cold saline. The homogenate was filtered through gauze and washed with saline by means of centrifugation until the supernatant was clear. The recipient serum (3 cc) was mixed with 1 cc of packed liver or kidney cells and subequently processed as with the red cell absorption described earlier. AGC was determined on the eventual supernatant, and the result compared to that obtained prior to absorption.

Absorption with homologous white blood cells. The sera of 7 post-transplant patients which had been proven to contain humoral antibodies were further studied by absorption against a panel of white cells obtained from 9 nonrelated donors. The donor blood (100 cc) was collected in EDTA solution and the white cells separated by a method modified from Payne (*23*) and Dausset (*9*). First, 20 ml 6% dextran (mol wt 200,000) in saline was added to the blood to promote red cell sedimentation; both the red cell pack and the

[7] Denver Serum Co., Denver, Colorado.

supernatant were collected separately. Both fractions were then saline-washed 4 times by means of centrifugation at room temperature.

The recipient serum (0.27 ml) was first absorbed against the red cell pack of the individual blood donors as described above. After this, the residual serum (0.25 ml) was added to a white cell pack (3–4 times 10^7 white blood cells) from the same blood donor, and incubated for 40 min at 37 C and then for 2 hr at 4 C. After centrifugation at 3000 rpm for 15 min, the supernatant was removed and its AGC measured.

The leukocyte-containing sediment was retained from 4 samples and also tested for AGC. It was saline-washed 4 times by centrifugation in the cold; 0.05 ml of anti-human globulin serum (Coombs serum) was added to the washed sediment. The mixture was incubated at 37 C for 1 hr and 4 C for another hour, centrifuged for 15 min at 3000 rpm and the supernatant collected. The supernatant was added to the globulin-coated latex and the agglutination reaction then read. Antiglobulin consumption of this sediment would indicate the presence of a humoral antibody which does not require reaction with sheep RBC stromata for its detection.

RESULTS

Humoral antibody during the first 4 postoperative months. Findings in the 10 patients studied before operation and during the first 4 post-transplant months are summarized in Table 1. Prior to operation the AGC test was positive in 2 cases. In one, a weak AGC was noted. The other patient (LD 92) had a 3+ AGC before exposure to the homograft. However, this positive reaction was present only when Coombs antiserum was employed; it was not present when pure anti-γG globulin was used as a substitute reagent and it therefore probably represents a different humoral antibody system as will be discussed subsequently. This patient had immediate urine excretion, and diuresed several hundred ml during the first 3½ postoperative hours. He then developed a violent rejection with rapidly developing oliguria, temperature of 41 C (106 F), and acute effusions into the joints of the arms and legs. He was treated with 200–400 mg per day prednisone for 2–3 weeks in addition to azathioprine, actinomycin C and homograft irradiation. After 4 days of virtual anuria, renal function resumed and continued until his death from pneumonitis 79 days postoperatively.

The antibody was detectable in all cases after operation. The timing of its appearance was variable, being detectable immediately only in the patient with the apparent accelerated rejection. In the others it was first noted from 2–22 days postoperatively. After its initial detection there was fluctuation in the titer, waxing and waning in several individual patients. The highest titer tended to occur some days after a clinical rejection was first diagnosed, although in one instance the antibody was first detected two months after the patient had been treated for graft repudiation. Such time relationships are given for each case in Table 1.

Humoral antibody after 4 months. Fourteen patients were studied from 4 to 27 months after homotransplantation, including 3 from the group de-

TABLE 1

Antiglobulin consumption in sera of 10 patients before and after renal homotransplantation

Case No.[a]	Time acute rejection diagnosed	Preop	Days After transplantation																										
			2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	35	40	45	50	55	60	65	70	80	90	100	110	120
CD 5 (NR)	Not clear (ATN)[b]	—	0		1+	1+	2+	3+		2+		2+			1+			1+											
CD 6 (NR)	Not clear (ATN)[b]	—	0	0			0							2+					1+			1+	3+	2+	4+	3	2+[c]			
LD 92 (NR)	2 hr	3+	1+		3+		3+	4+		2+		0			3+		1+	0		2+		2+	3+	4+	3+[d]					
94 (R)	4 days	0		0	0		2+		3+														3+		2+		1+	1+		1+
95 (R)	3 days (?)	1+		2+	2+	3+		3+		1+		0			1+	2+		1+		1+	3+	3+	3+	2+	2+	2+	2+	2+		1+
96 (R)	3 days (?)	0	0		0		0			0		0						0			1+		1+							1+
97 (R)	5 days	0	0	0			0		2+	2+			0	0				0		1+								2+		
98 (NR)	2 days	0	0		3+		1+		1+			1+		1+	1+		0	Died 35 days												
99 (NR)	3 days	0	0	1+		2+			2+		2+		1+	2+				3+						2+						
100 (R)	10 days	0			0		1+				3+			2+			2+			1+			1+							

[a] NR—non related donor; R—related donor.

[b] ATN—acute tubular necrosis.

[c] Died 93 days.

[d] Died 79 days.

TABLE 2

Antiglobulin consumption in sera of 14 patients 4 months or longer after renal homotransplantation

LD No.	Months after transplantation	BUN (mg/100 ml)	Antiglobulin consumption
25	26	19	2+
27	27	101	3+
45	25	23	0
48	24	21	1+
54	24	84	2+
60	22	31	2+
63	22	25	1+
67	10	35	1+
71	12	39	3+
89	10	43	2+
90	5	25	1+
94	5	26	3+
95	5	32	2+
96	4	26	1+

Controls

No. cases	Antiglobulin consumption				
	Negative cases	Positive cases			
		1+	2+	3+	4+
17[a]	14	3	0	0	0
14[b]	12	2	0	0	0
2[c]	2	0	0	0	0

[a] Patients on surgical ward.
[b] Healthy persons.
[c] Identical twin recipients.

scribed above. In each case the kidney was providing life-sustaining renal excretion but to a highly variable degree (Table 2) since function ranged from normal to poor. The determinations were done twice at closely-spaced intervals. The results therefore represent a spot check. Thirteen of the 14 patients exhibited positive AGC. The presence of the antibody could not be related to the quality of function or to either the benignancy or severity of the late course.

Humoral antibody in control cases. Thirty-three cases were available for analysis; 17 surgical cases, 14 normal healthy volunteers, and 2 recipients of identical twin transplants (Table 2). Twenty-eight of the 33 control patients had no AGC activity. The five exceptions all had a low (1+) titer.

The nature of the antibody. Since Coombs serum contains γM and γA as well as γG antibody, the humoral antibody under study could theoretically have belonged to any of the three systems. To clarify this question, 9 sera from 7 recipients known to have 2 or 3+ AGC employing Coombs sera were re-tested substituting pure anti-γG globulin as a reagent. In all instances the AGC was exactly the same with the alternative methods. These tests establish that the antibody under study is a γG globulin (78).

TABLE 3

Absorption of post-transplantation antibody against heterogeneic antigens[a]

Antibody	Sheep RBC	Ox RBC	Rabbit RBC	Guinea pig kidney	Horse serum
Post-transplantation	+	+	+	+	−
Forssman[b]	+	−	−	+	+
Serum sickness[b]	+	+	+	+	+
Infectious mononucleosis[b]	+	+	−	−	−

[a] + Indicates absorption.
[b] Data taken from (*24*).

The relation of the antibody to red cell antigens. The sera from 5 patients with known 2 or 3+ AGC were absorbed with red cells from the kidney donor, and had no loss of AGC as measured either with Coombs reagent or anti-G globulin. Finally, an additional serum from Patient CD 6 had unchanged anti-γG globulin consumption after absorption with the red cell stromata of 2 indifferent volunteers. These results indicate that the antibody did not appear in response to any accidentally transplanted donor red blood cells or to red cells from blood transfusions. To strengthen this conclusion, 4 of the same recipient sera were retested employing donor red cell rather than sheep red cell stromata for the anti-γG globulin consumption test. With this change in method, no anti-γG globulin consumption was demonstrable in 4 experiments nor in 2 other similar tests in which the red cells were provided by nonrelated healthy volunteers other than renal donors.

Heterologous reactions. Absorption of the recipient sera with horse serum and with red cells of the sheep, ox, and rabbit and with guinea pig kidney permitted its comparison with other antibodies which have known interspecies absorption profiles. It will be noted (Table 3) that the antibody under study has a different pattern of absorption than the Forssman antibody or the antibodies of serum sickness or infectious mononucleosis (*24*). The antibody under study was apparently not an anti-DNA factor (*10*). Twenty-one recipient sera tested had negative LE tests.

Absorption of antibody with liver and kidneys. A selective pattern of absorption was evident with different cadaveric kidneys (Table 4). Thus for the serum from LD 27, cadaveric kidney No. 1 absorbed all the antibody, kidney No. 2 absorbed a portion, and kidney No. 3 did not absorb any. In contrast, the antibody in the serum of LD 102 was not absorbed at all by kidneys No. 1 and 3 but was completely absorbed by kidney No. 2. The absorption by liver was apparently the same as for the kidney from the same donor (Table 4) as would be expected if both tissues possessed many common antigens. If either kidney No. 2 or 3 caused a reduction of or abolished AGC, absorption with the pooled livers from the same cadavers invariably caused the same effect.

Absorption of antibody with homologous white blood cells. A similar pattern of selective absorption by homologous white cells was apparent in 5 test sera. Some absorbed the antibody completely, some partially, and others not at all (Table 5), as judged by pre- and postabsorption AGC testing.

TABLE 4

Antiglobulin consumption tests of transplanted patient sera absorbed with human tissues

Case No.	Patient sera absorbed with	Antiglobulin consumption
LD 27	Unabsorbed	3+
	Kidney 1	0
	2	1+
	3	3+
	Liver 2, 3	1+
LD 71	Unabsorbed	2+
	Kidney 1	2+
	2	2+
	3	0
	Liver 2, 3	0
LD 99	Unabsorbed	3+
	Kidney 1	2+
	2	0
	3	0
	Liver 2, 3	0
LD 100	Unabsorbed	2+
	Kidney 1	0
	2	0
	3	0
	Liver 2, 3	0
LD 102	Unabsorbed	2+
	Kidney 1	2+
	2	0
	3	2+
	Liver 2, 3	0
CD 5	Unabsorbed	3+
	Kidney 1	0
	2	0
	3	0
	Liver 2, 3	0
CD 6	Unabsorbed	3+
	Kidney 1	1+
	2	0
	3	0
	Liver 2, 3	0

In 4 other recipient sera (Table 6) comparable results with AGC were first obtained. In these 4 sera, an AGC test was then performed upon the leukocyte-containing sediment (Table 6), omitting incubation with sheep red cell stromata and proceeding directly to the addition of Coombs reagent or anti-γG globulin. When absorption by the white cells occurs, the antibody attached to the leukocytes should be detectable in the sediment; the results with the sediment were therefore predicted to be a mirror image of those

TABLE 5

Antiglobulin consumption tests of recipient sera absorbed with human leukocytes

Case No.	Patient serum absorbed with[a]	Antiglobulin consumption
LD 94	Unabsorbed	3+
	Leukocyte 1	3+
	2	0
	3	3+
	4	0
LD 95	Unabsorbed	2+
	Leukocyte 1	0
	2	1+
	3	0
	4	0
LD 99	Unabsorbed	2+
	Leukocyte 1	0
	2	2+
	3	0
	4	2+
CD 5	Unabsorbed	3+
	Leukocyte 1	3+
	2	0
	3	3+
	4	0
CD 6	Unabsorbed	3+
	Leukocyte 1	3+
	2	0
	3	3+
	4	0

[a] Each serum was absorbed twice with leukocytes (2×10^7). Blood type of leukocyte donors: L_1 = 0−; L_2 = 0+; L_3 = A+; L_4 = A+.

with the supernatant. In most cases this prediction was fulfilled (Table 6), although in 3 instances of 19 a perfect reciprocity was not present in that a 3+ AGC test in the supernatant was accompanied by a 1+ direct test in the sediment, or vice versa.

The occasional discrepancies between the two methods were thought to be due to another humoral antileukocyte antibody in the serum of these patients, one which does not react with sheep red cell stromata. This possibility was proved in the sera of 4 patients known to have positive AGC tests. These 4 sera were absorbed twice against sheep red cell stromata, and the sediment which contains the antibody reactive against sheep cells was discarded. The supernatant was then absorbed twice with 50% human red cell pack and then incubated with 3×10^8 white cells from the same panel of donors as had provided the red cells. After centrifugation the sediment was tested by AGC. The results are summarized in Table 7. It will be noted that a weak titer antibody, differing from the principal subject of this study in that it was not

TABLE 6

Further absorption studies with homologous leukocytes[a]

Case No.	AGC of sera (supernatant) after absorption with leukocytes		AGC of leukocytes (sediment) after incubation with leukocytes	
	Patients' sera absorbed with leukocyte No.	AGC	Patients' sera incubated with leukocyte No.	AGC
LD 102	Unabsorbed	3+		
	Leukocyte 5	3+	5	0
	6	0	6	3+
	7	3+	7	1+
	8	0	8	3+
	9	0	9	
LD 103	Unabsorbed	3+		
	Leukocyte 5	0	5	3+
	6	3+	6	0
	7	0	7	3+
	8	3+	8	1+
	9	0	9	3+
CD 5	Unabsorbed	2+		
	Leukocyte 5	0	5	2+
	6	2+	6	0
	7	0	7	2+
	8	0	8	2+
	9	0	9	2+
CD 6[b]	Unabsorbed	3+		
	Leukocyte 5	0	5	3+
	6	0	6	3+
	7	3+	7	0
	8	1+	8	3+
	9	0	9	3+

[a] After absorption, antiglobulin consumption (AGC) was determined of both the patient's serum which was tested with the sheep red cell system (supernatant) and the white cell sediment which was tested directly with Coombs reagent or anti-γG globulin without sheep RBC (SRBCS). Failure in some instances to obtain exactly converse results with the supernatant and sediment suggests the presence of a second humoral antibody, one which does not react with SRBCS.

[b] Anti-γG globulin consumption tests. The other tests in this table and Table 5 were done with Coombs serum. Direct AGC tests of Leukocytes L_5, L_6, L_7, L_8 and L_9 were negative.

absorbed by sheep red cell stromata, was demonstrated in 7 of these 15 sediments. Furthermore, this extra antibody also exhibited individual specificity since it was absorbed by some but not all leukocytes in the testing panel.

Absorption of recipient serum with donor white cells and kidney and with recipient tissues. AGC detected by the sheep red cell system was 1–2+ in 3 patient sera. After absorption with donor white cell pack, AGC was absent. In patient CD 9, AGC was 2+. After absorption of the serum with his own spleen, kidney, and liver, AGC was still 2+. After absorption against the

TABLE 7

Demonstration of individual specificity of the second humoral antibody which does not react with sheep red blood cell stromata[a]

Case No.	Blood type of leukocyte donor	Recipient sera absorbed with sheep RBC stromata before testing	Recipient sera before absorption with sheep RBC stromata
LD 54	Control[b]		2+
	O	0	0
	A	2+	2+
	A	0	1+
LD 100	Control[b]		2+
	A	0	2+
	B	1+	1+
	O	1+	1+
LD 102	Control[b]		3+
	A	0	3+
	B	0	0
	O	1+	1+
CD 6_1	Control[b]		3+
	A	0	0
	B	1+	1+
	O	1+	3+
CD 6_2	Control[b]		2+
	O	1+	1+
	A	0	0
	A	0	1+

[a] The supernatant of patient's serum after absorption with sheep RBC stromata was incubated with the homologous leukocytes. AGC of the final leukocyte sediment was then determined with Coombs reagent. Unfortunately, this antibody was not looked for in patients before transplantation. It is not therefore established that its postoperative presence is related to the presence of the homograft.

[b] Antiglobulin consumption tests of recipient sera with stromata of sheep red cells.

homograft donor's other kidney, AGC was absent. The results indicate that the antibody is completely absorbed by tissues of the donor, and conversely that there is no absorption by recipient tissues.

DISCUSSION

These studies have shown that a humoral antibody which reacts with sheep red cells appears in the overwhelming majority of patients after renal homotransplantation. The antibody cannot be detected by direct leukoagglutination, and it is heat-stable at 56 C. It is a γG (7S) immunoglobulin which is different from the Forssman, infectious mononucleosis, or serum sickness antibodies by virtue of its specific reactivity characteristics. It is distinct from the rheumatoid factor which is a 19S antibody (*12*) and C-reactive protein which does not react with sheep red cell stromata (*15*). It is not likely to be a Wasserman antibody since all donors and recipients had

negative Wasserman tests. As proved by the absorption studies, it does not react against human red cell antigens but it does so selectively against antigens present in human white cells and other tissues. A. G. Bausset (personal communication) has pointed out that there is no simple explanation for the facts that the antigenic stimulation is provided by individual kidneys, that the resulting antibodies in each case react with a common sheep red cell antigen, and that the individual antibodies possess specific reactivity characteristics against other human tissues. He has suggested that one must assume either that the sheep red cells possess a variety of antigens each corresponding to a given human isoantigen, or that one sheep red cell antigen cross reacts with various human isoantigens.

It is evident that this humoral antibody which is so readily distinguished by its reactivity with sheep red cell antigens[8] is not the only circulating antibody which results from exposure to the homograft. The fact that some such antibodies do react with sheep red cell stromata but not with human red cells must be viewed only as a fortunate coincidence which facilitates their characterization. Preliminary observations in the present report strongly suggest that at least one other antibody is present in many of these patients; one which reacts neither with sheep nor human red blood cells, but which does selectively react with human leukocytes.

There is as yet no incontrovertible proof that the antibody systems described represent a response to histocompatibility antigen. Nevertheless, certain evidence suggest that this is the case. First, the sera of all but one of the transplanted patients had antiglobulin consumption. In contrast, the control studies of identical twin cases and routine surgical patients demonstrated a low incidence of the humoral antibody in the nonhomografted population. The control patients whose sera did exhibit antiglobulin consumption as well as the exceptional transplanted patient who possessed the antibody prior to operation had previously been transfused; Engelfriet and van Loghem (*11*) have noted that antiglobulin may be present in the serum under these circumstances in the absence of leukocyte agglutinins. The mechanism responsible for this finding in some transfused patients could be the same as after homotransplantation, namely sensitization by histocompatibility antigens.

The timing of the appearance of the antibody after homotransplantation was also suggestive of a response to homograft antigens. During the first few postoperative weeks or months its titer was variable, tending to reach peaks just after the clinically diagnosed rejection crises. Of importance, also, was the demonstration that some but not all fresh cadaveric kidneys were capable of absorbing the antibody obtained from any individual recipient; presumably such kidneys shared the same antigen as the homograft, and the others which did not absorb lacked the antigen. Finally, the most convincing evidence of

[8] The search for an anti-sheep red cell antibody in the human was prompted by the prior observation of one of us (Y.I.) that such an antibody appeared after canine renal homotransplantation. The presence of Forssman antigen in the dog kidney obscured the significance of this finding; fortunately the human kidney does not possess this antigen.

the specificity of at least the humoral antibody which reacts against sheep red cell stromata was its complete absorption by leukocytes of the renal donors or in one case by the other kidney of a cadaveric donor.

Although circulating antibodies have not yet been reported in the serum of human renal homograft recipients, their presence is not surprising. Since the early work of Clarkson and Gorer (*6*), a variety of humoral antibodies has been observed in animals and man, usually in response to skin grafts. These have been detected with hemagglutination (*6, 18*), leukoagglutination (*34, 36*), cytotoxicity (*2, 3*), passive serum transfer (*4, 30, 31, 32, 35*), agglutination of collodion particles (*22*), and antiglobulin consumption (*6, 7*) tests. Millipore chamber experiments have also provided evidence that humoral antibodies can cause homograft destruction in the absence of recipient cells (*17, 20, 21*). Altman's studies with a cytotoxic antibody which appeared after skin or kidney homografts to dogs (*1*) are particularly interesting. With serum from homograft recipients, he was able to passively confer homograft immunity to other animals against tissues from the original donor.

The antiglobulin consumption method which measures antibody unreactive in other tests (*26*) has been one of the most useful of these techniques. In an experiment resembling in design that of the present report, Colombani et al. (*7*) found a similar antibody in 10 of 27 subjects who received 2 or more skin grafts. In these patients who were not receiving immunosuppression, a positive antiglobulin consumption test developed more regularly with larger skin grafts. In their studies, the individual specificity of the antibody was also established by means of absorption against a panel of skin cells obtained from healthy donors.

The biologic implications of such a consistently detectable and highly characteristic antibody system in the sera of post-transplantation patients are many and obvious, but further comment will be confined to 2 specific points of interest. The first of these concerns the role of such antibodies in determining the course, particularly late, after transplantation. It has been noted that the patient who survives beyond the first few postoperative months often requires progressively lower doses of immunosuppressive agents as if some alteration had taken place in the host-homograft inter-relation (*28*). This position has been supported by the observation that rejection tends to be a spontaneously remittent phenomenon (*14, 29*). Murray (*19*) has attempted to analyze these events in terms of a change in either the host or the graft. The possibility that enhancement (*5, 16*) was the responsible mechanism seemed precluded by previous failure to demonstrate the necessary condition of an anti-homograft antibody (*25*). The results in the present study freshly raise enhancement as an attractive possible explanation for the two events which make clinical transplantation feasible—the reversibility of rejection, and the development of a relative host-graft nonreactivity. In this connection, it is important to note that virtually all (13 of 14) patients studied 4 months to more than 2 years after operation had the humoral antibody, an incidence comparable to that during the first 4 months.

A final practical point, previously stressed by Colombani et al. (*7*), concerns

the possible role of the above described individual-specific humoral antibody systems in facilitating development of tissue typing techniques. Chronic survivors after renal homotransplantation may prove to be an important source of typing sera.

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