Clinical Evaluation of Equine Antithymocyte Globulin in Recipients of Renal Allografts:

Analysis of Survival, Renal Function, Rejection, Histocompatibility, and Complications

ARNOLD G. DIETHELM, M.D.,* JOAQUIN S. ALDRETE, M.D.,* JUNE F. SHAW, M.D.,†
C. GLENN COBBS, M.D.,‡ MARSHALL W. HARTLEY, Ph.D.,§
WILLIAM A. STERLING, M.D.,|| JEAN McN. MORGAN, M.D.**

Equine antithymocyte globulin combined with azathioprine and prednisone as immunosuppressive therapy in 50 transplant recipients prolonged allograft survival and seemed to modify the severity of rejection episodes. Although nine patients died from a variety of causes, only three kidneys were lost to rejection, one of which was hyperacute. There were no serious untoward hematologic or systemic effects caused by the ATG, and all patients completed the course of therapy. Infection, a serious and frequent complication of transplant patients, was encountered no more often than in other transplant series not using ALG. The data pertaining to the clinical value of ATG, although suggestive in terms of its immunosuppressive effects, is still not conclusive; and a definitive answer to this question awaits further evaluation in a series of cadaveric recipients in a randomized-double-blind study.

From the Departments of Surgery, Medicine, Pathology and Clinical Pathology, The University of Alabama Medical Center and the Veterans Administration Hospital, Birmingham, Alabama 35294

THE INTRODUCTION of antilymphocyte globulin to clinical renal transplantation by Starzl [22] in 1967 stimulated other transplant centers to develop a variety of antilymphoid preparations which were combined with azathioprine and prednisone as immunosuppressive therapy for patients receiving renal allografts. In spite of strong experimental evidence supporting the immunosuppressive properties of antilymphocyte sera in prolonging skin allografts in rats,[29] mice[10] and in canine renal allografts,[13,18,23] the results from clinical trials regarding its immunosuppressive value are less conclusive. Evaluating the results of patients receiving renal transplants treated with antilymphocyte globulin in conjunction with azathioprine and prednisone is complicated by the many types of antilymphocyte sera preparations. The source of antigenic material has included lymphocytes from spleen,[23] thoracic duct,[27] thymus,[6,11] lymph nodes,[14] and lymphoblasts [17] from cell culture. Another factor in the preparation involves the host animal used to prepare the antisera. The horse,[9,14,17,22] goat [19] and rabbit [11] have all been used with varying immunization schedules.[12] The antisera (either crude globulin or the 7-S fraction) has been administered in dosage concentrations from 2–4 mg/kg/day to more than 20 mg/kg/day by either the intramuscular or intravenous route. The difficulty in assessing the immunosuppressive value of these products is enhanced when one considers the many variables encountered in the transplant patient. These include the histocompatibility differences between the donor and the recipient, the use of cadaver organs with various methods of preservation and the previous sensitization of recipients to antigens, some of which may not be detected by present methods of histocompatibility testing.

Submitted for publication June 28, 1973.
* Associate Professor of Surgery.
† Associate Professor of Clinical Pathology.
‡ Associate Professor of Medicine.
§ Professor of Pathology.
|| Assistant Professor of Surgery.
** Professor of Surgical Nephrology.

This study was initiated with the above difficulties in mind in an effort to determine the adjunctive value of equine antithymocyte globulin administered with prednisone and azathioprine to patients receiving allografts from living-related donors and cadavers. The results are analyzed according to patient survival, renal function, histocompatibility and the complications encountered, including rejection and infection.

Clinical Material and Methods

Patients

Forty-nine patients received 50 renal transplants between December of 1968, and December of 1971. The study period terminated December 1, 1972 with all living patients followed from 12 to 47 months. Thirty-nine patients obtained kidneys from related-living donors (RLD) with one patient receiving a second transplant. Eleven patients received cadaveric kidneys (CD) (See Table 1). Antithymocyte globulin (ATG) was administered to consecutive patients except for two patients when the supply was unavailable. Those patients were deleted from the study. Thirty-six patients were male and 13 female with an age distribution from 11 to 51 years (See Table 2). Chronic glomerulonephritis was the primary disease in 31 patients, chronic pyelonephritis in 12 patients and arteriolonephrosclerosis in three patients. Two patients had polycystic kidney disease, and one had congenital hypoplasia (Table 3).

Thirty-one of the 40 living patients had renal biopsy specimens taken from functioning kidneys at various intervals in the post-transplant course. All were examined by light microscopy and selected specimens studied by electron microscopy and immunofluorescent technique. Autopsies were performed on six of the nine patients who died, and two others had either a renal biopsy or transplant nephrectomy. Only one of the deceased patients did not have renal tissue for histologic evaluation. Nine patients, living and well with a serum creatinine of less than 2.0 and stable renal function, also did not have renal biopsies.

Transplant Procedure

All patients but one were prepared for transplantation by hemodialysis. The single exception was an 11-year-old boy with bilateral renal hypoplasia. Two patients retained their kidneys, and the remaining patients were nephrectomized prior to transplantation. The kidney was placed in the pelvis by the extraperitoneal approach utilizing the iliac vessels for reconstruction in every instance except one where the kidney was positioned within the abdominal cavity. A ureteroneocystostomy was performed in all patients.

TABLE 1. *Clinical Material*

Total Number of Transplants*	50
Total Number of Patients	49
Recipients of Related Donors (RLD)—39	
Parents 18	
Siblings 21	
Offspring 1	
Recipients of Cadaveric Donors (CD)—11	
Total Number of Patients Alive	40
Total Number of Patients Dead	9

* One patient received a second transplant.

TABLE 2. *Age of Patients at Transplant*

10–19	05
20–29	17
30–39	13
40–49	14
over 50	01
Total	50

Tissue Typing

Histocompatibility matching, performed by a modification of the cytotoxicity technique of Terasaki, was completed prospectively in all donor and recipient patients. The antigen profile was tested with antisera as well as screening for humoral antibodies in the recipient patient by the lymphocytotoxicity crossmatch.

Preparation and Immunosuppressive Activity

Horse antihuman thymocyte globulin (ATG) was prepared by injecting a suspension of 1×10^9 to 1×10^{10} human thymocytes into horses with 90% viability as determined by trypan blue. The first injection of thymocytes, combined with equal volumes of Freund's adjuvant was administered by the intramuscular route. A second and third injection of a similar number of thymocytes, suspended in saline, were given intravenously 21 and 28 days later. The horse was bled on day 35 and then allowed four weeks to recover before the final intravenous injection of thymocyte suspension. The second phlebotomy was performed seven days later with no horse receiving no more than two bleedings. The antithymocyte serum (ATS) was separated from the red cells and decomplemented at 56 C for 30 minutes. The hemagglutinin antibody was removed by two absorptions with human red cell stroma, and the globulin fraction prepared by ammonium sulfate precipitation (45%). The initial leukoagglutinin titer of 1:59,000 in the ATS was usually reduced to a titer between 1:2,000 and

TABLE 3. *Pathology*

Chronic Glomerulonephritis	31
Chronic Pyelonephritis	12
Arteriolonephrosclerosis	03
Polycystic Disease	02
Congenital Hypoplasia	01
Total No. Patients	49

1:20,000 after absorption with red cell stroma. The final hemagglutinin titer of all ATG prior to use was less than 1:32. Sterilization was achieved by high energy irradiation (performed by Dr. Kenneth Wright of the Massachusetts Institute of Technology, Boston). The immunosuppressive effect of ATG was tested in four Rhesus monkeys receiving skin allografts. The animals were treated with ATG, 2 mg/kg/day intramuscularly for 21 days (seven days before the skin graft and 14 days afterwards). This dosage schedule prolonged skin allografts in all animals with 80 to 90% viability of the skin for more than 21 days. The grafts sloughed four to nine days after discontinuing the ATG.

Immunosuppressive Management

All renal allograft recipients received azathioprine, prednisone and intramuscular ATG (2–4 mg/kg/day) as immunosuppressive therapy. Antithymocyte globulin was administered to patients receiving kidneys from related donors for seven days prior to transplantation and for 14 days post-transplantation for a total of 21 injections. Cadaveric recipients were given ATG by the same route for 28 days starting on the day of surgery. All patients were skin tested with horse serum to exclude individuals sensitive to the serum. Recipients of related-living donor kidneys were pretreated with azathioprine two days before transplantation (3 mg/kg/day) and continued post-transplantation with gradual reduction of the azathioprine to 1 to 2 mg/kg/day depending on the white blood cell count. Prednisone, 4 to 5 mg/kg/day started on the day of transplantation, was gradually reduced to 30 mg/day one month post-transplantation in patients receiving kidneys from related-living donors and to 40 mg/day in cadaveric recipients.

Acute rejection was defined as an increase in the BUN 10 mg/100 ml and in the serum creatinine 0.5 mg/100 ml above the previous stable level of renal function without evidence of ureteral obstruction. The usual clinical criteria of weight gain, hypertension, fever and increase in kidney size with tenderness were additional factors contributing to the diagnosis of rejection. Treatment of acute rejection included an increase in oral prednisone to 4–5 mg/kg/day with gradual reduction combined, on occasion, with intravenous methylprednisolone. Allograft irradiation, totaling 600 r to the midplane of the kidney, was given in 4 divided doses of 150 reach.

Results

Surviving Patients and Renal Function

Forty of the 49 patients receiving 50 renal transplants are alive from 12 to 47 months after surgery with an average followup of 24 months (See Table 4). Thirty-one of these patients received kidneys from related donors, and nine obtained kidneys from cadaveric origin. The surviving patients with functioning allografts were divided into three groups (Table 5): Group I—twenty-five patients with a serum creatinine of 1.5 mg/100 ml or less (RLD-18, CD-7); Group II—twelve patients with a serum creatinine between 1.6 and 2.0 mg/100 ml (RLD-10, CD-2) and Group III—three patients with a serum creatinine between 2.1 and 4.0 mg/100 ml (RLD-3). Five of the 40 patients with functioning allografts have evidence of chronic rejection (RLD-4, CD-1) characterized by decreasing renal function, proteinuria and histologic confirmation by biopsy.

Tissue Typing data was correlated with the status of renal function (Table 5). All seven patients considered to be HLA identical (A Match) maintained a serum creatinine of 1.5 mg/100 ml or less even though three of the seven had mild or moderate rejection episodes easily reversed with Prednisone. Seven of the eight patients with a B Match maintained excellent renal function and are also in Group I. The other B-Match patient is in Group II. It is significant to note that six of 16 patients with a D Match are also classified in Group I, indicating that two or more antigen mismatches may still be compatible with excellent renal function. Haplotype analyses were performed on 30 donor/recipient combinations with similar results to the phenotypic analysis. Again, patients with identical haplotype analysis maintained excellent renal function (Group I), whereas recipients with two haplotype differences have suboptimal renal function, some with evidence of chronic allograft rejection (Group II and III). Only one patient underwent hyperacute rejection; retrospective analysis indicated previous sensitization in this instance.

Patient Deaths and Transplant Failures

There were nine deaths and one transplant failure secondary to chronic rejection requiring retransplantation (Table 6). One patient (TX 11) sustained a hyperacute rejection, was returned to dialysis and died 11 months later following a nearly total small bowel resection for volvulus with gangrene. Three patients (TX 12, 19 and 20) died of myocardial infarction with severe coronary artery disease, 24, 16 and 3 months respectively after transplantation. One patient with chronic rejection (TX 10) died of septicemia at 18 months. Two patients (TX 41 and 54) died of cytomegalovirus with hepatic failure (one with normal renal function and one two months after transplant nephrectomy for rejection of a cadaveric kidney). One patient (TX 18) died of a pulmonary embolus following a gastric resection for a bleeding marginal ulcer six months after transplantation. Six of the nine patients

TABLE 4. *Analysis of Forty Patients with Functioning Kidneys*

TX. No.	Age	Donor	Tissue Typing	Duration of Function (mos)	BUN	CR	CRCL	24-hour Protein mg%	Azathioprine	Prednisone	Anti-Hypertensive
6	34	P	D	47	25	1.8	60	450	100	12.5	no
7	45	P	D	45	36	1.8	30	652	100	15	no
8	33	S	B	44	23	1.3	114	450	150	5	yes
9	50	S	B	44	14	0.7	102	470	150	5	yes
15	31	P	C	38	27	1.4	79	255	150	10	no
16	30	S	B	37	15	1.4	110	480	150	10	yes
17	22	P	D	37	38	2.2	45	770	100	10	yes
21	23	S	A	32	15	0.9	84	240	100	10	no
22	32	S	A	32	20	1.1	113	211	100	10	no
23	11	P	D	29	18	0.9	72	608	100	7.5	no
24	24	S	A	29	25	1.4	92	400	150	15	yes
26	45	Cd	C	29	17	1.0	124	353	75	15	yes
27	38	Cd	C	29	17	1.0	118	945	100	15	no
28	40	Cd	C	24	25	0.9	83	306	75	15	yes
29	27	P	C	24	27	1.5	92	784	150	12.5	no
30	25	P	D	24	36	2.2	34	207	100	25	no
31	33	Cd	D	24	22	1.0	56	1700	75	20	no
32	23	S	D	22	27	1.8	72	415	200	15	yes
33	23	P	D	21	23	1.4	78	250	75	10	no
34	38	Cd	B	21	21	1.1	123	200	100	20	no
35	39	Cd	D	21	27	1.3	147	129	100	20	yes
36	14	P	C	19	28	1.7	49	620	125	15	yes
37	24	Cd	D	19	24	2.0	107	552	150	25	yes
38	42	O	B	18	20	1.0	72	275	100	15	no
39	26	S	D	17	37	3.5	35	675	150	20	yes
40	42	Cd	D	17	27	1.0	77	120	150	17.5	yes
42	36	S	C	17	32	1.9	59	382	125	15	no
43	20	P	C	17	27	1.7	61	1793	150	15	yes
44	24	S	A	17	15	1.1	111	240	150	15	no
45	40	S	A	16	17	1.3	88	600	125	12.5	no
46	43	S	B	16	18	0.9	118	128	150	15	yes
47	26	S	B	15	13	1.2	97	450	125	15	no
48	25	S	B	14	24	1.9	58	257	150	15	yes
49	33	P	D	14	38	2.0	45	232	100	15	no
50	34	P	C	13	40	1.7	50	696	100	15	yes
51	24	S	A	13	17	1.2	122	100	150	15	no
52	17	P	C	13	23	1.0	74	202	125	17.5	no
53	27	P	D	13	39	1.7	60	1278	100	17.5	yes
55	20	Cd	D	12	41	1.6	98	936	175	25	yes
56	46	S	A	12	28	1.0	76	1543	150	17.5	no

who died were over the age of 40, and two were less than 20 years of age.

Pathologic Examination of Renal Allograft Tissue

Although renal biopsy specimens were obtained frequently during episodes or acute rejection, the histologic appearance seldom correlated with renal function except in Transplant 54 where cortical necrosis was present and necessitated transplant nephrectomy several days later. Renal tissue from patients in Group I (17 of 25, Table 5) showed the least amount of vascular injury and intestinal fibrosis on microscopic examination. The degree of mononuclear cell infiltration varied from patient to patient and at times appeared more prevalent than expected from the excellent renal function (Fig 1).

Complications

Rejection. There were 44 acute rejection episodes in 32 patients (Table 7). Twenty-eight rejections occurred in the first 60 days and 31 within 90 days after transplantation. Thirteen late rejection episodes developed after the third post-transplant month, nine of which were diagnosed after six months. These late rejections were

TABLE 5. *Comparison of Renal Function and Tissue Typing in Forty Living Patients*

Group	No. Pts.	Renal Function Cr	Source of Kidney RLD	Source of Kidney CD	Typing* A	Typing* B	Typing* C	Typing* D
I	25	1.5 or less	18		7	6	2	3
				7	0	1	3	3
II	12	1.6–2.0	10		0	1	4	5
				2	0	0	0	2
III	3	2.1–4.0	3		0	0	0	3
				0	0	0	0	0
Total	40		31	9	7	8	9	16

* A—Identical.
B—No group mismatch.
C—1 group mismatch.
D—2 or more group mismatches.

TABLE 6. *Analysis of Ten Patients with Transplant Failure or Death*

TX. No.	Age	Donor	Tissue Typing	No. Rejections	Duration of Function	BUN (at death)	CR (at death)	Azathioprine	Prednisone	Dead	Autopsy	Comments
5	23	P	D	2	24					no		Chronic Rejection Retransplant
10	14	P	C	1	18	105	4.1	75	17.5	yes	yes	Septicemia
11	48	S	C	1	0	Tx. Nephrectomy				yes	yes	Postoperative Intestinal Obstruction
12	43	S	C	1	24	14	1.0	150	17.5	yes	no	Myocardial Infarction
18	41	S	B	1	6	23	0.9	150	32.5	yes	yes	Pulmonary Embolus
19	42	S	D	1	3	29	2.7	100	30	yes	yes	Myocardial Infarction
20	39	CD	C	2	16	105	6.0	100	25	yes	yes	Myocardial Infarction
25	19	P	D	0	16	34	1.1	100	17.5	yes	no	Pneumonia (Pneumocystis)
41	48	S	B	1	10	26	1.3	100	25	yes	yes	Hepatitis (Cytomegalovirus)
54	45	CD	D	2	2	Tx. Nephrectomy				yes	no	Liver Failure, Pneumonia (Cytomegalovirus) 4 mos. P Tx.

often associated with prednisone reduction and easily reversed after increasing the steroid dosage. The post-transplant immunologic course was categorized into five types (Table 8): Type 0—no rejection (18 patients); Type 1—a rejection episode where the BUN remained less than 40 mg/100 ml and the creatinine less than 2 mg/100 ml (12 patients); Type 2—a rejection episode where the BUN increased to more than 40 mg/100 ml and the creatinine above 2 mg/100 ml (15 patients); Type 3—a rejection episode similar to Type 1 or 2 but with deranged renal function for more than 30 days (1 patient); Type 4—reversible rejection requiring dialysis (1 patient); Type 5—irreversible rejection, acute, hyperacute or chronic (3 patients). Thirty-nine of the 44 rejections were classified as Type 1 or Type 2. A single rejection crisis occurred in 23 patients. Two rejection crises developed in six patients, and three patients had three rejection episodes each. Thirteen of the 25 patients with excellent renal function (Group I, Table 5) had single rejection episodes all readily reversed with prednisone occasionally combined with x-ray therapy. Seven of the 12 patients with good renal function (Group II, Table 5) had one episode of allograft rejection, reversed with some difficulty. Five patients had two such crises requiring substantial steroid therapy and x-ray therapy. All three patients with fair renal function had two or three rejection episodes with subsequent unstable renal function requiring increased prednisone dosage for control (Group III, Table 5). One patient (TX 32) sustained acute rejection on the seventh post-transplant day and required two dialyses until renal function resumed. He is now 22 months post-transplantation with a creatinine of 1.8 mg/100 ml. Eighteen patients (RLD-12, CD-6) never experienced a rejection episode. Eight were a C or D Match (parent to offspring), six an A or B Match (sibling) and four received cadaveric kidneys (2-C Match, 2-D Match).

Infections. Eleven patients sustained an infection in the *early* (less than six weeks) post-transplant period (Table 9). In five patients, the process involved the surgical wound, two with perinephric extension. All of these patients recovered. Sixteen patients developed serious infections more than six weeks after transplantation, and three died. Ten of these late infections followed augmented immunosuppressive therapy for rejection. Gram-negative bacilli, the most common bacteria isolated, were the responsible organisms in 16 instances (Table 10). There were four patients who developed serious viral infections, and one died. Although all four patients with fungal infections recovered, the three patients with Pneumocystis carinii expired from pulmonary insufficiency (Table 10).

Malignancy. Four patients developed malignant tumors; three squamous cell carcinoma of the skin and one systemic Hodgkin's Disease. All tumors occurred two years or more after transplantation. Local excision was adequate treatment in the squamous cell tumors and chemotherapy was given in the patient with Hodgkin's Disease.

Skeletal Complications. Eighteen patients had skeletal complications, 14 of whom developed aseptic necrosis of the femoral head. Nine of these individuals required replacement with an Austin-Moore prosthesis and made a complete recovery. Three patients fell, sustaining intertrochanteric fractures, and one spontaneously fractured a pelvic ramus. Overt hyperparathyroidism could not be

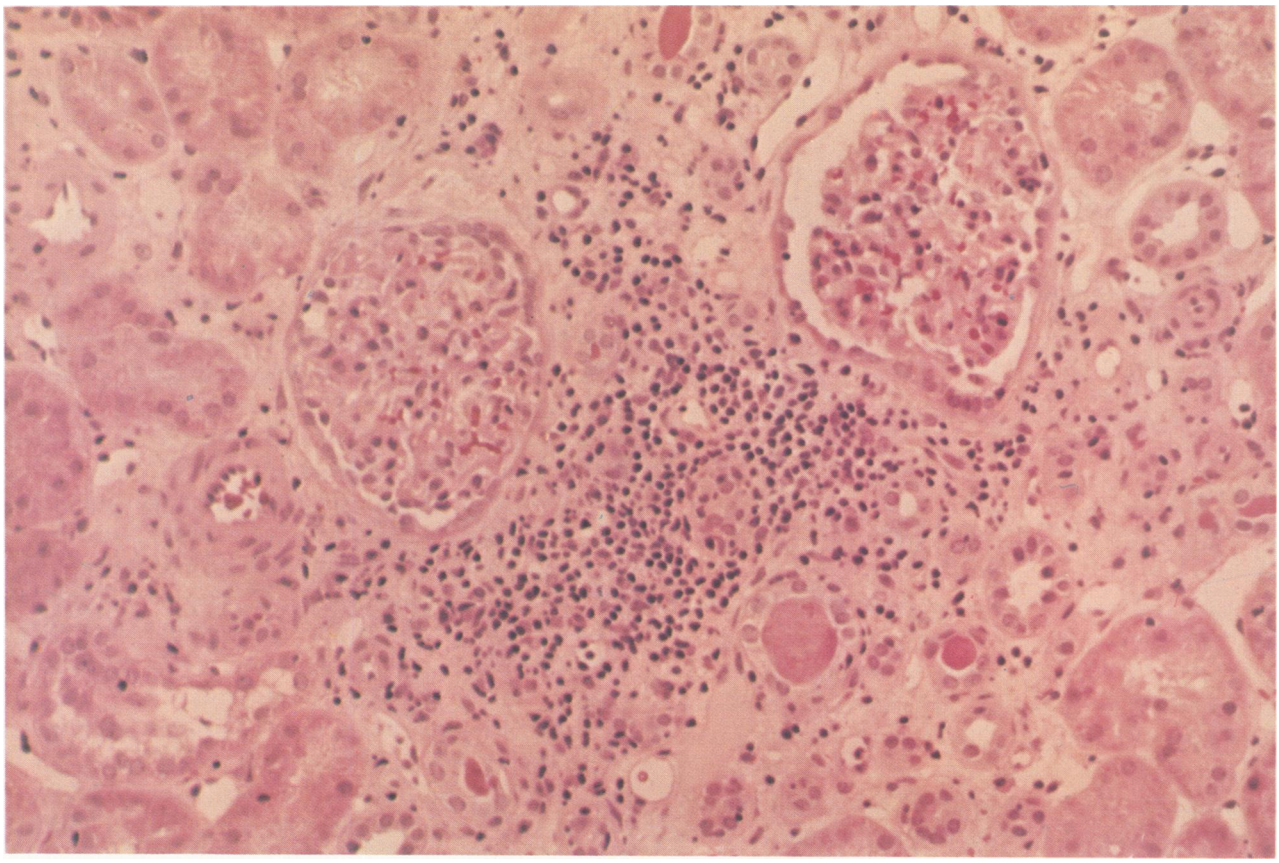

FIG. 1. A renal biopsy of Tx. 26, 29 months post cadaveric transplantation. The patient has maintained excellent renal function without rejection since transplantation in spite of the mononuclear cell infiltrate noted in this specimen.

implicated as a factor in these patients, and there was no definite correlation between the total steroid dosage and the development of aseptic necrosis (Table 9).

Urologic Complications. Five patients developed urologic complications including ureteral necrosis, ureteral perforation and vesico-cutaneous fistulae. None were fatal, and all patients recovered. Two patients with lymphoceles developed ureteral obstruction and hydronephrosis; both were cured after drainage.

Gastrointestinal Complications. Four patients had gastrointestinal complications; two with small intestinal obstruction secondary to adhesions, and one each with gastroduodenal and marginal ulceration with bleeding.

Psychiatric Complications. Two patients required hospitalization for two and four months for psychiatric problems. Both of these individuals recovered, and in neither could steroids be implicated as a causative factor.

Reactions to ATG. Erythema, induration and pain at the injection site were common reactions in all patients receiving ATG. Fever and leukocytosis were almost al-

TABLE 7. *Rejection Crises in 32 Patients*

Interval from Transplantation	No. of Crises
1–7 Days	1
8–30 Days	14
31–60 Days	13
61–90 Days	3
91–180 Days	4
181–365 Days	7
1–2 Years	1
3 Years	1
Total	44

TABLE 8. *Rejection Crises:* Effect upon Renal Function

Type*	No. Patients	No. Rejections
0	18	0
1	12	12
2	15	27
3	1	1
4	1	1
5	3	3

* Type 0—No evidence of rejection.
Type 1—BUN < 40; Cr < 2.0.
Type 2—BUN > 40; Cr > 2.0.
Type 3—As in 1 or 2 but with deranged function for more than 30 days.
Type 4—Reversible but requires dialysis.
Type 5—Irreversible rejection.
12 patients had more than one rejection episode.

TABLE 9. *Complications Excluding Rejection*

Type		No. Patients	
Infection			27
	Bacterial	17	
	Viral	5	
	Fungal	3	
	Protozoan	2	
Skeletal			18
	Aseptic necrosis	14	
	Intertrochanteric fracture	3	
	Pelvic ramus fracture	1	
Urologic			5
Gastrointestinal			4
	Intestinal obstruction	2	
	Gastroduodenal ulceration with bleeding	2	
Psychiatric			2
Lymphocele			2

A number of patients had two or more complications.

ways present and seemed to decrease after the first seven days. There were no anaphylactoid reactions in the 1,207 injections of ATG. Lymphopenia was consistently observed three to five days after the onset of the administration of the ATG and persisted in most instances until the ATG was discontinued. The hematocrit remained stable, and there was no evidence of thrombocytopenia. Lymph nodes adjacent to the iliac vein were biopsied during transplantation and consistently showed paracortical depletion.

Discussion

The critical question relating to the clinical value of equine ATG as an immunosuppressive agent prepared and administered to this series of 50 renal allograft recipients in conjunction with azathioprine and prednisone is difficult to answer. However, certain parameters can be examined in the clinical analysis of these transplant recipients. These include: 1) the quality and duration of renal function correlating these results with the histocompatibility differences between the donor and recipient, 2) the number and severity of rejection episodes, and 3) the frequency and type of infectious complications in the post-transplant course.

TABLE 10. *Microorganisms Causing Disease in Patients After Transplantation*

Organism	No. of Infections
Bacteria	
Diplococcus pneumoniae	2
Staphylococcus aureus	4
Escherichia coli	3
Pseudomonas	3
Other gram negative bacilli	10
Fungus	
Cryptococcus	3
Nocardia	1
Viruses	
Herpes virus hominis	2
Cytomegalovirus	2
Protozoa	
Pneumocystis carinii	3

Since the effects of antigenic disparity between donor and recipient patients can be modified by immunosuppressive agents, the prolonged survival of allografts reflects the efficacy of the immunosuppressive regimen. In this series 40 of 50 patients (80%) have functioning kidneys 12 to 47 months later. More important, only three patients (6%) lost their kidneys as a result of rejection. The remaining seven patients (14%) died with functioning kidneys, four of whom had normal renal function at death. The quality of renal function is encouraging in that 37 of the 40 patients (92%) with functioning kidneys (average 24 months) have a serum creatinine less than 2.0 mg%. If the A and B-matched patients are excluded from the analysis, 22 of 25 patients (eight CD and 14 RLD) have a serum creatinine less than 2.0 (88%). This group of patients represents those with the greatest degree of antigenic disparity.

Allograft rejection was noted 44 times in 32 patients; 18 patients were free from episodes of rejection. Only one patient (TX 54) developed irreversible acute fulminating rejection. Twelve of the rejections were classified as Type 1 (Table 8), and their rejections were easily reversed. This data supports the observation of others[11,15] in that ALG appears to modify the severity of acute rejection. The interval of the rejection crises from time of transplantation did not seem to differ from that noted in other transplant reports.

Infection either at the site of surgery or systemically results in part from the effects of immunosuppressive therapy upon the host defense mechanisms and has been noted in transplant patients receiving various anti-rejection protocols.[1,21] A prospective study of infection complicating renal transplantation has been previously reported from this center.[8] This report emphasized two patterns of post-transplantation infection. One occurred "early" and was associated with compromised anatomic barriers and the pretransplantation presence of *Staphylococcus aureus*. The other variety occurred "late," often following treatment for rejection and associated with significant abnormalities in humoral and cellular immunity. The findings of the present study confirm and amplify these conclusions. In particular, *early* infection has been much less common in the more recent patients, presumably because of greater experience with the procedures and the routine use of antistaphylococcal prophylaxis. Late infection occurring in proximity to augmentation of immunosuppressive therapy has continued to occur but in fewer patients. A recent report by Anderson *et al.*[1] has elevated risk factors in transplanted patients and

noted that the recent decline in infectious death at their center has been due to: 1) the accumulation of extensive experience with infection; and 2) a decrease in the use of high dose prednisone therapy associated with the introduction of antilymphocyte globulin. It has been our impression that the prednisone "sparing effect" of antilymphocyte globulin has decreased the incidence of late infection.

The untoward effects of the ATG, limited to pain, erythema and induration at the site of injection and fever, were similar to those observed previously.[22] In no instance was an anaphylactoid response observed, and all patients completed their course of treatment. The effect of antithymocyte globulin contaminated with antiglomerular basement membrane antibodies upon the glomerular basement membrane of the renal allograft was reported by Thiel.[26] He noted deposition of anti-GBM antibodies by immunofluorescent technique although light and electron microscopy gave no evidence of glomerulonephritis, and the histologic appearance was indistinguishable from transplant glomerulopathy in recipients not treated with ALG. Busch *et al.*[3] observed that horse IgG was present on renal tissue from two patients receiving equine ALG prepared against thoracic duct lymphocytes. One of these two patients developed nephritis possibly secondary to glomerular basement membrane antibody. Additional support regarding this complication has been presented by Chase *et al.*,[4] who demonstrated ultrastructural changes in kidneys of monkeys receiving intramuscular injections of human antithymocyte globulin. Wilson *et al.*[28] reported that lymphocytes from whole organs, i.e. thymus, spleen and lymph node, produced antilymphocyte sera containing glomerular basement membrane antibodies, whereas sera prepared against thoracic duct cells or cultured lymphoblasts did not. A complete immunoflourescent study has not been completed in this series; and therefore, the question of whether or not equine ATG is deposited with regularity upon the glomerular basement membrane remains to be determined. However, examination of renal biopsy material by light microscopy on 17 of 25 patients with excellent renal function (Group I, Table 5) did not reveal evidence of glomerular injury.

The most serious pulmonary complications in this group of patients have been *Pneumocystis carinii* pneumonia, occurring in three patients, two of whom had associated disseminated cytomegalovirus infection. Two of the patients with pneumocystis became ill with normal renal function 10 months and 16 months respectively after transplantation. Cytomegalovirus infection occurred in two additional patients without pneumocystis pneumonia. Both continued to demonstrate the virus even though they remained asymptomatic. The serious nature of these two microorganisms, separate and combined, has been reported.[5,7,21] Herpes simplex, also recognized by others,[16] was most commonly seen in the first three weeks and usually related to the fever associated with the administration of ATG. Warts, considered by Spencer[20] to be associated with viruses, were observed in three patients. Fungal and nocardial infections were recognized but less frequently than that noted by Bach *et al.*[2]

Malignant tumors have been reported in some 7% of all renal transplant recipients[25] with the suggestion that antilymphocyte sera might stimulate the growth of tumors in humans as in animals. The results in this study, although interesting, are too premature for drawing conclusions about the oncologic properties of ALG.

The clinical assessment of ATG in this series of patients, as in those reported by others,[6,11,14,19,22,24] implies that when combined with prednisone and azathioprine, rejection is either avoided or decreased in severity. This statement, although suggestive, cannot be proven conclusively until a double-blind-randomized study has been completed. Such a study still must take into account all of the variables mentioned earlier and analyze the data accordingly. The cadaveric patient, not previously sensitized, appears to be most suitable for such a study.

References

1. Anderson, R. J., Schefer, L., Olin, D. B. and Eickoff, T. C.: Infectious Risk Factors in the Immunosuppressed Host. Am. J. Med., **54**:453, 1973.
2. Bach, M. C., Adler, J. L., Bremen, J., Peng, K. F., Sahyoun, A., Schlesinger, R. M., Madras, P. and Monaco, A. P.: Influence of Rejection Therapy on Fungal and Nocardial Infections in Renal Transplant Recipients. Lancet, **1**:180, 1973.
3. Busch, G. J., Birtch, A. G., Lukl, P., Kobayashi, K., Galvanek, E. G. and Carpenter, C. B.: Human Renal Allografts. Glomerular Deposits of Horse Immunoglobulin G and Nephritis Following Administration of Antilymphocyte Globulin. Hum. Pathol., **2**:299, 1971.
4. Chase, W. H., Paine, L. D., Lamoureux, G. and Taylor, H. E.: Ultrastructural Study of the Glomerulonephritis Produced by Antilymphocyte Globulin in Monkeys. Lab. Invest., **27**:393, 1972.
5. Craighead, J. E., Hanshaw, J. B. and Carpenter, C. B.: Cytomegalovirus Infection after Renal Allotransplantation. JAMA, **201**:725, 1967.
6. Davis, R. C., Nabseth, D. C., Olsson, C. A., Idelson, B. A., Schmitt, G. W. and Mannick, J. A.: Effects of Rabbit ALG on Cadaver Kidney Transplant Survival. Ann. Surg., **176**: 521, 1972.
7. Fine, R. N., Grushkin, C. M., Malekzadeh, M. and Wright, H. T.: Cytomegalovirus Syndrome Following Renal Transplantation. Arch. Surg., **105**:564, 1972.
8. Harris, J. A., Diethelm, A. G., Mason, K. N. and Cobbs, C. G.: Infection Complicating Renal Homotransplantation. Ala. J. Med. Sci., **8**:107, 1971.
9. Iwasaki, Y., Porter, K. A., Amend, J. R., Marchioro, T. L., Zühlke, V. and Starzl, T. E.: The Preparation and Testing of Horse AntiDog and AntiHuman Antilymphoid Plasma

or Serum and its Protein Fractions. Surg. Gynecol. Obstet., **124**:1, 1967.

10. Levey, R. H. and Medawar, P. B.: Some Experiments on the Action of Antilymphoid Antisera. Ann. NY Acad. Sci., **129**:164, 1966.
11. Mannick, J. A., Davis, R. C., Cooperband, S. R., Glasglow, A. H., Williams, L. F., Harrington, J. T., Cavallo, T., Schmitt, G. W., Idelson, B. A., Olsson, C. A. and Nabseth, D. C.: Clinical Use of Rabbit AntiHuman Lymphocyte Globulin in Cadaver Kidney Transplantation. N. Engl. J. Med., **284**:1109, 1971.
12. Monaco, A. P.: Antilymphocyte Serum. *In* Transplantation J. S. Najarian and R. L. Simmons, editors. Philadelphia, Lea & Febiger, 1972.
13. Monaco, A. P., Abbot, W. M., Othersen, H. B., Simmons, R. L., Wood, M. L., Flax, M. H. and Russell, P. S.: Antiserum to Lymphocytes: Prolonged Survival of Canine Allografts. Science, **153**:1264, 1966.
14. Monaco, A. P., Schlesinger, R. and Sayhoun, A.: Experience with Equine AntiHuman Lymph Node Lymphocyte Serum in Renal Transplantation. ALG Therapy and Standardization Workshop, Behring Institute Research Communications, **51**:111, 1972.
15. Monaco, A. P.: Summary Statement of Kidney Transplantation Session. ALG Therapy and Standardization Workshop, Behring Institute Research Communications, **51**:272, 1972.
16. Montgomerie, J. Z., Croxson, M. C., Becroft, D. M. O., Doak, P. B. and North, J. D. K.: Herpes Simplex Virus Infection after Renal Transplantation. Lancet, **2**:867, 1969.
17. Najarian, J. S. and Simmons, R. L.: The Clinical Use of Antilymphocyte Globulin. N. Engl. J. Med., **285**:158, 1971.
18. Pichlmayr, R., Brendel, W. and Zenker, R.: Production and Effect of Heterologous Anti-Canine Lymphocyte Serum. Surgery, **61**:774, 1967.
19. Sheil, A. G. R., Kelly, G. E., Storey, B. G., May, J., Kalowski, S., Mears, D., Rogers, J. H., Johnson, J. R., Charlesworth, J. and Stewart, J. H.: Controlled Clinical Trial of Antilymphocyte Globulin in Patients with Renal Allografts from Cadaver Donors. Lancet, **1**:359, 1971.
20. Spencer, E. S. and Andersen, H. K.: Clinically Evident, Nonterminal Infections with Herpes Viruses and the Wart Virus in Immunosuppressed Renal Allograft Recipients. Br. Med. J., **251**, 1971.
21. Starzl, T. E.: Experience in Renal Transplantation. Philadelphia, W. B. Saunders, 1964.
22. Starzl, T. E., Marchioro, T. L., Hutchinson, D. E., Porter, K. A., Cerilli, G. J. and Brettschneider, L.: The Clinical Use of Antilymphocyte Globulin in Renal Homotransplantation. Transplantation, **5**:1100, 1967.
23. Starzl, T. E., Marchioro, T. L., Porter, K. A., Iwasaki, Y., and Cerilli, G. J.: The Use of Heterologous Antilymphoid Agents in Canine Renal and Liver Homotransplantation and in Human Renal Homotransplantation. Surg. Gynecol. Obstet., **124**:301, 1967.
24. Starzl, T. E., Porter, K. A., Andres, G., Halgrimson, G., Hurwitz, R., Giles, G., Terasaki, P. I., Penn, I., Schroter, G. T., Lilly, J., Starkie, S. J., and Putnam, C. W.: Long Term Survival after Renal Transplantation in Humans: with Special Reference to Histocompatibility Matching, Thymectomy, Homograft Glomerulonephritis, Heterologous ALG and Recipient Malignancy. Ann. Surg., **172**:437, 1970.
25. Starzl, T. E., Penn, I., Putnam, C. W., Groth, C. G. and Halgrimson, C. G.: Iatrogenic Alterations of Immunologic Surveillance in Man and Their Influence on Malignancy. Transplant Reviews, **7**:112, 1971.
26. Thiel, G., Gudat, F., Moppert, J., Zollinger, H. U., Brunner, F., Enderlin, F., and Harder, F.: Follow-Up of Glomerular Changes in Man after Intravenous Administration of Antilymphocyte Globulin Containing Anti-Glomerular Basement Membrane Antibodies (anti GBM-Ab) ALG Therapy and Standardization Workshop, Behring Institute Research Communications, **51**:242, 1972.
27. Traeger, J., Carraz, M., Fries, D., Perrin, J., Saubier, E., Bernhardt, J. P., Revillard, J. P., Bonnet, P., Archimbaud, J., and Brochier, J.: Studies of Antilymphocyte Globulin Made from Thoracic Duct Lymphocytes. Transplantation Proc., **1**:455, 1969.
28. Wilson, C. B., Dixon, F. J., Fortner, J. G., and Cerilli, G. J.: Glomerular Basement Membrane—Reactive Antibody in Antilymphocyte Globulin. J. Clin. Invest., **50**:1525, 1971.
29. Woodruff, M. F. A. and Anderson, N.: Effect of Lymphocyte Depletion by Thoracic Duct Fistula and Administration of Antilymphocyte Serum on the Survival of Skin Homografts in Rats. Nature, **200**:702, 1963.